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INTRAVENOUS SELF-ADMINISTRATION OF MORPHINE AND COCAINE: A COMPARATIVE STUDY

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The aim of the present study was to estimate differences between patterns of morphine and cocaine use in Sprague-Dawley rats. This was done by first developing a set of conditions under which both drugs would be consistently self-administered over time. Subsequently rats were studied in groups of three, with only one rat actively self-administering morphine or cocaine while others two receiving yoked injections of either the drug or saline. With the exception of the 0.056, 0.1, 0.3 and 1.0 mg/kg/inj. training-dose regimens, intravenous *(iv)* self-administration of morphine was acquired at the dose of 0.56 mg/kg/inj. and subsequently maintained by rats. In contrast to morphine self-administration, rats rapidly acquired cocaine self-administration behavior at either the 0.3 or 0.56 injection dose and showed typical inverted U-shaped dose-response curves with maximal responding occurring at the injection dose of 0.3 mg/kg. With the "yoked" pairs of subjects, the rate of responding of the animal actually self-administering the drug was significantly higher than that of a paired animal which passively received injection whenever the first animal self-administered the drug. Thus, both morphine and cocaine served as a positive reinforcer of self-administration behavior under the fixed ratio 5 schedule of reinforcement. However, the 0.56 mg/kg injection dose of morphine resulted in an acquisition curve that was markedly, temporally delayed relative to the injection dose of cocaine. Finally, cocaine maintained higher rates of responding for its delivery than morphine. These differences between self-administration patterns of morphine and cocaine may provide significant information about the nature of drug reinforcement and dependence.

Key words: *morphine, cocaine, self-administration, reinforcement*

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INTRODUCTION

Both opiates (morphine, heroin) and psychomotor stimulants (e.g. cocaine, amphetamine) have potent effects on motivated – that is, goal directed – behaviors [2, 15, 19]. It is generally accepted that drugs abused by humans can function as reinforcers under laboratory conditions [14]. Several experimental techniques utilizing both operant and classical conditioning paradigms provide tools to evaluate drug reinforcement in animals. These methods include drug self-administration, conditioned place preference, and facilitation of intracranial electrical self-stimulation. The techniques differ in several fundamental aspects.

Self-administration is one of the most powerful tools for investigating the manner in which neurochemical and neuropharmacologic processes influence behaviors related to drug reinforcement. In general, a particular behavior or class of behaviors (nose poke, lever press, alley running) emitted by the experimental subject is maintained by the reinforcing stimulus (e.g. oral, intravenous (*iv*), or intracranial drug administration). In other words, the presentation of the reinforcing stimulus engenders, maintains and controls behavior. Inherent in this definition of reinforcement is the contingent relationship between behavior and stimulus presentation (drug administration). Thus, operant responding consists of a three-term contingency between environment stimuli that occasion responding, the response, and the contingent delivery of an environmental event that increases the probability or frequency of the response [14]. The concept of contingency represents an important difference between conditioned place preference and self-administration that is often ignored when comparing results from the two paradigms. Indeed, place conditioning relies on the formation of a classically conditioned association between drug effect and environment and is commonly used to approximate affective states associated with abused drugs [1]. However, the utility of the conditioned place preference procedure is hindered by the lack of dose-dependent effects of tested drugs (i.e. low to moderate doses increase responding and higher doses result in dose-related decreases) [17], and more importantly by the fact that drug administration in this paradigm is not dependent on a response emitted by the subject. Therefore, a strict interpretation of the definition of reinforcement indicates that conditioned place preference is simply not a measure of the reinforcing effects of drugs, but instead may yield insight into conditioned stimulus effects of abused compounds.

Essential to the evaluation of the drug reinforcement in animals is the measurement of the reinforcing efficacy of drugs. Reinforcing efficacy is the degree to which a drug or any other reinforcement maintains behavior. It is not useful to discuss reinforcing efficacy as an absolute value. Rather, reinforcement is discussed in terms of its relative reinforcing efficacy when compared with reinforcement established by other stimuli, different amounts of the same substance, or the same substance under different conditions. The aim of the present study was to estimate the reinforcing efficacy of morphine when compared with cocaine in Sprague-Dawley rats.

MATERIALS and METHODS

Animals

Male Sprague-Dawley rats (Collegium Medicum, Jagiellonian University in Kraków, Poland) weighing approximately 300 g at the start of the experiment were individually housed in a temperature- and humidity-controlled environment under a 12-h light/dark cycle (lights on at 7:00 p.m.). Food and water were available *ad libitum* in the home cage. Rats were trained and tested between 10:00 a.m. and 5:00 p.m. All experimental procedures were conducted in accordance with the guide for care and use of laboratory animals and reviewed by the 2nd Local Animal Care and Use Committee of the Warsaw School of Medicine, Warszawa, Poland.

Drugs

Cocaine hydrochloride and morphine hydrochloride were obtained from Polfa Kutno S.A. (Kutno, Poland) and dissolved in sterile physiological saline. Drug concentration was adjusted daily according to the weight of each rat in order to provide injections of 0.056, 0.1, 0.3, 0.56 or 1.0 mg/kg in a volume of 110 μl/kg over a 2-s period.

Surgery

Catheters were implanted into the right jugular vein under Calypsol (ketamine 50 mg/ml) anesthesia (1.5 ml/kg, *ip*). A small incision was made to

the right of the midline of the neck and the external jugular vein was isolated and opened. A silastic catheter was inserted into the vein and anchored into the neck muscles by sutures. The other end of the catheter was threaded subcutaneously around to the animal's back to exit the skin through a small opening near the midscapular region. A stylet cap was inserted into the distal end of the catheter protruding from the animal's back to prevent its clogging and maintain a closed system. To protect the catheter from being pulled out while the rat was in the self-administration chamber the tether spring was used. It was attached to the harness with Velcro at one end and the swivel at the other. A minimum of seven days of recovery was allowed before initiation of the experiments. On each day following surgery, catheters were flushed with a 0.1 ml saline solution containing heparin (1.25 units/ml) and gentamicin (0.16 mg/kg) to maintain their patency. Catheter patency was tested periodically or whenever an animal displayed behavior outside baseline parameters with the ultrashort-acting barbiturate anesthetic methohexital (10 mg/kg, *iv*) for loss of conscious within 5 s. In addition, the patency of all catheters was verified at the end of the experiment.

Apparatus

Self-administration sessions were conducted in twelve standard operant chambers (Coulbourn Instruments, Allentown, PA) equipped with two nose-poke operanda. Responding on one of the holes (defined as "active") resulted in drug delivery to the animal when schedule requirements were met, whereas responding on the other hole (defined as "inactive") was recorded but not reinforced. Each nose poke produced a brief feedback tone. A house light was on during drug availability but was turned off during the entire injection and time-out periods. The injector system consisted of a fluid swivel (Alice King Chatham, Hawthorne, CA) that was mounted on top of each chamber. One end of the swivel was connected *via* polyethylene tubing encased in a protective stainless steel spring tether to the animal's catheter while the other end of the swivel was connected *via* polyethylene tubing to the fixed speed infusion pump (Razel Scientific Instruments, Stamford, CT). The operant chambers were enclosed in ventilated, sound-attenuating cubicles and controlled by an IBM compatible computer using the Coulbourn Instruments WinLinc Behavioral Experiment Control Software package.

Cocaine self-administration procedure

Rats were allowed to acquire self-administration of cocaine at one of two doses: 0.3 or 0.56 mg/kg/inj. Sessions were conducted Monday to Friday and were 2 h in duration. At the beginning of each session, a priming injection (0.3 or 0.56 mg/kg/inj.) was automatically delivered, which has been shown to elicit drug-seeking in rats with drug self-administration experience [3] and reports of "wanting" or "craving" in experienced human drug users [12]. Once responding was initiated, the number of responses required to produce each injection was gradually increased over a two-week period to a final value of FR-5 (every fifth response produced an injection). Following each injection there was a 30-s time-out period during which responding was recorded but had no programmed consequences. The daily 2-h access to cocaine was continued until the number of active-hole responses per session stabilized to within ±15% for five consecutive days. After approximately three weeks, saline was substituted for cocaine for 6 days followed by testing of cocaine injection doses ranging from 0.1 to 1.0 mg/kg/inj.

Morphine self-administration procedure

Rats were allowed to acquire self-administration of morphine at one of five doses: 0.056, 0.1, 0.3, 0.56 or 1.0 mg/kg/inj. Sessions were conducted Monday to Friday and were 2 h in duration. At the beginning of each session, a priming injection (0.056, 0.1, 0.3, 0.56 or 1.0 mg/kg/inj.) was automatically delivered, which has been shown to elicit drug-seeking in rats with drug self-administration experience [3] and reports of "wanting" or "craving" in experienced human drug users [12]. Once responding was initiated, the number of responses required to produce each injection was gradually increased over a seven-week period to a final value of FR-5 (every fifth response produced an injection). Following each injection there was a 30-s time-out period during which responding was recorded but had no programmed consequences. The daily 2-h access to morphine was continued until the number of active-hole responses per session stabilized to within ± 15% for five consecutive days. After approximately nine weeks, saline was substituted for morphine for 8 days followed by testing of morphine at two injection doses: 0.56 and 0.3 mg/kg/inj. With the 0.56 mg/kg training dose

regimen, animals did not survive the full period of testing and, thus, a dose-response curve was not constructed.

Yoked self-administration procedure

Rats were tested simultaneously in groups of three, with two rats serving as yoked controls that received an injection of either 0.56 mg/kg morphine or saline which was not contingent on responding each time a response-contingent injection of 0.56 mg/kg morphine was self administered by the third paired rat. Additional naive rats received noncontingent injections of cocaine (0.3 mg/kg/inj.) or saline as a consequence of a contingent cocaine injection (0.3 mg/kg/inj.) by a subject in the self-administration condition. Unlike self-administering rats, nose-poke responses by the yoked rats were recorded but had no programmed consequences.

Statistical analysis

Data are presented as group means, and error bars show the standard errors of the means. Data were analyzed using multifactorial analysis of variance (ANOVA) for repeated measures (effects between active and inactive hole responding, effects between sessions, interaction between nose-poke responding and sessions) and *post-hoc* Student's *t*-test comparisons were performed to locate differences between group means.

RESULTS

Acquisition and maintenance of cocaine self-administration

Figure 1 shows the average number of active and inactive hole responses for rats that were allowed to acquire self-administration of cocaine at one of two doses. The number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 11th session of training.

For the data from the 0.3 mg/kg/inj. dosage (top panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,8) = 25.9$, $p < 0.001$] over the 16 sessions [$F(15,120) = 16.4$, $p < 0.001$]. In addition, a significant interaction between nose-poke responding and sessions was found [$F(15,120) = 17.4$, $p < 0.001$]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 7–16 ($p < 0.01$). When saline was substituted for cocaine (sessions 17–22), the number of active nose-pokes decreased progressively over sessions. A one-factor ANOVA for repeated measures indicated significant differences in sessions 17–22 of extinction [$F(6,28) = 13.8$, $p < 0.001$]. Substitution of saline for cocaine did not produce any significant change in the number of inactive nose-pokes [$F(6,28) = 0.96$, n.s.]. Following the extinction test, animals were given access to cocaine at the dose of 0.3 mg/kg/inj. A two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,8) = 30.1$, $p < 0.001$]. The effect of session [$F(5,40) = 1.2$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(5,40) = 1.33$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 23–28. When saline was substituted for cocaine again, a one-factor ANOVA for repeated measures indicated significant differences in sessions 29–34 of extinction [$F(6,28) = 14.7$, $p < 0.001$]. Substitution of saline for cocaine did not produce any significant change in the number of inactive nose-pokes [$F(6,28) = 0.77$, n.s.]. Following this extinction test, animals were given access to cocaine injection doses ranging from 1.0 to 0.1 mg/kg/inj. With the dose of 1.0 mg/kg/inj. (sessions 35–39), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,8) = 46$, $p < 0.001$]. The effect of session [$F(4,32) = 0.44$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(4,32) = 0.27$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 35–39. With the dose of 0.56 mg/kg/inj. (sessions 40–47), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,8) = 328$, $p < 0.001$]. The effect of session [$F(7,56) = 1.1$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(7,56) = 0.9$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 40–47. With the dose of 0.3 mg/kg/inj. (sessions 48–52), a two-factor ANOVA for repeated measures indicated a significant effect between active

and inactive hole responding [F(1,8) = 95, p < 0.001]. The effect of session [F(4,32) = 0.4, n.s.] as well as the interaction between nose-poke responding and sessions [F(4,32) = 0.26, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 48–52. With the dose of 0.1 mg/kg/inj. (sessions 53–57), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [F(1,8) =

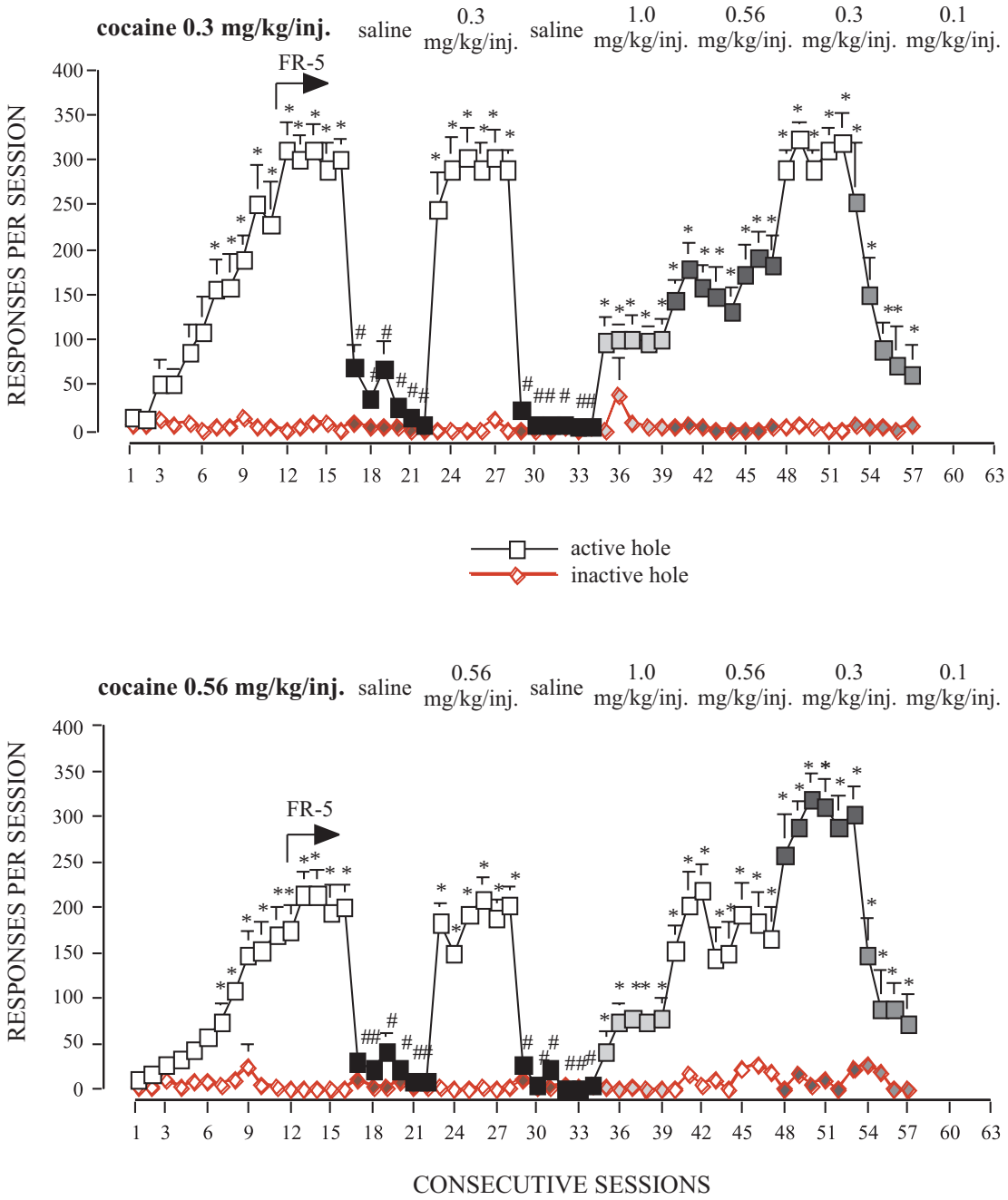

Fig. 1. The mean number (SEM) of responses on the active and inactive holes for various doses of cocaine and saline on each of the daily two-hour sessions. Rats (n = 5–6) were trained to self-administer cocaine at one of two doses: 0.3 mg/kg/inj. (top panel) and 0.56 mg/kg/inj. (bottom panel). The arrow indicates the period when morphine self-administration was maintained under the final FR-5 schedule of reinforcement. Asterisks (*) denote significant differences (p < 0.01) between active and inactive nose-pokes. Number symbols (#) denote significant differences (p < 0.01) in active nose pokes from baseline (sessions 16 or 28) during substitution of saline

11, $p < 0.05$] over the 5 sessions [$F(4,32) = 5.8$, $p < 0.001$]. In addition, a significant interaction between nose-poke responding and sessions was found [$F(4,32) = 5.4$, $p < 0.001$]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 53–57.

For the data from the 0.56 mg/kg/inj. dosage (bottom panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,10) = 27.6$, $p < 0.001$] over the 16 sessions [$F(15,150) = 17.3$, $p < 0.001$]. In addition, a significant interaction between nose-poke responding and sessions was found [$F(15,150) = 18.3$, $p < 0.001$]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 7–16 ($p < 0.01$). When saline was substituted for cocaine (sessions 17–22), the number of active nose-pokes decreased progressively over sessions. A one-factor ANOVA for repeated measures indicated significant differences in sessions 17–22 of extinction [$F(6,35) = 5.8$, $p < 0.001$]. Substitution of saline for cocaine did not produce any significant change in the number of inactive nose-pokes [$F(6,35) = 0.73$, n.s.]. Following the extinction test, animals were given access to cocaine at the dose of 0.56 mg/kg/inj. A two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,10) = 116$, $p < 0.001$]. The effect of session [$F(5,50) = 2.1$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(5,50) = 1.99$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 23–28. When saline was substituted for cocaine again, a one-factor ANOVA for repeated measures indicated significant differences in sessions 29–34 of extinction [$F(6,35) = 15$, $p < 0.001$]. Substitution of saline for cocaine did not produce any significant change in the number of inactive nose-pokes [$F(6,35) = 0.97$, n.s.]. Following this extinction test, animals were given access to cocaine injection doses ranging from 1.0 to 0.1 mg/kg/inj. With the dose of 1.0 mg/kg/inj. (sessions 35–39), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,10) = 74$, $p < 0.001$]. The effect of session [$F(4,40) = 0.2$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(4,40) = 1.27$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 35–39. With the dose of 0.56 mg/kg/inj. (sessions 40–47), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,8) = 44$, $p < 0.001$]. The effect of session [$F(7,70) = 0.46$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(7,70) = 0.28$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 40–47. With the dose of 0.3 mg/kg/inj. (sessions 48–52), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,10) = 69$, $p < 0.001$]. The effect of session [$F(4,40) = 2.2$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(4,40) = 1.3$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 48–52. With the dose of 0.1 mg/kg/inj. (sessions 53–57), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [$F(1,10) = 22$, $p < 0.001$]. The effect of session [$F(4,40) = 2.9$, n.s.] as well as the interaction between nose-poke responding and sessions [$F(4,40) = 1.5$, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 53–57.

Acquisition and maintenance of morphine self-administration

Figure 2 shows the average number of active and inactive hole responses for rats that were allowed to acquire self-administration of morphine at one of five doses. For the 0.56 mg/kg/inj. dosage group, the number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 38th session of training.

For the data from the 0.056 mg/kg/inj. dosage (1st panel), a two-factor ANOVA for repeated measures revealed significant effects between sessions [$F(54,648) = 2.97$, $p < 0.01$] and interaction between nose-poke responding and sessions [$F(54,648) = 1.7$, $p < 0.01$]. The effect of active and inactive hole responding [$F(1,12) = 2.1$, n.s.] failed to reach significance.

For the data from the 0.1 mg/kg/inj. dosage (2nd panel), a two-factor ANOVA for repeated

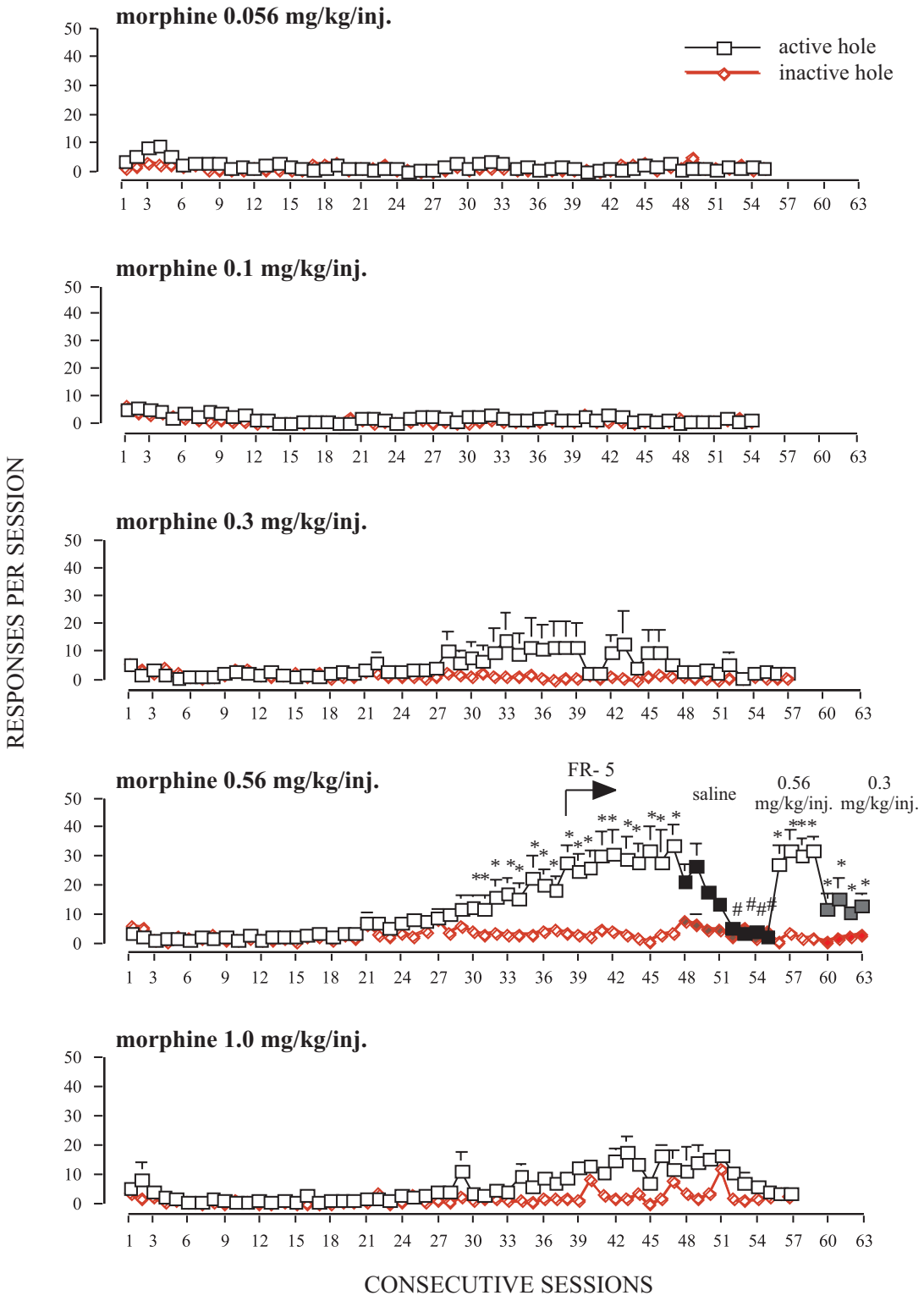

Fig. 2. The mean number (SEM) of responses on the active and inactive holes for various doses of morphine and saline on each of the daily two-hour sessions. Rats (n = 6–8) were trained to self-administer morphine at one of five doses: 0.056 mg/kg/inj. (1st panel), 0.1 mg/kg/inj. (2nd panel), 0.3 mg/kg/inj. (3rd panel), 0.56 mg/kg/inj. (4th panel) and 1.0 mg/kg/inj. (5th panel). The arrow indicates the period when morphine self-administration was maintained under the final FR-5 schedule of reinforcement. Asterisks (*) denote significant differences ($p < 0.01$) between active and inactive nose-pokes. Number symbols (#) denote significant differences ($p < 0.01$) in active nose pokes from baseline (session 47) during substitution of saline

measures revealed significant effects between sessions [F(53,530) = 4.8, p < 0.01]. The effect of active and inactive hole responding [F(1,10) = 1.95, n.s.] as well as the interaction between nose-poke responding and sessions [F(53,530) = 3.6, p < 0.01] failed to reach significance.

For the data from the 0.3 mg/kg/inj. dosage (3rd panel), a two-factor ANOVA for repeated measures did not reveal significant effects between active and inactive hole responding [F(1,10) = 0.88, n.s.] over the 57 sessions [F(56,560) = 0.96, p < 0.01]. In addition, no significant interaction between nose-poke responding and sessions was found [F(56,560) = 1.2, n.s.].

For the data from the 0.56 mg/kg/inj. dosage (5th panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [F(1,14) = 7.93, p < 0.01] over the 47 sessions [F(46,644) = 10.7, p < 0.001]. In addition, a significant interaction between nose-poke responding and sessions was found [F(46,644) = 9.2, p < 0.001]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 30–47 (p < 0.05). When saline was substituted for morphine (sessions 48–55), the number of active nose-pokes decreased progressively over sessions. A one-factor ANOVA for repeated measures indicated significant differences in sessions 52, 53, 54 and 55 of extinction [F(8,63) = 8.3, p < 0.001]. Substitution of saline for morphine did not produce any significant change in the number of inactive nose-pokes [F(8,63) = 0.96, n.s.]. Following the extinction test, animals were given access to morphine at the dose of 0.56 mg/kg/inj. A two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [F(1,14) = 12.1, p < 0.01]. The effect of session [F(3,42) = 1.4, n.s.] as well as the interaction between nose-poke responding and sessions [F(3,42) = 0.28, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 56–59. When animals were given access to morphine at the dose of 0.3 mg/kg/inj. (sessions 60–63), a two-factor ANOVA for repeated measures indicated a significant effect between active and inactive hole responding [F(1,14) = 3.8, p < 0.05]. The effect of session [F(3,42) = 0.38, n.s.] as well as the interaction between nose-poke responding and sessions [F(3,42) = 0.07, n.s.] failed to reach significance. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 60–63.

For the data from the 1.0 mg/kg/inj. dosage (6th panel) a two-factor ANOVA for repeated measures revealed significant effects between sessions [F(56,560) = 2.4, p < 0.01] and interaction between nose-poke responding and sessions

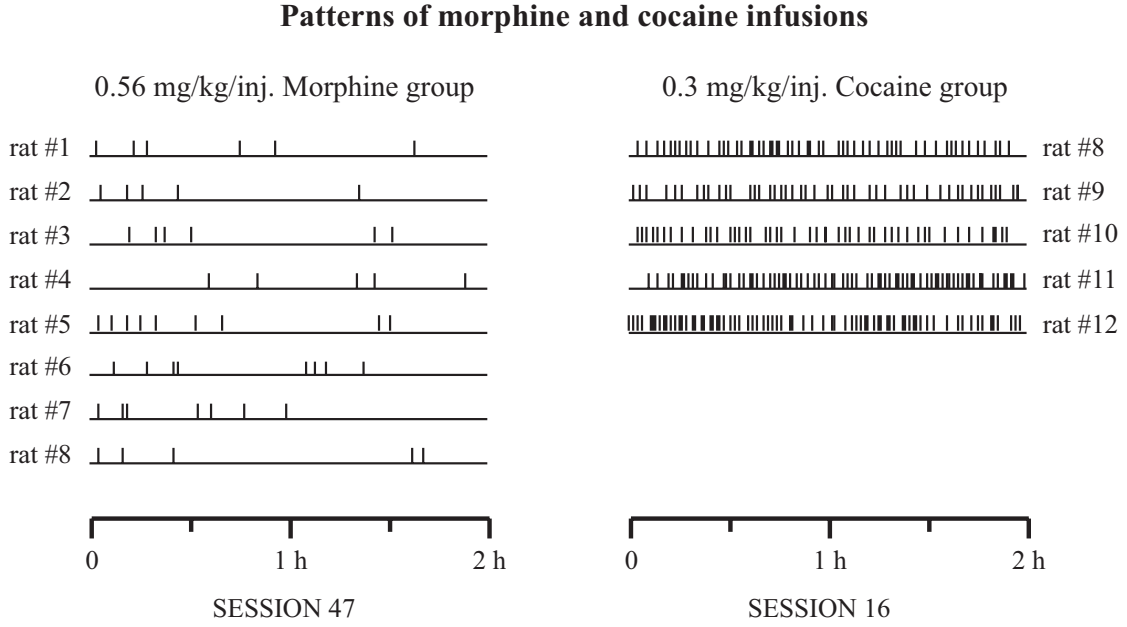

Fig. 3. Typical event records for self-administered injections of morphine and cocaine. The horizontal axis denotes time and each vertical mark represents a single drug infusion delivered after completion of the FR-5 schedule requirement

[F(56,560) = 1.8, p < 0.01]. The effect of active and inactive hole responding [F(1,10) = 3.08, n.s.] failed to reach significance.

Pattern of morphine and cocaine infusions

Figure 3 illustrates spacing of self-administered injections of the 0.56 mg/kg dose of morphine (left panel) or the 0.3 mg/kg dose of cocaine (right panel) on the FR-5 schedule of reinforcement. Morphine self-administration was characterized by alternating irregular periods of drug intake and abstinence during the 2-h session. Cocaine-reinforced responses were equally distributed during the 2-h session.

Dose-response curves for cocaine self-administration

Figure 4 shows the dose-response functions for cocaine self-administration maintained under the final FR-5 schedule of reinforcement.

With the 0.3 mg/kg/inj. training-dose regimen (left panel), the dose-effect curve for cocaine had an inverted-U shape and maximal responding for the active hole occurred at the 0.3 mg/kg/inj. dose [F(4,20) = 26, p < 0.001]. Individual mean comparisons with 0.3 mg/kg/inj. – dose data revealed significant decreases in responding for cocaine at 0.1, 0.56 and 1.0 mg/kg/inj. and for saline (p < 0.01). No statistically significant difference was observed in the number of inactive nose-pokes (data not shown).

With the 0.56 mg/kg/inj. training-dose regimen (right panel), the dose-effect curve for cocaine had an inverted-U shape and maximal responding for the active hole occurred at the 0.3 mg/kg/inj. dose [F(4,25) = 21.4, p < 0.001]. *Post-hoc* comparisons with 0.3 mg/kg/inj. – dose data revealed significant decreases in responding for cocaine at 0.1, 0.56 and 1.0 mg/kg/inj. and for saline (p < 0.01). No statistically significant difference was observed in the number of inactive nose-pokes (data not shown).

Yoked self-administration procedure: contingent vs. noncontingent cocaine administration

Figure 5 shows the average number of active and inactive hole responses for rats actively self-administering cocaine and receiving yoked injections of either cocaine or saline. The number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 15th session of training.

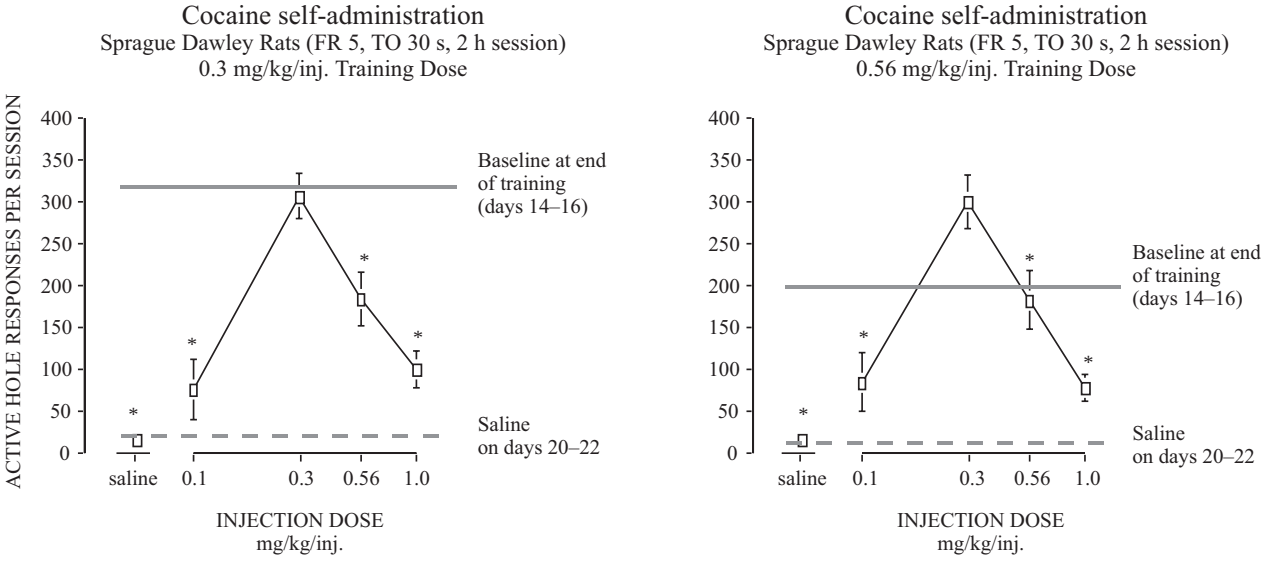

Fig. 4. Dose-response curves for cocaine self-administration under the FR-5, time-out 30-s schedule of reinforcement. Each point represents the mean (SEM) number of responses on the active hole over the last three sessions of testing at a particular dose of cocaine or saline. Asterisks (*) denote significant differences (p < 0.01) in the number of active hole responses from the 0.3 mg/kg/inj. unit dose. Solid horizontal line indicates the mean baseline responding for cocaine over the last three sessions of training. Dotted horizontal line indicates the mean responding for saline over the last three sessions of extinction

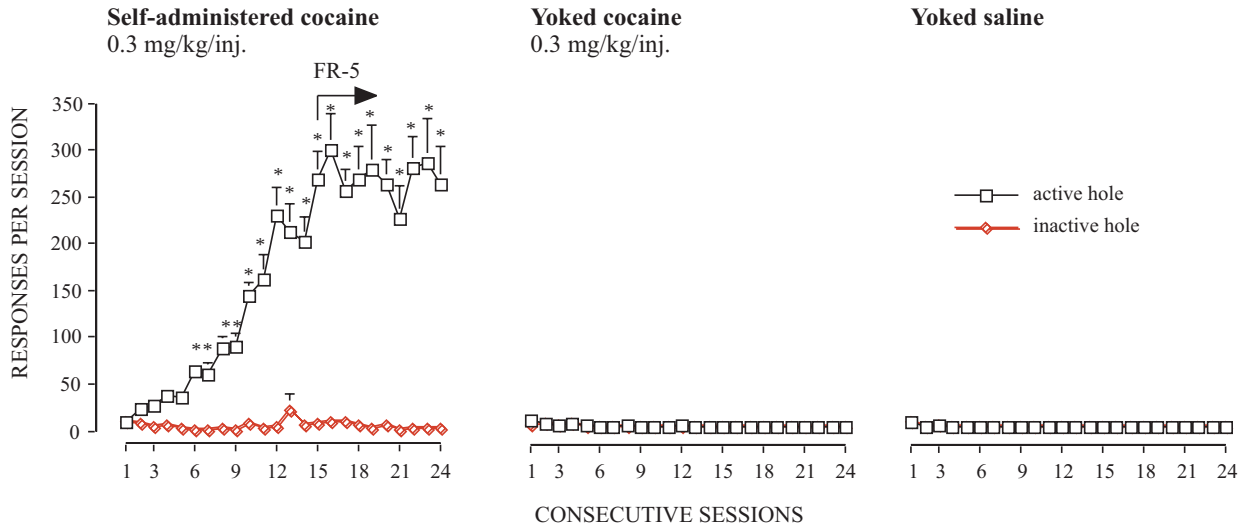

Fig. 5. The mean number (SEM) of responses in the active and inactive holes for rats that were allowed to acquire self-administration of cocaine at a dose of 0.3 mg/kg/injection (n = 7) and their littermates that received yoked infusions of cocaine (n = 7) or saline (n = 7) during each of the daily two hour sessions. The arrow indicates the period when cocaine self-administration was maintained under the final FR-5 schedule of reinforcement. Asterisks (*) denote significant differences ($p < 0.01$) between active and inactive nose-pokes

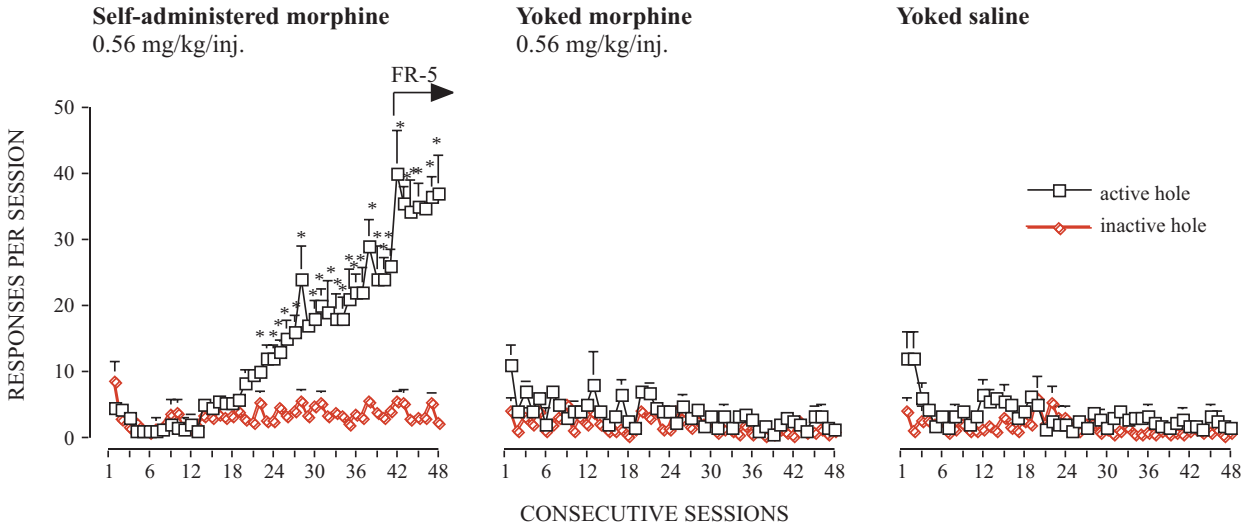

Fig. 6. The mean number (SEM) of responses in the active and inactive holes for rats that were allowed to acquire self-administration of morphine at a dose of 0.56 mg/kg/injection (n = 7) and their littermates that received yoked infusions of morphine (n = 7) or saline (n = 7) during each of the daily two hour sessions. The arrow indicates the period when morphine self-administration was maintained under the final FR-5 schedule of reinforcement. Asterisks (*) denote significant differences ($p < 0.01$) between active and inactive nose-pokes

For the data from the cocaine self-administration group (left panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [$F(1,12) = 102$, $p < 0.001$] over the 24 sessions [$F(23,276) = 27$, $p < 0.001$]. In addition, there was an overall significant interaction between nose-poke responding and sessions [$F(23,276) = 26.7$, $p < 0.001$]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 6–24 ($p < 0.01$).

For the data from the yoked cocaine group (middle panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [$F(23,276) = 7.2$, $p < 0.001$]. The effect between

active and inactive hole responding [F(1,12) = 2.2, n.s.] as well as the interaction between nose-poke responding and sessions [F(23,276) = 2.9, n.s.] failed to reach significance.

For the data from the yoked saline group (right panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [F(23,276) = 14.4, $p < 0.001$]. The effect between active and inactive hole responding [F(1,12) = 0.65, n.s.] as well as the interaction between nose-poke responding and sessions [F(23,276) = 0.3, n.s.] failed to reach significance.

Yoked self-administration procedure: contingent vs. noncontingent morphine administration

Figure 6 shows the average number of active and inactive hole responses for rats actively self-administering morphine and receiving yoked injections of either morphine or saline. The number of responses required to produce each injection was increased over days, reaching a final value of 5 (fixed-ratio 5 schedule of drug injection; FR-5) by the 42nd session of training.

For the data from the morphine self-administration group (left panel), a two-factor ANOVA for repeated measures revealed significant effects between active and inactive hole responding [F(1,12) = 87, $p < 0.001$] over the 48 sessions [F(47,564) = 13.8, $p < 0.001$]. In addition, there was an overall significant interaction between nose-poke responding and sessions [F(47,564) = 11.7, $p < 0.001$]. *Post-hoc* analysis revealed that a significant preference for the active hole occurred on sessions 23–48 ($p < 0.01$).

For the data from the yoked morphine group (middle panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [F(47,564) = 2.8, $p < 0.001$]. The effect between active and inactive hole responding [F(1,12) = 0.12, n.s.] as well as the interaction between nose-poke responding and sessions [F(47,564) = 1.27, n.s.] failed to reach significance.

For the data from the yoked saline group (right panel), a two-factor ANOVA for repeated measures indicated a significant effect of sessions [F(47,564) = 3.45, $p < 0.001$]. The effect between active and inactive hole responding [F(1,12) = 0.7, n.s.] as well as the interaction between nose-poke responding and sessions [F(47,564) = 0.39, n.s.] failed to reach significance.

DISCUSSION

The present data confirm earlier reports that both morphine and cocaine can serve as a positive reinforcer in rats *via* the *iv* route of administration [22, 25]. The use of procedures employing response-dependent drug administration, instead of response-independent drug administration, is critical to furthering our understanding of the neurobiologic underpinnings of drug reinforcement. There is general concordance between substances that are abused by humans and those that are self-administered in the laboratory by experimental animals [10]. A variety of species have been shown to acquire behaviors maintained by the delivery of drug injections under a variety of schedules of reinforcement [17]. The self-administration procedure allows the study of both the primary reinforcing effects of drugs and the conditioned reinforcing effects of associated stimuli [7, 8]. Finally, since addiction offers an interesting model of motivated – that is goal directed behavior – the self-administration procedure allows the separation of motivational aspects of drugs of abuse from their other effects using yoked pairs of animals [20, 21]. While not a disadvantage of the self-administration procedure *per se*, over interpretation of data is a common pitfall. Reinforcement does not explain drug addiction: simply, it allows for quantification of the initiation and maintenance of a response occurring in the presence of specific stimuli and resulting in the presentation of the reinforcing stimulus. Other factors such as learning, memory, and performance may alter both the acquisition and expression of reinforcement.

Reinforcing effects of cocaine

Rats rapidly acquired cocaine self-administration behavior at one of two doses per injection (0.3 or 0.56 mg/kg) and showed typical inverted U-shaped dose-response curves. Acquisition of self-administration, defined as development of a significant preference for responding in the active hole that resulted in cocaine injection vs. the inactive hole, occurred more readily at the lower dose per injection of 0.3 mg/kg cocaine. The higher 0.56 mg/kg injection dose of cocaine resulted in an acquisition curve that was temporally delayed relative to the lower injection dose of 0.3 mg/kg. These findings with cocaine injection does not support the hypothesis developed with non-drug reinforcers that

reinforcement efficacy and speed of acquisition of a response to obtain a reinforcing agent are directly related [11]. Following the initial acquisition period, drug intake remained relatively constant. Rats showed stability in their daily intake and there were no indications of any further changes in sensitivity (tolerance or sensitization) to cocaine's reinforcing effects as would be indicated by an increase or a decrease in its rate of self-administration.

Under fixed-ratio of reinforcement, injections of cocaine and a variety of other drugs which function as positive reinforcers appear to be equally spaced across the experimental session [6, 24, 26] but this is a function of dose and access conditions and training history [5, 18]. In the present study, event records of fixed-ratio cocaine self-administration showed stability of intake within sessions; cocaine self-administration was characterized by equally spaced injections during the 2-h session.

Since intermittent exposure of rats to cocaine causes sensitization to its locomotor activity and behavioral stereotypy effects [13], it would not be surprising to find that cocaine self administration over the 16-day acquisition period could result from increases in general behavioral activity rather than from a specific reinforcement effect, particularly at the higher training dose of 0.56 mg/kg/inj. with a daily average intake of 22.4 mg/kg cocaine. Indeed, at the end of each session, hyperlocomotion with prolonged multiple stereotypical behaviors, including continuous downward-directed sniffing, licking, side-to-side head waving, and forepaw movement was observed when the daily cocaine intake was 20 mg/kg or higher. However, in the rats with the 0.3 mg/kg/inj. training dose of cocaine, average daily intake was lower (17.8 mg/kg) and the alterations in behavioral activities described above were less expressed, but selective responding in the active hole was acquired and subsequently maintained over time with cocaine.

Several approaches to ascertaining that drug self-administration is specifically due to the reinforcing effects of a drug are to show that drug injections maintain higher rates of responding than vehicle injections, that responding is sensitive to changes in drug dose, and that there is selective maintenance of drug-reinforced behavior [17]. When saline was substituted for cocaine, responding decreased markedly over several sessions and higher rates of responding occurred when injections of cocaine were reinstated. Secondly, when the dose per injection of cocaine was varied there were dose-related changes in responding. Responding in both groups of rats was maintained almost exclusively in the active hole, with responding increasing slightly in the inactive hole only during the first few days of extinction. Finally, the present experiment utilized a "yoked" procedure in which rats receive noncontingent (response-independent) infusions of cocaine or saline as a consequence of a contingent (response-dependent) cocaine infusion by a subject in the self-administration condition. Higher rates of responding were found in a group in which cocaine was contingent upon responding compared to one in which the drug was administered passively. Thus, self-administration of cocaine in the present experiment did appear to be maintained by the response-drug infusion contingency, and, therefore, was a reinforcement effect.

In animal drug self-administration studies, response rates are usually an inverted U-shaped function of drug dose and, within the range of doses which maintain drug self-administration responding in rats, rate of responding and frequency of injection are usually inversely related to injection dose [16]. In the present study, typical inverted U-shaped dose-response curves were obtained in rats that initiated cocaine responding at either the 0.3 or 0.56 injection dose, with maximal responding occurring at an injection dose of 0.3 mg/kg. Decreasing per injection dose below 0.3 mg/kg or increasing dose per injection to 1.0 mg/kg produced a decrease in the number of responding down to the saline level. When dose-response curves were constructed, mean intake of cocaine remained relatively constant while dose per injection was varied tenfold from 0.1 to 1.0 mg/kg doses.

Reinforcing effects of morphine in comparison with cocaine

With the exception of the 0.056, 0.1, 0.3 and 1.0 mg/kg/inj. training-dose regimens, self-administration of morphine was acquired (as defined by significant differences in responding in active vs. inactive holes) and subsequently maintained by rats. Nose-poke responding was a function of contingent morphine injection because (i) there was selective responding in an active vs. an inactive hole, (ii) morphine injections maintained higher rates of responding that saline injections, (iii) responding was sensitive to changes in morphine dose, and, finally, (iv) with the pairs of subjects, the rate of respond-

ing of the animal actually self-administering morphine was significantly higher than that of a paired animal which passively received injection whenever the first animal self-administered morphine. Thus, morphine served as a positive reinforcer of self-administration behavior with maximal responding occurring at an injection dose of 0.56 mg/kg under the present experimental conditions.

The characteristics of acquisition and pattering of self-infusions are distinct for the opiate drugs and for the stimulant cocaine. The 0.56 mg/kg injection dose of morphine resulted in an acquisition curve that was temporally delayed relative to the injection dose of cocaine. While cocaine-reinforced responses were equally distributed, morphine self-administration was characterized by alternating irregular periods of drug intake and abstinence during the 2-h session. Finally, cocaine maintained higher rates of responding for its delivery than morphine. The rate of opiate self-administration is evidently determined by plasma levels of the drug [15]. For example, pretreatment of animals with morphine, codeine, or meperidine reduces *iv* self-administration of morphine. In contrast, pretreatment with naloxone increases the response rate of morphine self-administration to a rate similar to that seen during morphine abstinence. It is evident from these studies that the animals learn to regulate with some accuracy the amount of morphine that they require [15].

The present results are consistent with previous reports that the total numbers of responses reinforced by cocaine were markedly higher than the number of operant responses maintained by heroin [4]. It has been postulated that separate neuronal systems mediate the reinforcing effects of opiates and psychostimulant drugs [4]. Studies with selective receptors antagonists suggest that dopaminergic receptors are involved in the mediation of reinforcing effects of psychostimulants [3] while opiate receptors are implicated in the neuronal mediation of opiate drug reinforcement [9, 23]. Ettenberg et al. [4] reported that non-selective dopamine receptor antagonist, alpha flupentixol, did not increase *iv* self-administration responding for heroin, nor did naltrexone increase cocaine-reinforced operant responding. The specificity with which the two antagonists exerted their behavioral effects suggests that separate neuronal substrates are responsible for the reinforcing actions of heroin and cocaine.

Thus, in the present experiment drug reinforcement was established rapidly with cocaine that was absorbed quickly, and this drug yielded faster acquisition rates than morphine that was absorbed more gradually and had less intense effects, suggesting that cocaine had a greater reinforcing efficacy that morphine. These differences between self-administration patterns of opiate and cocaine may provide significant information about the nature of drug reinforcement and dependence.

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