*Pharmacology Biochemistry & Behavior*, Vol. 24, pp. 1429–1440, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

# Neuropharmacological Assessment of Cocaine Self-Administration Into the Medial Prefrontal Cortex[1]

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GOEDERS, N. E., S. I. DWORKIN AND J. E. SMITH. *Neuropharmacological assessment of cocaine self-administration into the medial prefrontal cortex.* PHARMACOL BIOCHEM BEHAV **24**(5) 1429–1440, 1986.—Neuronal systems involved in the initiation of reinforcement following the response-contingent delivery of cocaine into the medial prefrontal cortex were investigated. Dose-effect analyses demonstrated that different concentrations of cocaine result in distinguishable patterns of self-administration which could be empirically determined by measuring the relative frequency distribution of the interinfusion intervals. The substitution of equimolar *d*-amphetamine or lidocaine resulted in rates and patterns of responding similar to vehicle or a low dose of cocaine, suggesting that reinforcement occurs from actions on specific receptors rather than through a local anesthetic neuronal blockade or through properties of a general psychomotor stimulant. The co-infusion of equimolar concentrations of sulpiride attenuated intake and produced patterns of responding similar to those seen after decreasing the cocaine dose consistent with an excitatory role for $D_2$ dopaminergic receptors in these processes. Sulpiride and cocaine may act at separate sites since the decreased intake was not reversed by increasing the concentration of cocaine. $D_1$ dopaminergic, muscarinic-cholinergic and beta-noradrenergic receptor antagonists either did not modulate drug-intake or had minimal effects. Cocaine reinforcement may result in part from an activation of $D_2$ receptors initiating neuronal activity in pathways or circuits mediating reinforcement processes.

Intracranial self-administration Cocaine reinforcement Medial prefrontal cortex Dopamine

THE illicit use of cocaine has rapidly increased during the last decade [23]. Behavioral studies have demonstrated that the response-contingent administration of the drug is reinforcing and will maintain long and complex sequences of behavior by animals and humans under various schedules of reinforcement [14, 15, 21, 22, 24, 44, 56]. Self-administration is thought to result from a drug-induced activation of receptors which initiate reinforcing neuronal activity. Although the effects of the drug on neurotransmission are complex, the primary neurochemical action appears to be an inhibition of biogenic amine neurotransmitter uptake into presynaptic nerve endings. Cocaine decreases the reuptake of norepinephrine [10, 25, 52], dopamine [26, 35, 41] and serotonin [54,58] with no effect on release except at high concentrations [8,53]. In addition, dopamine and acetylcholine turnover rates are increased [5,43] while norepinephrine and serotonin are decreased [2,16] following cocaine administration. While it has yet to be conclusively elucidated whether or not any of these effects on neurotransmission are involved in the neuronal events responsible for the reinforcing properties of cocaine, intravevous self-administration experiments suggest an important function for dopaminergic neurons. The administration of pimozide [9], haloperidol [7] and alpha-flupenthixol [13] (dopaminergic receptor antagonists) modulate cocaine-maintained responding by rats and rhesus monkeys in a dose-related manner, suggesting an attenuation of reinforcing efficacy. Moreover, 6-hydroxydopamine lesions of the nucleus accumbens disrupt cocaine self-administration [47,48] as do similar lesions of the ventral tegmental area where the cell bodies for the mesolimbic/mesocortical dopaminergic neuronal system are localized [49]. However, the magnitude of attenuation after the ventral tegmental area lesion does not correlate with decreases in neurotransmitter content in the nucleus accumbens suggesting that dopaminergic innervations of other structures (i.e., medial prefrontal cortex or olfactory tubercle) are necessary for reinforcement [49].

The identification of specific cocaine binding to presynaptic dopaminergic nerve terminals in brain tissue [33, 45, 51] and the inhibition of this binding by endogenous peptides [27,46] suggest that specific receptors may initiate reinforcing neuronal activity following the response-contingent delivery of the drug. The initiation and mediation of this activity may be through separate and distinct neuronal populations. Different reinforcers may ultimatley result in the activation of a subset of neuronal pathways participating in reinforcement circuits (demonstrated with electrical brain stimulation reinforcement), with the distinction between these stimuli partially dependent upon the discrete inputs activated. The magnitude of reinforcement may be related to

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the magnitude, duration and spread of neuronal activity within these circuits, which may in turn be directly related to the modulation of this activity by different variables (e.g., deprivation, satiation, behavioral history, etc.). Identification of sites where the drug initiates reinforcing neuronal activity is necessary for a complete understanding of the basic mechanisms of cocaine reinforcement. The direct intracranial self-administration of cocaine into the medial prefrontal cortex of rats has been recently demonstrated [17], suggesting reinforcement-relevant cocaine receptors to be localized there. The experiments reported here were designed to investigate the neurotransmitter receptors responsible for this behavior using neuropharmacological methodologies. Individual dose-response relationships were evaluated to assess the effects of dopaminergic, cholinergic and noradrenergic neurotransmitter receptor antagonists on drug-intake. A sympathomimetic and local anesthetic drug were also substituted to determine if the self-administration was specific for cocaine.

## METHOD

### *Subjects*

Ten experimentally naive male Fischer 344 rats that were initially 90 to 150 days old were used in this experiment. The animals were housed in individual cages on a reversed 12-hour light-dark cycle (light onset at 20:00 hr) with unlimited access to food (Purina Rat Chow) and water.

### *Apparatus*

Rectangular, Plexiglas (with metal end panels) operant-conditioning chambers (29×27×30 cm) contained inside ventilated sound-attenuated enclosures were used for behavioral sessions. A red stimulus light was located 4 cm above the lever (V3-101 Micro Switch) which was positioned in the center of the side panel 5 cm from the floor. The conditioning chamber also contained a tone source and a white light mounted 6.5 cm to the left of the red light.

Intracranial microinfusions were delivered with an electrolytic microinfusion system [1,6] which mounted directly onto the guide cannula. Microinjections were produced by passing a direct current between a silver anode and platinum cathode in an air-tight drug reservoir with the resulting evolution of hydrogen gas forcing a reproducible amount of drug solution out through a 28-gauge injection cannula extending 0.5 mm beyond the tip of the guide cannula. The amount of solution injected was directly proportional to the volume of hydrogen gas evolved, which was in turn proportional to the current intensity and duration. This system was calibrated to deliver consistent 100±7 nanoliter volumes with a 200 $\mu$A injection current over 5 seconds [19]. A small quiescent current (6 $\mu$A) prevented the redissolution of hydrogen evolved during the experimental session. A flexible spring-covered lead connected the microinjection system to a two-channel mercury commutator (Mercotac, Inc.) which was counterbalanced and attached to the conditioning chamber to permit relatively unrestrained movement of the animal during testing.

### *Surgery*

Sodium pentobarbital anesthesia (50 mg/kg, IP) was used during surgery. The animals were stereotaxically implanted with unilateral 22-gauge guide cannulae (Plastic Products Co.) into the right medial prefrontal cortex (10.05 mm anterior to lambda, 0.6 mm from the midline, 2.1 mm ventral to dura) [36] using a modification of a previously described procedure [42]. The guide cannulae were inserted 2.1 mm below the surface of the brain and secured to the skull using dental acrylic and 00 stainless steel screws. A 28-gauge dummy cannula (Plastic Products Co.), extending 0.5 mm beyond the tip of the guide cannula, was inserted into each to prevent infections or formation of obstructions and remained in place except during testing. The animals were injected with penicillin G procaine suspension (75,000 units, IM) and were allowed a minimum of two weeks to recover.

TABLE 1

DOSING SEQUENCE FOR EQUIMOLAR ANTAGONIST ADMINISTRATION

| Rat No. | Dosing Sequence |
|---|---|
| 14-A | sulpiride, atropine, SCH 23390 |
| 18-A | atropine, propranolol |
| 18-C | sulpiride, atropine, propranolol |
| 20-C | sulpiride, SCH 23390 |
| 20-E | SCH 23390 |
| 23-A | propranolol |

### *Procedure*

The rats were tested for intracranial self-administration every third day at the start of their active cycles (08:00 to 09:00). Sessions were terminated either after 8 hours or after 40 injections were delivered to minimize potential cumulative cytotoxic effects. A red light above the response lever was illuminated at the start of the session. Each response in the presence of the red light resulted in a 5-second infusion of 100 nanoliters of drug. During the infusion, the red light was extinguished, the white light illuminated, and a tone presented. Each microinfusion was followed by a 30-second time-out period during which the stimulus lights were both extinguished and responses monitored but having no scheduled consequences. Experimental sessions were controlled by a Rockwell Aim 65 Microcomputer with a Microcomputer Control System (Micro Interfaces, Inc.).

### *Drugs*

The composition of the vehicle (artificial cerebrospinal fluid (CSF)), expressed as grams/liter, was: NaCl, 8.10; KCl, 0.25, $CaCl_2$, 0.14; $MgCl_2$, 0.11, $NaHCO_3$, 1.76, $NaH_2PO_4$, 0.07; urea, 0.13; glucose, 0.61; pH 5.7. The initial dose of cocaine HCl (Mallinckrodt) investigated in all subjects was 50 pmol/100 nl infusion. When lever-pressing stabilized for at least three consecutive sessions at this dose, the concentration was increased or decreased by 5 to 25 pmol with the magnitude of each dose manipulation determined by comparing the effects of previous changes in concentration on self-administration. If the behavior was sensitive, then the dose was modified by only 5 pmol. If rates of self-administration were less affected, then larger manipulations were made (15 to 25 pmol). Dose-response curves were determined for each animal in this manner with a minimum of three sessions between each dose change until drug-intake decreased to vehicle levels at both the higher and lower concentrations. Equimolar concentrations of *d*-amphetamine sulfate (Sigma) or lidocaine HCl (Astra) and nine times equimoar concentra-

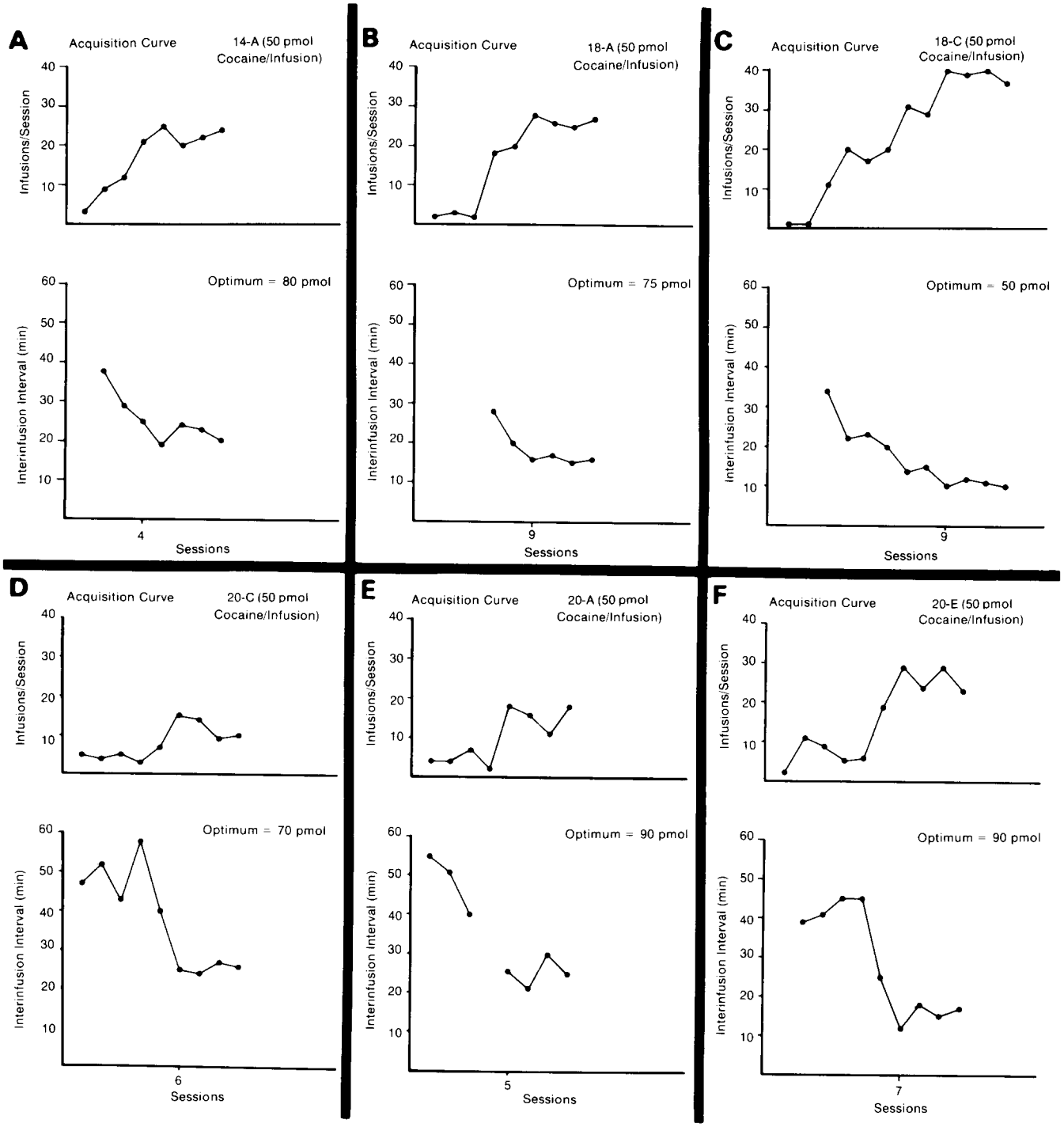


FIG. 1. Acquisition curves for responding maintained by microinfusions of cocaine (50 pmol/100 nl) into the medial prefrontal cortex for the six rats (A thru F) that completed the experimental protocol. Infusions per session and the average interinfusion intervals are shown for each rat. Interinfusion intervals were not calculated (and are not shown in the figure) when less than three infusions were delivered during a session since these data do not represent actual rates of self-administration. Animal identification numbers (i.e., 14-A) are listed as well as the session number when stable rates of self-administration were obtained (i.e., the fourth session for rat 14-A). The optimum dose of cocaine derived for each animal in subsequent dose-response evaluations (i.e., 80 pmol for animal 14-A) is also presented since maximal rates of self-administration were not always maintained by the training dose (50 pmol). For example, the average number of infusions resulting from the training dose was 25±3 for rat 14-A compared to 40±0 (the maximum number allowed) at 80 pmol.

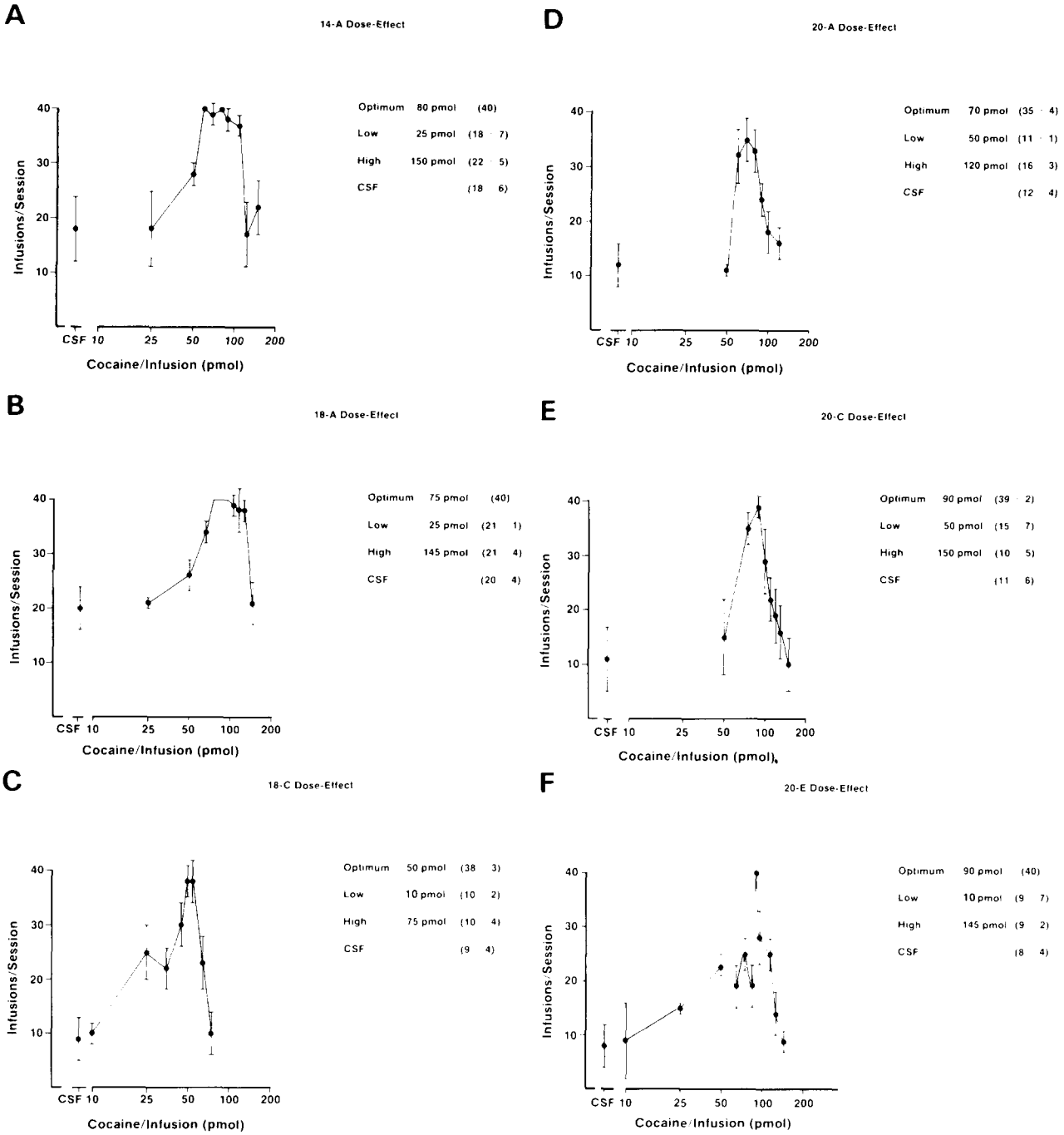


FIG. 2. Dose-response curves for the intracranial self-administration of cocaine into the medial prefrontal cortex of the six rats (A–F) that completed the experimental protocol. Animal identification numbers, (i.e., 14-A) and the average number of infusions (±standard deviations) resulting from exposure to CSF and low, optimum and high doses of cocaine are listed for each animal.

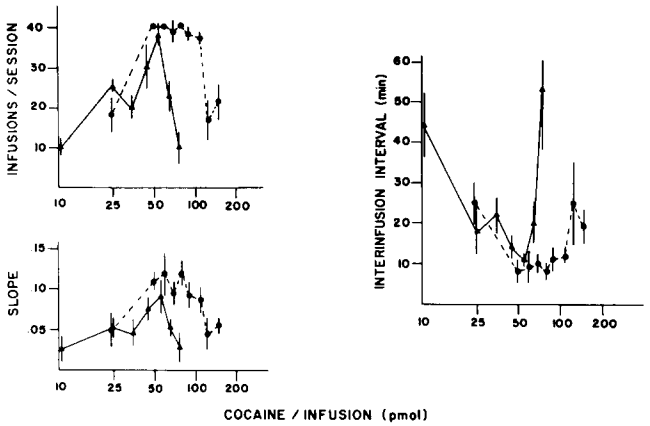


FIG. 3. Dose-response curves for the intracranial self-administration of cocaine into the medial prefrontal cortex of two rats, 14-A (●) and 18-C (▲), expressed as total infusions per session, the slope of the plot of the cumulative infusion intervals and the mean interinfusion interval. Points are means (±standard deviations) for three sessions at each concentration. Corresponding changes in each parameter occurred when the concentration was increased or decreased from that resulting in maximum rates of drug-intake.

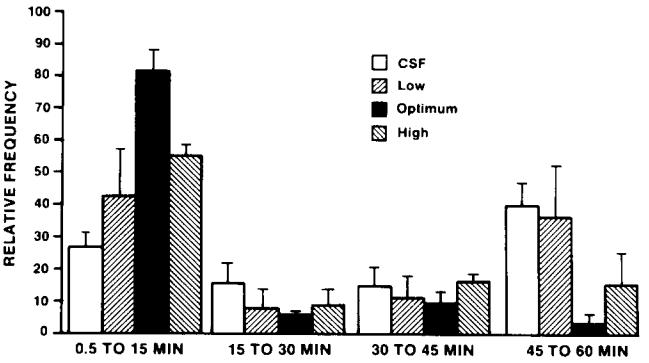


FIG. 4. Mean relative frequency of the interinfusion intervals for the intracranial self-administration of cocaine into the medial prefrontal cortex of six rats. Optimum represents the concentration of cocaine that resulted in maximum rates and consistent drug-intake (50 to 90 pmol), while low and high represent the lowest (10 to 50 pmol) and highest (75 to 150 pmol) concentrations of the drug respectively that maintained responding in each animal. Values are the mean percentage of total interinfusion intervals that occurred during each 15 min division (±standard error of the mean) for three days of stable responding at each dose for each of the 6 animals. The significance of differences between the doses and vehicle were assessed using Tukey's all pairwise comparisons among treatment means (Table 2) and were most evident in the first and fourth divisions. In the first division, all comparisons among the relative frequency distributions were significantly different ($p<0.05$), while in the fourth division, all comparisons except low vs. CSF and optimum vs. high were significantly different ($p<0.05$). The patterns of self-administration are clearly different for CSF and these doses of cocaine.

tions of licocaine HCl were substituted twice in two rats for the optimum dose of cocaine (the dose resulting in the highest and most stable rates of drug-intake).

Equimolar concentrations of the $D_2$ dopaminergic receptor antagonist sulpiride (Delagrange), the $D_1$ dopaminergic receptor antagonist SCH 23390 (Schering), the beta-noradrenergic receptor antagonist dl-propranolol HCl (Sigma) or the muscarinic cholinergic receptor antagonist atropine sulfate (Sigma) were administered in a random order (Table 1) with the optimum dose of cocaine. Each antagonist was included in the microinjection systems of three rats for two experimental sessions which were separated by a minimum of three sessions of stable responding maintained by the optimum cocaine dose (variations in drug-intake <10%). If an attenuation of self-administration was observed, the concentration of cocaine was doubled and the effects of the antagonist again determined. If no change in intake occurred, then the concentration of the antagonist was doubled and the effects again determined.

### *Data Analysis*

Several parameters of self-administration were monitored, including the total number of infusions, the average duration between successive microinjections (mean interinfusion intervals) and the slope of the plot of the cumulative infusion intervals. These slopes were calculated using a linear regression analysis for the best fit between the data points generated by comparing the infusion number with the total elapsed time since the first infusion and were representative of the rate of self-administration (i.e., the larger the slope, the greater the rate). The relative frequency of the interinfusion intervals was also determined as an empirical measure of the pattern of self-administration. The intervals were counted separately in four 15-minute divisions (0 to 15 minutes, 15 to 30 minutes, 30 to 45 minutes and 45 to 60 minutes) and a frequency distribution was generated for each session. Relative frequency was calculated by dividing the number of infusions in each division by the total number of infusions taken in that session and multiplying by 100. The significance of difference in the relative frequency distribution of interinfusion intervals for the various treatment conditions was evaluated with a two-factor randomized analysis of variance [11] followed by Tukey's pairwise comparisons among treatment means [31]. These parameters were used to correlate changes in self-administration with the experimental manipulations.

## RESULTS

### *Dose-Effect*

All ten rats intracranially self-administered cocaine, with an average of six behavioral sessions required to engender stable rates of self-administration (Fig. 1). Four rats dislodged their guide cannulae before the completion of the dose-response sessions. An average of 62 sessions were necessary to complete dose-response studies for the remaining six animals and an additional 40 sessions for the drug substitution and antagonist experiments.

Self-administration was dose-related with maximal rates of responding obtained with 50 to 90 pmol/infusion (Fig. 2). Dose-response relationships were unique for each animal with responding maintained over either narrow or wide ranges in different subjects. Large changes in drug-intake often occurred after increasing or decreasing the concentration by as few as 5 or 10 pmol. Rates of self-administration declined as the dose was either increased or decreased from optimum levels. Such dose changes were accompanied by decreases in the number of infusions, increases in the aver-

TABLE 2

STATISTICAL ANALYSIS OF THE EFFECTS OF COCAINE DOSE ON THE MEAN RELATIVE FREQUENCIES OF THE INTERINFUSION INTERVALS FOR INTRACRANIAL SELF-ADMINISTRATION INTO THE MEDIAL PREFRONTAL CORTEX

| Interval | ANOVA[1] F(3,24) | Cocaine Dose | Comparison[2] Cocaine Dose High | Low | Optimum |
|---|---|---|---|---|---|
| 1 | | CSF | 8.13 | 4.16 | 15.01 |
| (0–15 min) | 244.8588† | Optimum | 6.89 | 10.86 | — |
| | | Low | 3.97 | — | — |
| 2 | | CSF | 0.67* | 1.58* | 2.08* |
| (15–30 min) | 5.1718‡ | Optimum | 1.41* | 0.50* | — |
| | | Low | 0.91* | — | — |
| 3 | | CSF | 3.25 | 3.15 | 6.10 |
| (30–45 min) | 37.2662† | Optimum | 2.85* | 2.95 | — |
| | | Low | 0.10* | — | — |
| 4 | | CSF | 6.27 | 1.13‡ | 8.72 |
| (45–60 min) | 103.2983† | Optimum | 2.45* | 7.59 | — |
| | | Low | 5.14 | — | — |

Mean relative frequency values are the percentage of total interinfusion intervals that occurred during each 15 min division (Fig. 4). Statistical analysis consisted of a [1]completely randomized 2-factor analysis of variance [11] followed by [2]Tukey's all pairwise comparisons among treatment means [31] for N=6.

*Non-significant comparisons at $p<0.05$ ($t=2.95$).

†$p<0.0005$.

‡$p<0.01$.

age interval between infusions, and decreases in the slope of the cumulative infusion plot (Fig. 3). Thus, these measures could not be used to distinguish between high and low doses. However, the mean relative frequency of interinfusion intervals was sensitive to drug dose and demonstrated that various doses of cocaine resulted in significantly different and quantifiable patterns of intake (Fig. 4; Table 2). When a high dose was delivered, 55% of the interinfusion intervals were shorter than 15 minutes long while only 42% of the intervals were this short with low doses and 26% with CSF substitution. In contrast, only 15% of the intervals were longer than 45 minutes with high doses compared to 36% with low concentrations and 40% with CSF substitutions. The behavioral effects of the response-contingent intracranial delivery of high, low and optimum concentrations of cocaine and CSF could therefore be most readily distinguished by calculating the percentage of total interinfusion intervals that were shorter than 15 minutes (division 1) and the percentage longer than 45 minutes (division 4).

*Amphetamine and Lidocaine Substitutions*

The substitution of equimolar concentrations of amphetamine or lidocaine or a nine-fold higher molar concentration of lidocaine for the optimum dose of cocaine resulted in drug-intake similar to vehicle (Fig. 5). Moreover, the patterns of responding resulting from either drug substitution resembled those seen when either a low dose of cocaine or CSF was delivered.

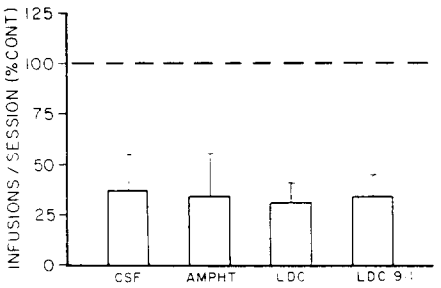


FIG. 5. Effects of substituting artificial cerebrospinal fluid, equimolar concentrations of amphetamine or lidocaine and a 9 to 1 higher molar concentration of lidocaine for cocaine on intracranial self-administration into the medial prefrontal cortex. Values are means (±standard error of the mean) for double determinations in two rats and are expressed as the percentage of baseline drug-intake maintained by the concentration of cocaine resulting in maximum rates (100%).

TABLE 3

STATISTICAL ANALYSIS OF THE EFFECTS OF NEUROTRANSMITTER RECEPTOR ANTAGONISTS ON THE MEAN RELATIVE FREQUENCIES OF THE INTERINFUSION INTERVALS FOR INTRACRANIAL COCAINE SELF-ADMINISTRATION INTO THE MEDIAL PREFRONTAL CORTEX ON THE DAY OF ANTAGONIST ADMINISTRATION

| Antagonist | ANOVA[1] F(4,15) | Comparison[2] Cocaine Dose High | Low | Optimum | CSF |
|---|---|---|---|---|---|
| Sulpiride | | | | | |
| Interval 1 | 114.7816† | 4.69 | 0.60* | 12.50 | 5.95 |
| Interval 4 | 32.2571† | 4.53 | 0.28* | 7.09 | 1.81* |
| SCH 23390 | | | | | |
| Interval 1 | 156.5836† | 6.71 | 10.89 | 2.89* | 17.05 |
| Interval 4 | 36.9988† | 0.93* | 5.53 | 5.53 | 7.75 |
| Atropine | | | | | |
| Interval 1 | 181.3651† | 8.40 | 13.73 | 0.88* | 19.25 |
| Interval 4 | 78.0140† | 2.99* | 9.03 | 0.70* | 12.25 |
| Propranolol | | | | | |
| Interval 1 | 122.3824† | 6.16 | 11.44 | 1.04* | 15.48 |
| Interval 4 | 98.6302† | 1.87* | 11.18 | 2.18* | 11.43 |

Mean relative frequency values are the percentage of total interinfusion intervals that occurred during each 15 min division (Fig. 7). Statistical analysis consisted of a [1]completely randomized 2-factor analysis of variance [11] followed by [2]Tukey's all pairwise comparisons among treatment means [31] for N=3 for each antagonist.

*Non-significant comparisons at $p<0.05$ ($t=3.09$).

†$p<0.0005$.

*Neurotransmitter Antagonists*

The neurotransmitter receptor antagonists were investigated in a random order (Table 1) with each drug tested in at least one antagonist-naive animal. Sulpiride significantly decreased drug-intake to 47% of baseline (Fig. 6) ($p<0.001$) and generated patterns of self-administration (mean relative frequency distributions) that were consistent with those seen when the concentration of cocaine was decreased from op-

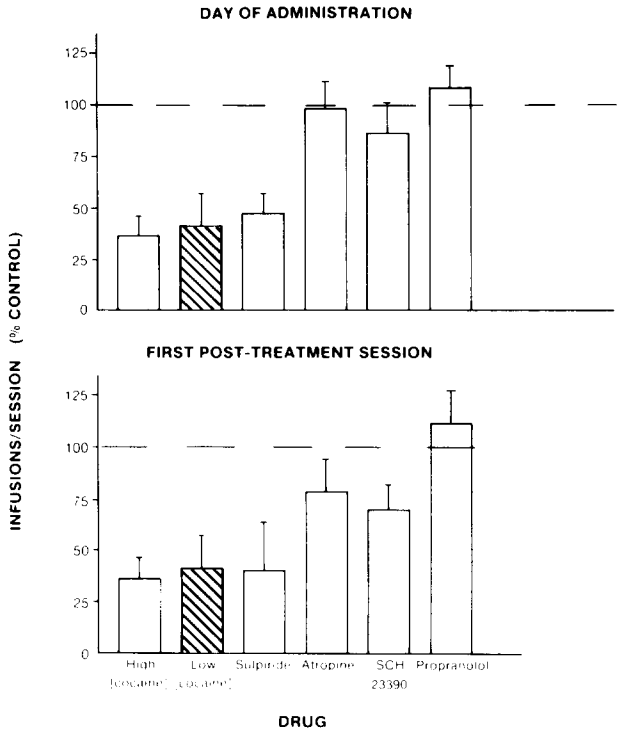


FIG. 6. Effects of including equimolar concentrations of receptor antagonist with the optimum dose of cocaine (i.e., the dose resulting in maximum rates of drug-intake) on intracranial self-administration into the medial prefrontal cortex. Values are means (±standard error of the mean) for double determinations in three rats and are expressed as the percentage of baseline drug-intake maintained by the optimum dose (100%) on the day of antagonist administration and the first post-treatment session (3 days later). The significance of the differences between means was determined with Student's *t*-tests. The intake of cocaine was significantly decreased with the addition of sulpiride on the day of administration ($p<0.001$) and the next session ($p<0.001$). Atropine and SCH 23390 had no effect the day of administration, but significantly decreased intake the next session ($p<0.05$ and $p<0.01$, respectively). Propranolol had no effect.

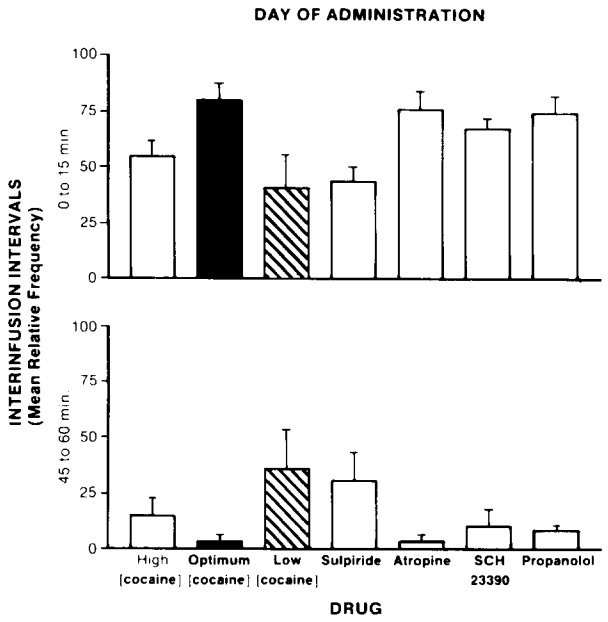


FIG. 7. Mean relative frequency of interinfusion intervals for the intracranial self-administration of cocaine into the medial prefrontal cortex in the first division (0 to 15 min) and fourth division (45 to 60 min). Optimum represents the cocaine dose that resulted in maximum rates of drug-intake, low and high represent the lowest and highest concentrations of the drug that maintained responding and sulpiride, atropine, SCH 23390 and propranolol represent the effects of including equimolar concentrations of each antagonist with the optimum dose of cocaine. Values are the mean percentage of total interinfusion intervals that occurred during each 15 min division (±standard error of the means) for double determinations in three rats on the day of antagonist administration. The differences between the patterns of intake generated by each antagonist and the different concentrations of cocaine were assessed with Tukey's all pairwise comparisons among treatment means with a $p<0.05$ critical value=3.09 (Table 3). Patterns of self-administration seen with sulpiride are similar to the low dose or CSF, but significantly different than the high or optimum doses. On the other hand, the patterns of drug-intake with atropine, SCH 23390 or propranolol were similar to the optimum or high doses, but significantly different than CSF or the low dose of cocaine.

timum levels (Fig. 7; Table 3). This effect persisted for two sessions (6 days), but drug-intake returned to baseline by the third post-treatment session (9 days). Doubling the concentration of cocaine did not alter the sulpiride effect, suggesting a non-competitive action at separate receptor sites.

The administration of the other antagonists did not alter drug-intake directly. Atropine had no effect on rates or patterns of self-administration during the session it was delivered, but significantly decreased drug-intake ($p<0.05$) during the first post-treatment session (3 days later) with patterns of responding (mean relative frequency distributions) consistent with those seen with higher doses of cocaine (Fig. 8; Table 4). These effects dissipated by the second post-treatment session and were not altered by doubling the concentration of atropine. Similar results were observed with equimolar and twice equimolar concentrations of SCH 23390. Propranolol, however, did not affect the rates or patterns of self-administration at any concentration tested. Phenoxybenzamine was tested in one animal with the data similar to those observed with propranolol, but was not studied further to avoid potential cytotoxic effects because of its insolubility in artificial CSF. Histological analyses [34] showed the guide cannulae to be in the medial prefrontal cortex (Fig. 9).

## DISCUSSION

The response-contingent intracranial delivery of cocaine to receptors in the medial prefrontal cortex is reinforcing. Lever-pressing was rapidly engendered in experimentally naive animals suggesting that reinforcement occurs with little or no delay. Two-lever choice discrimination procedures have shown the self-administration to be directed to responding and not a non-specific effect of the drug on motor activity [17]. Histological examination indicated that repeated exposure to intracranial microinfusions did not damage brain tissue, a finding consistent with the stable baselines of self-

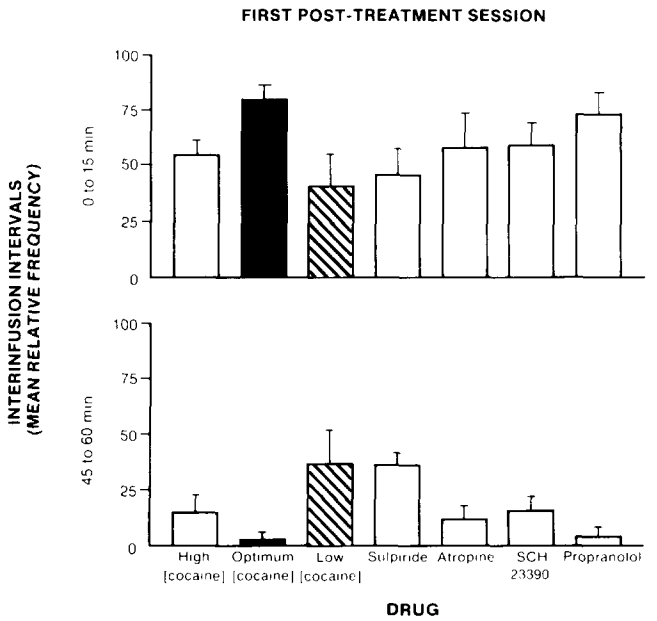


FIG. 8. Mean relative frequency of the interinfusion intervals for the intracranial self-administration of cocaine into the medial prefrontal cortex in the first division (0 to 15 min) and fourth division (45 to 60 min) during the first post-drug session (3 days later). Optimum represents the cocaine dose that resulted in maximum rates of drug-intake, high and low represent the highest and lowest concentrations of the drug that maintained responding and sulpiride, atropine, SCH 23390 and propranolol represent the effects of including equimolar concentrations of each antagonist with the optimum dose of cocaine. Values are the mean percentage of total interinfusion intervals that occurred during each 15 min division (±standard error of the means) for double determinations in three rats during the first session after antagonist administration (3 days later). The differences between the patterns of intake generated by each antagonist and the different concentrations of cocaine were assessed using Tukey's all pairwise comparisons among treatment means with a $p<0.05$ critical value of 3.09 (Table 4). Patterns of intake after sulpiride were still more similar to the low dose of cocaine or CSF. Although atropine and SCH 23390 did not affect drug-intake on the day of administration (Fig. 7), the patterns of intake were similar to the high dose three days later. Propranolol did not result in delayed effects, with patterns of intake still similar to the optimum dose.

TABLE 4

STATISTICAL ANALYSIS OF THE EFFECTS OF NEUROTRANSMITTER RECEPTOR ANTAGONISTS ON THE MEAN RELATIVE FREQUENCIES OF THE INTERINFUSION INTERVALS FOR INTRACRANIAL COCAINE SELF-ADMINISTRATION INTO THE MEDIAL PREFRONTAL CORTEX ON THE FIRST SESSION AFTER ANTAGONIST ADMINISTRATION

| Antagonist | ANOVA[1] F(4,15) | Comparison[2] Cocaine Dose High | Low | Optimum | CSF |
|---|---|---|---|---|---|
| Sulpiride | | | | | |
| Interval 1 | 114.3568† | 3.75 | 1.57* | 11.62 | 6.96 |
| Interval 4 | 34.1573† | 5.58 | 1.42* | 8.08 | 0.62* |
| SCH 23390 | | | | | |
| Interval 1 | 125.3625† | 2.33* | 6.40 | 7.00 | 12.39 |
| Interval 4 | 32.0187† | 1.08* | 3.47 | 3.51 | 5.66 |
| Atropine | | | | | |
| Interval 1 | 145.1465† | 0.97* | 6.49 | 8.63 | 12.20 |
| Interval 4 | 59.5084† | 0.28* | 5.51 | 3.83 | 8.61 |
| Propranolol | | | | | |
| Interval 1 | 132.9182† | 6.72 | 12.16 | 0.69* | 16.31 |
| Interval 4 | 105.4048† | 3.35 | 12.55 | 0.66* | 12.79 |

Mean relative frequency values are the percentage of total interinfusion intervals that occurred during each 15 min division (Fig. 8). Statistical analysis consisted of a [1]completely randomized 2-factor analysis of variance [11] followed by [2]Tukey's all pairwise comparisons among treatment means [31] for N=3 for each antagonist.

*Non-significant comparisons at $p<0.05$ ($t=3.09$).

†$p<0.0005$.

administration observed for more than 120 behavioral sessions (>400 days) in six of the animals. The individual differences in the concentration that produced maximum rates of drug-intake, the distinctly different dose-response curves generated for each rat and the locations of the guide cannulae within the medial prefrontal cortex (Fig. 9) suggest reinforcement-relevant receptor subpopulations for cocaine to be restricted to specific loci within this brain region. *In vivo* experiments assessing drug diffusion have shown that non-contingently infused [$^3$H]-cocaine was localized within 1 mm of the injection site in the medial prefrontal cortex [20], further suggesting that the reinforcing effects are restricted to this region. Cocaine appears to be rapidly metabolized and cleared from the injection site since only 2.5% of the infused radioactivity was recovered from each brain following 40 microinfusions delivered on a schedule equivalent to maximal rates of self-administration [20]. A decreased concentration of cocaine at reinforcement-relevant receptors would be necessary for the initiation of the behavioral sequence that results in subsequent self-administration since the animal may attempt to maintain an optimum concentration of the drug at these receptors [18]. Although there was no significant correlation between the location of the cannulae within the medial prefrontal cortex and rates of drug-intake, a trend suggests higher concentrations to be necessary for maximal rates of self-administration into deeper layers of the cortex (Fig. 9).

Significant variations in drug-intake often occurred after small changes in concentration. Increasing or decreasing the dose from optimum levels similarly decreased rates of self-administration, producing inverted U-shaped dose-effect relationships. However, the relative frequency distributions of interinfusion intervals (measures of the patterns of drug-intake) were significantly different with CSF or high, optimum or low doses. Greater than 75% of the interinfusion intervals were shorter than 15 minutes at the optimum concentration, with less than 5% longer than 45 minutes. Other doses shifted the patterns of responding to identifiable frequency distributions.

The response-contingent infusion of cocaine into the medial prefrontal cortex appears to be a reinforcer as a result of interactions with discrete neuronal receptors. The neuronal activity produced by the intracranial administration of cocaine into the frontal cortex may be one of the antecedent neurobiological conditions necessary for the drug to function as a reinforcer. Equimolar concentrations of amphetamine did not maintain responding above vehicle levels and likely do not result in the initiation of this antecedent neuronal activity at this concentration in this brain region. Although

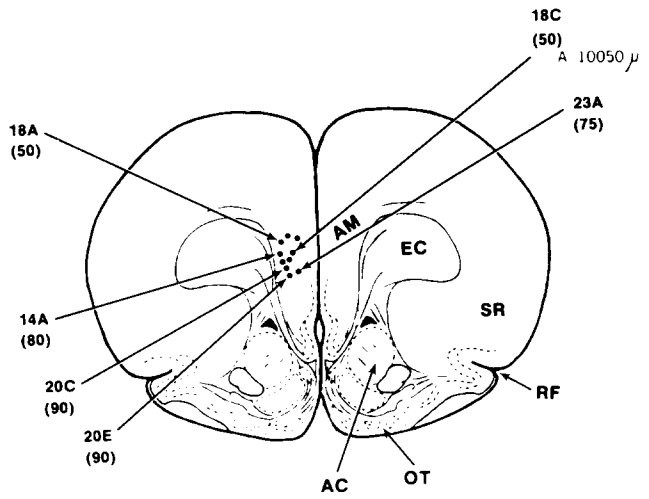


FIG. 9. Location of the injection cannulae tips in the medial prefrontal cortex of the ten animals intracranially self-administering cocaine. The animal identification numbers and dose of cocaine resulting in the highest rate and most consistent patterns of intake (optimum dose) are shown for the six rats that completed the dose-effect phase of the experiment. The remaining four animals self-administered cocaine but did not complete the dose-effect relationships because of cannulae displacement. Abbreviations: AC, nucleus accumbens; AM, anteromedial dopaminergic system (medial prefrontal cortex); EC, external capsule; OT, olfactory tubercle; RF, rhinal fissure; SR, suprarhinal dopaminergic system (sulcal prefrontal cortex) [38].

complete dose-response curves were not investigated with amphetamine, response patterns were consistent with those seen after decreasing the cocaine dose, suggesting that lower amphetamine concentrations may not be reinforcing. It is also unlikely that a higher dose would maintain self-administration since the drug has a broader spectrum of action than cocaine. Amphetamine inhibits the neuronal reuptake of catecholamines [29], releases newly synthesized catecholamines [3,40] and may be a partial catecholamine receptor agonist [4], while cocaine appears to inhibit catecholamine reuptake at physiological concentrations [37]. In addition, cocaine is not self-administered into the nucleus accumbens at any concentration tested (0.025 to 5 nmol) [17], while amphetamine is, but at much higher concentrations (4.8 nmol) than cocaine in the medial prefrontal cortex [30]. Amphetamine has only $^1/_{10}$ the affinity for the cocaine binding site as cocaine [27], but a specific binding site for amphetamine has also been recently identified [28]. These data suggest that even though the two drugs have similar behavioral actions, the initiation of neuronal antecedents of reinforcement may result from different neurobiological events. A more complete analysis of this possibility is currently under investigation. Lidocaine was chosen to determine if cocaine reinforcement was mediated through a non-specific blockade of neuronal conduction instead of another local anesthetic which may also have reinforcing properties (e.g., procaine, chloroprocaine; [32,57]). If the agent also resulted in reinforcement through properties unrelated to its local anesthetic effect, then this determination would not be possible. The two doses of lidocaine did not maintain responding, suggesting that the reinforcing properties of cocaine are not the result of a non-specific local anesthetic blockade.

Intracranial self-administration results in the delivery of the drug directly onto a discrete population of receptors. Experimental manipulations to characterize these receptors should occur at these same discrete binding sites. Systemic administration of neurotransmitter receptor antagonists may not result in the desired concentration at the brain loci under study, even at behaviorally debilitating doses. For example, sulpiride (100 mg/kg, IP) accumulates mainly in brain regions such as the hypothalamus, medulla oblongata and cerebellum, rather than in the prefrontal cortex [39]. Even if the antagonist were to reach the target brain locus in sufficient amounts, such high concentrations could non-specifically interfere with ICSA as a result of peripheral effects not associated with reinforcement (e.g., alterations in metabolism, etc.). For neuropharmacological assessments the antagonist should be intracranially co-infused to increase the probability that the effects of both drugs are localized at the same receptor populations. However, a specific antagonist for the cocaine binding has not been identified and cocaine and various neurotransmitter receptor antagonists may not compete for the same binding sites. Since large changes in rates of ICSA were often seen after relatively small changes in cocaine concentration, competition curves similar to those observed for the opiate receptor with methionine enkephalin and naloxone [18] may not be currently possible. Relative frequency of the interinfusion intervals is a reliable empirical measure of the effects of changes in drug concentration on ICSA. Significant differences in relative frequency distributions were obtained with the high, low or optimum doses of cocaine or vehicle for each rat, and these data were reliable both within animal or between all six rats. It was therefore possible to determine whether a high or low dose of cocaine was delivered by calculating the percentage of total interinfusion intervals that occurred during successive 15 min divisions during the session. This measure demonstrated whether an experimental manipulation resulted in patterns of behavior similar to those seen when the dose of cocaine was increased or decreased from optimum levels indicating whether the antagonist attenuated or potentiated cocaine-initiated neuronal activity. For example, if the effects of cocaine are receptor-mediated, then decreasing the concentration of the drug would decrease the number of receptors occupied and result in specific changes in neuronal activity in discrete brain loci. If an antagonist produced similar changes in this neuronal activity, the animal should respond as though the concentration of cocaine had been decreased and exhibit corresponding changes in the rates and patterns of self-administration.

The involvement of dopaminergic neurotransmitter receptors in the processes responsible for intracranial cocaine self-administration were delineated with the behavioral procedures. The relative excitatory or inhibitory roles of these systems were suggested by comparing the rates and patterns of responding after administration of specific neurotransmitter receptor antagonists with those resulting from changes in the dose of cocaine. $D_2$-dopaminergic receptors in the medial prefrontal cortex appear to be excitatory to these processes since equimolar concentrations of sulpiride reduced self-administration to vehicle levels with patterns of responding consistent with decreasing the cocaine dose. The effects of blockade of $D_2$ receptors did not appear to be the result of a non-specific decrease in response rates since noncontingent

intracranial infusions of sulpiride into the medial prefrontal cortex of rats responding on fixed-interval 2-minute schedules of food reinforcement did not modify rates or patterns of responding [12]. Cocaine and sulpiride may act at different sites since the attenuation of self-administration after $D_2$ receptor blockade could not be reversed by increasing the cocaine dose. The effects of sulpiride persisted for more than 6 days. Rates and patterns of drug-intake during the 2 sessions after this antagonist probe suggest that a decreased number of $D_2$ receptors were available.

The $D_1$-dopaminergic receptor antagonist, SCH 23390, and the muscarinic cholinergic receptor antagonist, atropine, had either no or minimal direct effects on intracranial self-administration. However, the blockade of these receptors resulted in a delayed attenuation of drug-intake with patterns of responding resembling an increased dose of cocaine (i.e., a potentiation of cocaine-initiated neuronal activity). While it is unclear why these effects occur, it is possible that they are receptor-mediated. For example, the chronic blockade of $D_1$ receptors from repeated self-infusions during the antagonist probe which did not affect the reinforcing efficacy of cocaine could have resulted in a receptor supersensitivity, the effects of which were not seen until the next experimental session (3 days later). This sensitization could result from a non-specific effect if $D_1$ and $D_2$ receptors are localized on the same neuron and if blockade of one class of receptors produced increases in both types of receptors. If this were to occur, then the increased number of $D_2$ binding sites could result in a potentiation of the effects of cocaine and produce neuronal activity comparable to that resulting from a higher dose even though the synaptic concentration of dopamine was similar to that before the antagonist probe. Muscarinic cholinergic receptor blockade could have resulted in indirect delayed effects on the reinforcing efficacy of cocaine through similar mechanisms. If these receptors are localized on some of the same postsynaptic neurons as dopaminergic receptors, then chronic blockade with atropine could result in a non-specific compensatory increase in both cholinergic and dopaminergic receptors with the net result similar to that described above for the $D_1$ antagonist. Noradrenergic systems in the medial prefrontal cortex may not be involved in cocaine reinforcement, while blockade of muscarinic cholinergic and $D_1$-dopaminergic systems result in delayed, indirect effects that are not currently understood.

The data from this investigation are consistent with previous research suggesting an important role for the mesolimbic/mesocortical dopaminergic system in cocaine reinforcement. Lesions of the dopaminergic innervations of the nucleus accumbens attenuate intravenous cocaine self-administration [48]. Similar lesions of the ventral tegmental area also disrupt the intravenous self-administration of cocaine [49]. However, as previously stated, the degree of attenuation does not correlate with the extent of dopamine depletion in the nucleus accumbens, suggesting that damage to dopaminergic fibers terminating in other brain regions may be responsible for these effects. The nucleus accumbens could be involved in the mediation of cocaine reinforcement rather than its initiation since this region will not maintain intracranial self-administration [17]. The receptors through which the drug exerts its direct effects to initiate reinforcing neuronal activity may be localized in other brain regions including the medial prefrontal cortex. However, to date, only two other brain regions (nucleus accumbens and ventral tegmental area) have been adequately assessed for this activity [17].

The current dopamine hypothesis of psychomotor stimulant reinforcement proposes that the mesolimbic dopaminergic terminals and dopaminoceptive cells localized in the nucleus accumbens are essential for the expression of the reinforcing effects of both amphetamine and cocaine [55]. However, direct evidence suggests that this brain region is not involved in the initiation of reinforcing neuronal activity following cocaine administration [17]. The interaction of cocaine with presynaptic receptors associated with reuptake processes in the medial prefrontal cortex could result in an increased synaptic concentration of dopamine without changing the level of activity in the presynaptic dopaminergic neurons. The initiation of the reinforcing effect of cocaine, therefore, results from the activation of $D_2$ receptors localized postsynaptically to these dopaminergic innervations, activating neuronal systems that mediate cocaine reinforcement processes in which other dopaminergic and non-dopaminergic neurons may directly participate. This antecedent neuronal activity may be necessary for cocaine infusions to engender and maintain behavior.

Reinforcement resulting from the response-contingent delivery of cocaine appears to be initiated in part through an action on $D_2$ dopaminergic receptors in the medial prefrontal cortex. This is consistent with recent reports showing $D_2$ dopaminergic receptor antagonists to increase intravenous cocaine self-administration in a dose-related manner [50] while $D_1$ antagonists result in unsystematic or no effects [58]. The reinforcing neuronal activity produced by the intracranial administration of cocaine into the frontal cortex may be initiated by direct effects through interactions with postsynaptic $D_2$ receptors or indirectly as a result of a presynaptic inhibition of dopamine reuptake where cocaine receptors are proposed to be located [33]. The inability of higher doses of cocaine to overcome the $D_2$ blockade suggests action through sites not directly associated with the $D_2$ receptors. The loci of the reinforcing action is currently being directly evaluated with 6-hydroxydopamine lesions at the self-administration site.

Intracranial self-administration combined with neuropharmacological and neurobiological methodologies are clearly useful in identifying brain loci where the initiation of drug reinforcement occurs and for discriminating these sites from mediation systems which may be more related to general reinforcement processes.

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