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Dose-dependent characterization of the rewarding and stimulant properties of cocaine across intraperitoneal and intravenous routes of administration

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ORIGINAL INVESTIGATION

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Dose-dependent characterization of the rewarding and stimulant properties of cocaine following intraperitoneal and intravenous administration in rats

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Abstract Dose-dependent differences in the rewarding and stimulant properties of cocaine administered intravenously (IV) and intraperitoneally (IP) were compared. Six 2-day conditioning trials were conducted over consecutive days. Rats received cocaine and were placed into a compartment on one day of the trial, and were directly placed into a different compartment without drug on the other day. Rats were exposed to the compartments for either 20 or 40 min. The effects of cocaine on stimulant behaviors, including locomotion and stereotypies, were compared following the first and last injection. After conditioning, three tests were given with 1 rest day intervening each: (1) conditioned place preference (CPP) was measured as an increase in the amount of time animals spent in the injection compartment relative to the noninjection compartment when given access to both, (2) conditioned activity (CA) was measured as an increase in stimulant behaviors in cocaine-treated animals relative to saline controls following an injection of saline in the injection compartment and (3) context-independent sensitization was measured as an increase in stimulant behaviors following an injection of cocaine in the noninjection compartment relative to the animals' behavior following the first injection. Cocaine did not reliably produce sensitization of locomotion under any of the conditions examined. Cocaine produced sensitization of headbobbing that was more robust following IP administration than it was following IV administration. In both cases, sensitization of headbobbing involved a context-independent component. Cocaine produced CPP and CA with both routes of administration. CPP was established more readily with 40-min relative to 20-min exposures following IV administration, whereas CA was more prevalent with 20-min relative to 40-min exposures. This study provides a thorough characterization of the behavioral effects of cocaine administered IV and a new efficient method for assessing the effects of cocaine on conditioned and unconditioned behaviors following repeated administration.

Key words Cocaine · Dose-response · Intraperitoneal · Intravenous · Repeated administration · Sensitization · Locomotion · Stereotypy · Conditioned place preference · Conditioning · Rat

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Introduction

Studies examining oral, subcutaneous and intraperitoneal (IP) administration of cocaine have demonstrated that the dose-response curves for locomotor activity and stereotypy vary depending on the route of administration (Lau et al. 1991; Yeh and Haertzen 1991). The stimulant properties of cocaine following intravenous (IV) administration, however, have not been well characterized. The neurochemical changes following IV and other routes of administration have been reported to be different. These differences include 2-[^{14}C]deoxyglucose utilization (Porrino 1993) and dopamine and serotonin neurotransmission (Spyraki et al. 1987; Broderick 1992). In humans, IV administration of cocaine produces greater physiological changes and more intense subjective effects relative to intranasal administration (Resnick et al. 1977). Since the current cocaine abuse epidemic involves routes of administration that rapidly distribute drug to the brain, including IV administration, it is important to characterize the behavioral effects of cocaine administered IV.

The stimulant properties of cocaine, including both locomotor activity and stereotypy, are often sensitized following repeated administration (Downs and Eddy 1932; Post and Rose 1976; Kilbey and Ellinwood 1977). Although the mechanisms of sensitization are not well understood, it has been suggested that conditioned, as well as unconditioned components, may be involved (Ellinwood and Kilbey 1980 ; Post et al. 1987; Zahniser and Peris 1991). Specifically, cocaine-induced sensitization may involve Pavlovian conditioning, in which the drug initiates neural events that serve as unconditioned

stimuli and elicit unconditioned responses, such as locomotion or stereotypy. The drug is administered in the presence of environmental cues which serve as conditioned stimuli. Following the drug-environment pairing(s), exposure to the environment may elicit conditioned responses, such as conditioned locomotion or stereotypy. These conditioned activities (CA) may be evident as behavioral activation in animals tested in the drug-paired environment in the absence of drug (Barr et al. 1983; Beninger and Herz 1986), or as sensitized responses to the activational effects of the drug in animals that had received the drug in the test environment relative to animals that had received the drug in their home cage (Hinson and Poulos 1981; Post et al. 1981). Sensitization observed using the latter procedure is referred to as context-dependent sensitization. Sensitization effects that occur regardless of the test environment are referred to as context-independent. In order to understand the mechanisms of sensitization, it is important to examine the contribution of context-dependent and context-independent factors. Post et al. (1992) have suggested that the contribution of contextual factors to cocaine-induced sensitization may vary depending on the dose that is administered. Specifically, administering low doses of cocaine produce sensitization that is context-dependent, whereas administering high doses and/or increasing the number of injections produces sensitization that is context-independent. This hypothesis was developed based on a review of several studies and has not been tested systematically.

Drug-associated stimuli may acquire incentive motivational properties through classical conditioning that can be assessed using the place conditioning paradigm. In this paradigm, the drug is administered in a distinct environment. After several pairings the environment becomes associated with the drug effects, thereby acquiring incentive-motivational properties. Thus, the environmental stimuli elicit approach (i.e., conditioned place preference; CPP) or avoidance (i.e., conditioned place aversion) depending on whether rewarding or aversive properties of the drug have been conditioned. Several investigators have reported cocaine-CPP (e.g., Mucha et al. 1982; Spyraki et al. 1982; Bardo et al. 1986), and have noted different dose-response curves depending on the route of administration (Nomikos and Spyraki 1988; Mayer and Parker 1993). CPP is established using a drug-environment pairing procedure similar to that used to establish conditioning to the stimulant properties of cocaine. Previous research, however, has examined either CPP or CA in separate experiments when it is possible to examine both behaviors in a single experiment. The advantages of this approach include efficiency and the ability to assess both types of conditioning in the same animal and possible relationships between these conditioned responses.

The present study was designed to characterize dose-dependent differences in the stimulant and rewarding properties of cocaine following repeated IV and IP administration. A procedure was used that allowed CPP, CA, and context-independent sensitization to be measured in a single experiment. Six 2-day conditioning trials were conducted over consecutive days. Rats were confined to a compartment immediately following an injection on one day, and were confined to a distinctively different compartment without injection on the other day. Sensitization was assessed by comparing locomotion and stereotypy following the first and last injection, and was defined as an increase in the behavioral response following the sixth relative to the first injection. CPP was assessed following conditioning by allowing the rats free access to both compartments simultaneously, and was defined as an increase in the amount of time animals spent in the injection compartment relative to the noninjection compartment. CA was assessed subsequently by measuring locomotion and stereotypy following a saline injection in the injection compartment, and was defined as an increase in these behaviors in cocaine-treated animals relative to saline controls. Lastly, context-independent sensitization was assessed by measuring locomotion and stereotypy following a challenge injection of cocaine in the noninjection compartment, and was defined as an increase in these behaviors relative to the first injection of cocaine. Since the behavioral effects of cocaine administered IV have a more rapid onset and termination relative to IP administration, it was hypothesized that the optimal length of exposure to the compartment may vary depending on route of administration. In order to examine this hypothesis, the rats were exposed to the compartments for either 40 or 20 min following IV administration.

Materials and methods

Animals and surgical procedure

Male Sprague-Dawley rats were housed under standard laboratory conditions and were handled for 3–5 days prior to surgery and/or conditioning. They were anesthetized using pentobarbital (50 mg/kg, IP) coadministered with atropine sulfate (10 mg/kg, IP). One end of the catheter was inserted into the right jugular vein and the other end exited the rat's back through a backplate assembly as described by Weeks (1962). After surgery, the rats were administered a mixture of heparinized saline (30 U/ml), streptokinase (4 mg) and ticarcillin disodium (400 mg) daily, and caps were placed over the catheters between injections.

Verification of catheter patency

To test catheter patency, the rats were administered Brevital (0.17 mg/0.1 ml, IV) prior to and following the conditioning phase of the experiment. Only animals anesthetized by the brevital were included in the statistical analyses (i.e., this dose of Brevital is only potent enough to anesthetize rats when administered IV).

Apparatus

Behavioral testing was conducted in rectangular Plexiglas chambers 76×24×30 cm high. Each chamber consisted of two equal-sized compartments separated by a removable partition. One compartment had pine scented bedding beneath a wire mesh floor and all but the front wall were white. The other compartment had cedar scented bedding beneath a bar grid floor and all but the front wall were black. The front wall of the chambers was transparent to allow direct observation of the rats' behavior. On the CPP test

Table 1 Experimental design and procedure

Experiment	Conditioning procedure (days 1–12)[a]		CPP (day 14) Free access to both compartments	Conditioned activity (day 16) Injection compartment	Context-independent sensitization (day 18) Noninjection compartment
	Injection compartment	Noninjection compartment			
1. IP administration 40 min exposure:	Saline (n=7)	No injection	No injection	Saline	Saline
	Cocaine 10 mg/kg (n=8)	No injection	No injection	Saline	Cocaine 10 mg/kg
	Cocaine 20 mg/kg (n=9)	No injection	No injection	Saline	Cocaine 20 mg/kg
	Cocaine 40 mg/kg (n=9)	No injection	No injection	Saline	Cocaine 40 mg/kg
2. IV administration 40 min exposure:	Saline (n=13)	No injection	No injection	Saline	Saline
	Cocaine 0.3 mg/kg (n=11)	No injection	No injection	Saline	Cocaine 0,3 mg/kg
	Cocaine 1.0 mg/kg (n=11)	No injection	No injection	Saline	Cocaine 1.0 mg/kg
	Cocaine 3.0 mg/kg (n=11)	No injection	No injection	Saline	Cocaine 3.0 mg/kg
	Cocaine 4.2 mg/kg (n=8)	No injection	No injection	Saline	Cocaine 4.2 mg/kg
3. IV administration 20 min exposure:	Saline (n=8)	No injection	No injection	Saline	Saline
	Cocaine 0.3 mg/kg (n=8)	No injection	No injection	Saline	Cocaine 0.3 mg/kg
	Cocaine 1.0 mg/kg (n=9)	No injection	No injection	Saline	Cocaine 1.0 mg/kg
	Cocaine 3.0 mg/kg (n=9)	No injection	No injection	Saline	Cocaine 3.0 mg/kg
	Cocaine 4.2 mg/kg (n=9)	No injection	No injection	Saline	Cocaine 4.2 mg/kg
	Cocaine 5.6 mg/kg (n=11)	No injection	No injection	Saline	Cocaine 5.6 mg/kg

[a] The order of placement and assignment of compartments as injection vs. noninjection were counterbalanced

days, the solid partition was replaced by a similar partition that contained an opening in the center (8×8 cm high), which allowed the rat free access to both compartments simultaneously. Preliminary experiments have demonstrated that rats exhibit equal preference for the two compartments. In each compartment, locomotor activity was recorded in 10-min intervals by a computer-automated relay system which consisted of two sets of photocells and detectors mounted opposite each other such that the emitted photobeams were 27 cm apart and 4 cm above the floor.

Experimental design and conditioning procedure

The design and conditioning procedure are summarized in Table 1. Six 2-day conditioning trials were conducted over 12 consecutive days. Rats were administered saline or their respective dose of cocaine and confined to one compartment, and on the other day they were confined to the other compartment without injection for either 20 or 40 min. The order of placement and the compartment paired with injection were counterbalanced. Two days after the last conditioning trial, the rats were tested for CPP. Rats were allowed free access to both compartments for 15 min. Two days later, the rats were tested for CA. Rats were administered saline were confined to their injection compartment. Two days later, the rats were tested for context-independent sensitization. Rats were administered their respective dose of cocaine and confined to their noninjection compartment. Locomotor activity was measured each day of the experiment and was defined as the number of times the individual photobeams were interrupted consecutively by the rat crossing from one end of the compartment to the other. Stereotypic headbobbing, head-down sniffing, rearing, grooming and convulsions were measured by recording their presence every 10 s for the duration of the test period. These behaviors were measured following the first and last injection during conditioning, and during the tests for CA and context-independent sensitization by an observer who was unaware of the animals' previous treatment.

Statistics

Locomotor activity following the first injection, the conditioned activity test and the context-independent sensitization test were analyzed using repeated measures ANOVAs with dose as a between-subjects factor and 10-min interval as a within subjects factor. Locomotor activity following repeated administration of cocaine was analyzed using repeated measures ANOVAs with dose as a between subjects factor and injection day as a repeated measure. Pairwise comparisons were made between groups using Fisher LSD tests. Stereotypies and grooming were analyzed using nonparametric Kruskal-Wallis ANOVAs. Subsequent pairwise comparisons were made using Mann-Whitney U-tests for between groups comparisons and Wilcoxon signed-ranks tests for within subjects comparisons. CPP data were analyzed using nonparametric Wilcoxon signed-ranks tests to compare differences in time spent in the injection and noninjection compartments for each group.

Table 2 Toxicity measures

	Dose of cocaine	Number of rats that died	Number of rats that convulsed
Experiment 1	10 mg/kg	0	1
	20 mg/kg	0	2
	40 mg/kg	2	2
Experiment 2	4.2 mg/kg	1	5
Experiment 3	4.2 mg/kg	1	2
	5.6 mg/kg	2	6

Results

Cocaine toxicity

With both routes of administration, high doses of cocaine produced convulsions and death. These toxic effects are summarized in Table 2. The deaths occurred following the fifth or sixth IP administration and following the third or fourth IV administration.

Behavioral measures following acute and repeated administration of cocaine

With both routes of administration, dose and time-dependent changes in locomotor activity were observed following acute administration of cocaine (data not shown). The most robust increase in locomotor activity occurred in the first and second 10-min intervals regardless of the route of administration (P<0.05, dose by 10-min interval

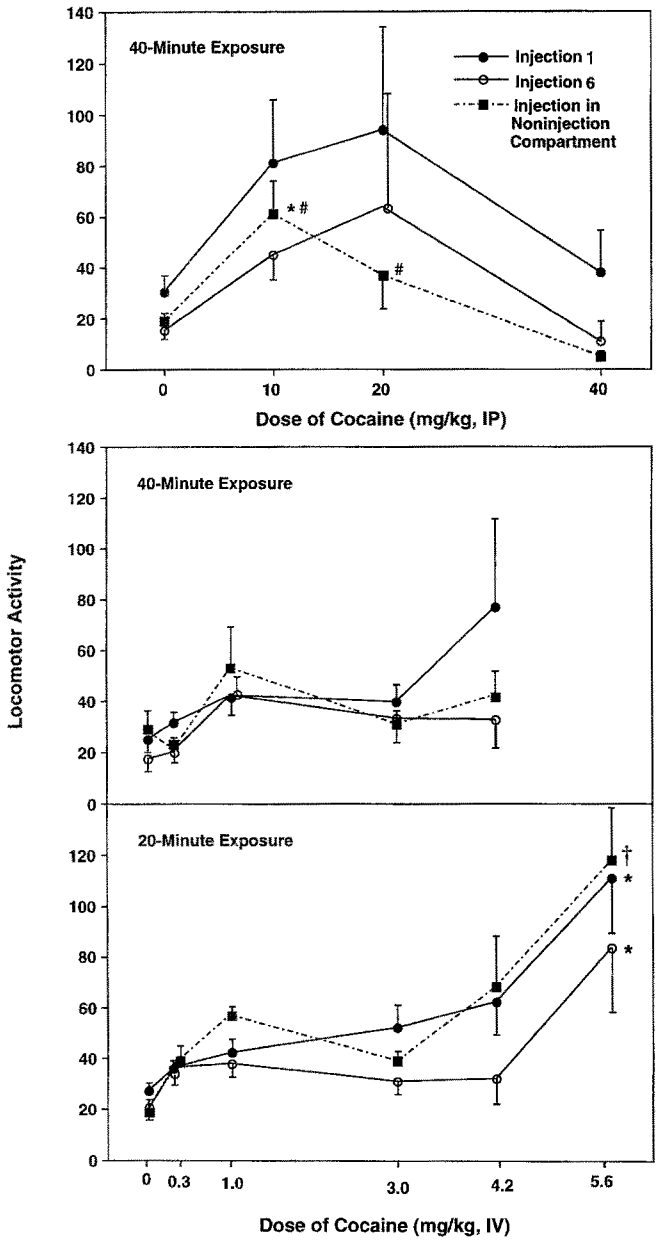

Fig. 1 Locomotor activity (±SEM) following injections 1 and 6 in the injection compartment, and following a challenge injection in the noninjection compartment (i.e., test for context-independent sensitization) in animals receiving cocaine administered either IP (*top panel*) or IV (*middle and bottom panels*). An *asterisk* (*) represents a significant difference from saline controls, a *number sign* (#) represents a significant difference from animals receiving 40 mg/kg, IP, and a *dagger* (†) represents a significant difference from all groups, Fisher LSD tests, $P<0.05$

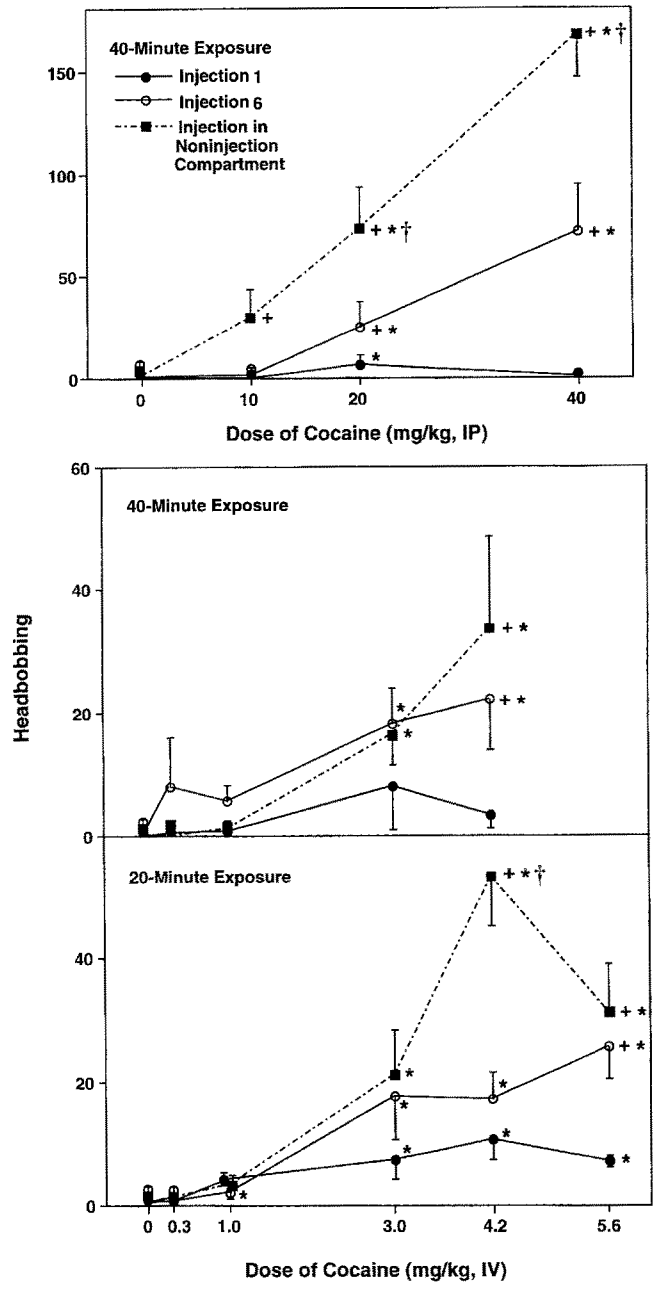

Fig. 2 Stereotypic headbobbing following injections 1 and 6 in the injection compartment, and following a challenge injection in the noninjection compartment (i.e., test for context-independent sensitization) in animals receiving cocaine administered either IP (*top panel*) or IV (*middle and bottom panels*). The presence of headbobbing was recorded every 10 s for the duration of the test period (i.e., 20 or 40 min). Values represent the number of observation periods (±SEM) during which headbobbing was present. An *asterisk* (*) represents a significant difference from saline controls and a *dagger* (†) represents a significant difference from all groups, Mann-Whitney *U*-tests, $P<0.05$. A *plus sign* (+) represents a significant difference relative to the first injection within a given group, Wilcoxon signed-ranks tests, $P<0.05$

interaction F ratios). Following IP administration, a low dose of cocaine (10 mg/kg) produced more locomotor activity relative to saline controls in the first 10-min interval (Fisher LSD test, $P<0.05$). In contrast, following IV administration, high doses of cocaine (3.0–5.6 mg/kg, IV) produced more locomotor activity relative to saline controls in the first and second 10-min intervals (Fisher LSD test, $P<0.05$).

Figure 1 illustrates locomotor activity following repeated IP or IV administration of cocaine. There was no change in cocaine-induced locomotion following the sixth injection relative to the first in any of the groups for either route of administration. In rats receiving IP administration of cocaine, an analysis of total activity collapsed across injection days revealed that although there

Table 3 Effects of cocaine following injection 1, 6 and the injection in the "noninjection" compartment on stereotypy[a]

	Dose of cocaine (mg/kg)	Time-sampled behaviors					
		Head-down sniffing			Rearing		
		Injection 1	Injection 6	Noninjection compartment	Injection 1	Injection 6	Noninjection compartment
Experiment 1	0	63.10±7.3	41.00±7.0	61.00±9	6.60±1.7	3.60±0.8	6.90±3.0
	10	108.00±9	123.00±14*	119.00±21*	14.00±3.8	6.10±1.8	5.20±1.5+
	20	91.00±13	101.00±18*	109.00±21*	4.90±1.3	0.50±0.3+*	1.90±0.8
	40	81.00±20	50.00±16+	14.00±5+*	9.80±6.8	0.00±0*	0.00±0*
Experiment 2	0	37.60±5.90	37.20±11.8	36.80±18.1	7.40±1.5	5.60±1.4	3.00±1.0
	0.3	65.50±15.3	34.50±6.10	67.50±19.7	12.00±2.4	4.70±1.5	5.00±1.4
	1.0	100.60±24.5*	129.80±18.9*	105.40±9.4*	15.00±3.5	11.40±1.6*	8.20±2.5
	3.0	93.40±11.5*	142.20±15.3+*	148.60±12.7+*	19.40±8.20	9.80±5.6	4.00±1.1
	4.2	76.70±10.6*	145.80±19.4+*	128.30±18.2+*	12.30±5.2	3.10±1.2	6.40±1.8
Experiment 3	0	70.60±4.5	44.70±4.5	67.80±8.2	10.30±1.6	4.50±1.5+	6.70±1.3
	0.3	83.50±1.4*	76.20±3.3*	84.50±5.40	10.80±1.9	10.00±2.2*	11.00±1.8
	1.0	60.70±8.6	77.30±5.7*	77.20±5.90	11.30±2.6	5.80±1.2	11.80±2.1
	3.0	66.60±8.7	84.40±5.5*	88.00±12.2	6.20±1.9	1.40±0.2+	1.00±0.5*
	4.2	59.60±8.9	74.10±9.9*	74.80±9.20	11.10±4.5	1.80±1.1+	1.30±0.7+*
	5.6	50.30±9.2	65.40±8.5	68.80±5.50	6.50±1.3	8.60±5.1	8.30±4.8

[a] Values represent the mean number of time-sampled observations (i.e., presence of behavior every 10 s) of head-down sniffing and rearing ±SEM

* Represents a significant difference from control group Mann-Whitney *U*-test, $P<0.05$

\+ Represents a significant difference from injection 1, Wilcoxon signed rank test, $P<0.05$

were no group differences [$F(3, 33)=1.72$, $P<0.18$], there was a strong trend for rats administered 20 mg/kg, IP to exhibit an increase in locomotor activity relative to saline controls (Fisher LSD test, $P<0.05$). In rats given 40-min exposures, repeated IV administration of cocaine produced a significant dose by injection day interaction [$F(20, 240)=2.4$, $P<0.01$]. Following the first injection, there were no dose-dependent differences in locomotor activity. Following the second-fifth injections, however, rats administered 1.0–4.2 mg/kg, IV exhibited an increase in locomotor activity relative to saline controls (data not shown; Fisher LSD test, $P<0.05$). Following the third to fifth injections, rats administered 1.0 mg/kg, IV exhibited an increase in locomotor activity relative to the first injection (Fisher LSD test, $P<0.05$). However, there was no significant difference in locomotor activity following the sixth injection relative to the first in any group. In rats given 20-min exposures, repeated IV administration of cocaine produced a significant dose by injection day interaction [$F(25, 225)=2.7$, $P<0.01$]. Rats administered 5.6 mg/kg, IV exhibited an increase in locomotor activity relative to saline controls following each injection and also exhibited an increase in their locomotor activity following the second injection relative to the first (Fisher LSD test, $P<0.05$). There were no significant changes in locomotor activity in any other dosage groups.

Figure 2 illustrates time-sampled observations of headbobbing following IP or IV administration of cocaine. With both routes of administration, cocaine produced an increase in headbobbing following acute administration that was significant relative to saline controls at doses of 20 mg/kg, IP and 1.0–5.6 mg/kg, IV (Mann-Whitney *U*-test, $P<0.05$). Sensitization of headbobbing following repeated administration of cocaine was also evident with both routes of administration. Rats administered doses of 20–40 mg/kg, IP and 4.2–5.6 mg/kg, IV exhibited an increase in headbobbing following the sixth injection relative to the first (Wilcoxon signed-ranks test, $P<0.05$).

Table 3 illustrates time-sampled observations of head-down sniffing following IP or IV administration of cocaine. Acute administration of cocaine produced an increase in head-down sniffing that was significant relative to saline controls at doses of 0.3–4.2 mg/kg, IV (Mann-Whitney *U*-test, $P<0.05$). In experiments 1 and 3, doses of 10 and 20 mg/kg, IP and 1.0–4.2 mg/kg, IV did not alter head-down sniffing following the first injection, but produced a significant increase in head-down sniffing relative to saline controls following the sixth injection (Mann-Whitney *U*-test, $P<0.05$). This is likely due to an habituation-induced decrease in baseline head-down sniffing in controls, resulting in increased sensitivity for detecting cocaine-induced head-down sniffing. The dose of 40 mg/kg, IP, however, produced a decrease in head-down sniffing following the sixth injection relative to the first (Wilcoxon signed-ranks test, $P<0.05$). This decrease was probably due to the high level of stereotypic headbobbing in this group following the sixth injection competing with expression of head-down sniffing behavior. Sensitization of sniffing was only evident in rats given repeated IV administration and 40-min exposures. In these animals, sensitization of head-down sniffing was evident at doses of 3.0–4.2 mg/kg, IV as an increase in head-down sniffing following the sixth injection relative to the first (Wilcoxon signed-ranks test, $P<0.05$). These

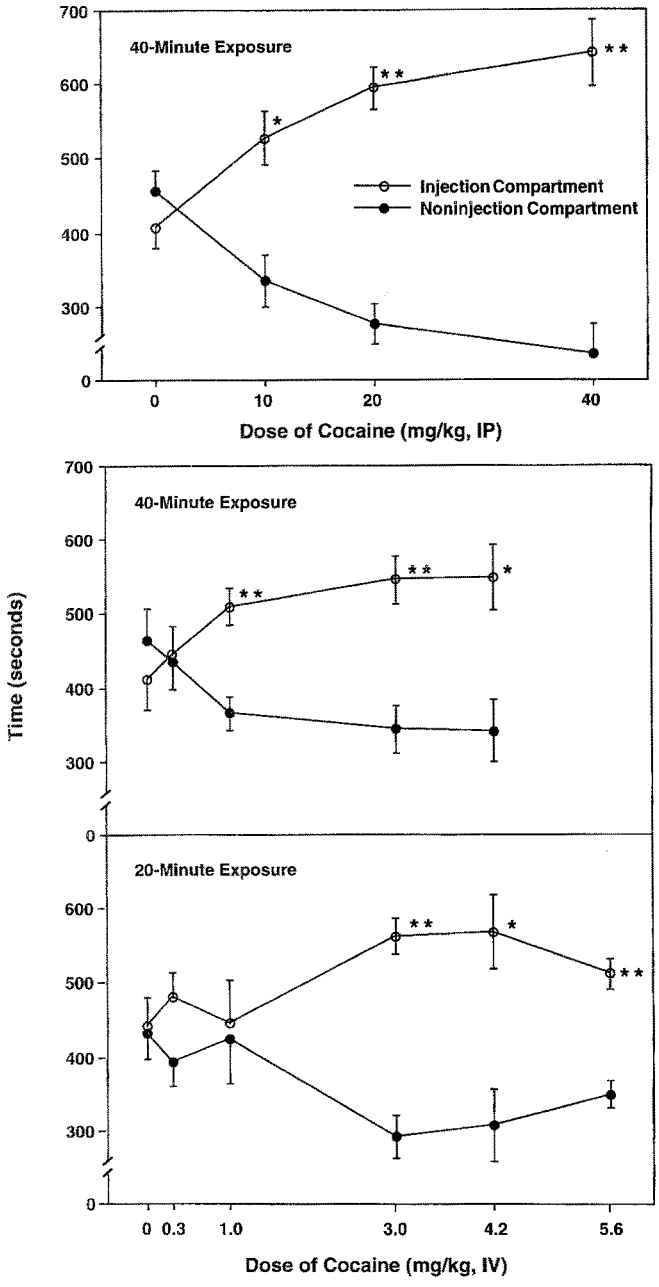

Fig. 3 The amount of time (s±SEM) spent in the injection compartment (*open circles*) and the noninjection compartment (*solid circles*) during the 15-min test for CPP in rats receiving various doses of cocaine administered IP (*top panel*) or IV (*middle and bottom panels*). An *asterisk* (*) represents a significantly greater amount of time spent in the injection compartment relative to the noninjection compartment, Wilcoxon signed-ranks tests, $*P<0.05$, $**P<0.01$

doses produced a 52% and 90% increase in sniffing, respectively.

Table 3 also illustrates time-sampled observations of rearing following IP or IV administration of cocaine. With both routes of administration, there were no changes in rearing following acute administration of cocaine. In general, there was a trend for decreases in rearing following repeated administration. Significant decreases were observed following the sixth injection relative to the first in rats administered 20 mg/kg, IP and 0, 3.0–4.2 mg/kg, IV (Wilcoxon signed-ranks test, $P<0.05$), and were likely due to habituation of exploratory behavior since saline controls also exhibited decreases in rearing.

With both routes of administration, cocaine produced a decrease in grooming that was significant relative to saline controls at all doses administered IP and at doses of 3.0–5.6 mg/kg, IV (data not shown; Mann-Whitney *U*-test, $P<0.05$).

Conditioned place preference

Figure 3 illustrates the amount of time rats spent in the injection and noninjection compartments on the CPP test day. Each dose of cocaine administered IP produced an increase in the amount of time rats spent in the injection compartment relative to the noninjection compartment (Wilcoxon signed-ranks test, $P<0.05$). Doses of 1.0–4.2 mg/kg, IV produced a significant cocaine-CPP with 40-min exposures, whereas doses of 3.0–5.6 mg/kg, IV produced a significant cocaine-CPP with 20-min exposures (Wilcoxon signed-ranks test, $P<0.05$). There were no significant differences in locomotor activity in the injection and noninjection compartments during the CPP test within or between groups (data not shown).

Conditioned activities

Table 4 illustrates locomotor activity and time-sampled stereotypies following administration of saline in the injection compartment (i.e., test for CA). With both routes of administration, cocaine-conditioned rats exhibited an increase in locomotion relative to saline controls. Rats receiving IP administration of cocaine exhibited an increase in locomotor activity across all 10-min intervals relative to saline controls at doses of 10 and 20 mg/kg, IP (Fisher LSD test, $P<0.05$). In rats given 40-min exposures, repeated IV administration of cocaine produced a significant dose by 10-min interval interaction [$F(12, 153)=3.2$, $P<0.01$]. Rats administered 1.0 mg/kg, IV exhibited an increase in locomotor activity relative to saline controls during the first and second 10-min intervals (data not shown; Fisher LSD test, $P<0.05$). In rats given 20-min exposures, repeated IV administration of cocaine produced an increase in locomotor activity across both 10-min intervals relative to saline controls at doses of 1.0 and 5.6 mg/kg, IV (Fisher LSD test, $P<0.05$). In general, rats given 20-min exposures exhibited more CA than rats given 40-min exposures. In rats given 40-min exposures, there were no significant differences from controls in stereotypies or grooming, although there was a trend for an increase in headbobbing, head-down sniffing and rearing. Rats given 20-min exposures, however, exhibited an increase in headbobbing at doses of 4.2–5.6 mg/kg, IV and an increase in head-down sniffing at doses of 0.3,

Table 4 Effects of a saline challenge in the injection compartment on locomotor activity[a] and stereotypy[b]

	Dose of cocaine given during conditioning	Locomotion	Headbobbing	Head-down Sniffing	Rearing
Experiment 1	0 mg/kg	21.3±3.5			
	10 mg/kg	41.6±6.3*			
	20 mg/kg	49.3±4.7*			
	40 mg/kg	32.3±5.2			
Experiment 2	0 mg/kg	26.6±6.4	1.2±0.8	36.2±12.8	3.6±0.93
	0.3 mg/kg	25.2±5.6	0.0±0	52.7±7.37	5.5±1.5
	1.0 mg/kg	57.3±14.2*	1.4±0.6	88.4±14.8	8.0±3.8
	3.0 mg/kg	29.0±5.2	5.4±3.1	69.6±12.1	9.6±1.9
	4.2 mg/kg	30.4±7.5	5.2±1.6	60.0±12.1	9.3±2.0
Experiment 3	0 mg/kg	19.2±3.4	1.1±0.4	50.0±6.2	7.1±1.6
	0.3 mg/kg	24.8±2.9	2.3±1.1	73.8±4.8*	7.8±1.5
	1.0 mg/kg	33.7±7.9*	3.8±2.7	61.0±3.7	9.6±2.6
	3.0 mg/kg	28.4±1.9	1.0±0.4	78.4±4.9*	10.6±1.7
	4.2 mg/kg	31.8±5.0	6.6±2.1*	67.0±4.3*	6.8±1.7
	5.6 mg/kg	37.5±5.3*	7.3±1.8*	58.0±6.5	10.2±1.4

[a] Values represent the mean number of crosses ±SEM from one side of the compartment to the other

[b] Values represent the mean number of time-sampled observations (i.e., presence of behavior every 10 s) of each behavior ±SEM

* Represents a significant difference from control group, Fischer test, $P<0.05$ for locomotion and Mann-Whitney U-test, $P<0.05$ for stereotypies

3.0 and 4.2 mg/kg, IV relative to saline controls (Mann-Whitney U-test, $P<0.05$).

Context-independent sensitization

Figures 1–2 and Table 1 illustrate behaviors measured following a challenge injection of cocaine in the noninjection compartment (i.e., test for context-independent sensitization). There were no changes in locomotor activity on this test day relative to the first injection day at any dose of cocaine administered IP or IV. In contrast, there was an increase in headbobbing relative to the first injection day in rats challenged with 20–40 mg/kg, IP and 4.2–5.6 mg/kg, IV (Wilcoxon signed-ranks test, $P<0.05$), suggesting that this sensitization involved context-independent changes. Sensitization of headbobbing was more robust following repeated IP administration than it was following repeated IV administration, evident as a greater percent increase on this test day relative to the first injection. Specifically, the dose of 40 mg/kg, IP produced the maximal increase in headbobbing of 16 600%, whereas the dose of 4.2 mg/kg, IV produced the maximal increase in headbobbing of 915%. The increase in headbobbing in rats administered 40 mg/kg, IP was accompanied by a decrease in head-down sniffing relative to saline controls (Mann-Whitney U-test, $P<0.05$) and relative to the first injection (Wilcoxon signed-ranks test, $P<0.05$; see Fig. 4). There was also an increase in head-down sniffing on this test day relative to the first injection day in rats administered 3.0–4.2 mg/kg, IV (Wilcoxon signed-ranks test, $P<0.05$), suggesting this sensitization involved context-independent changes. With both routes of administration, cocaine produced a decrease in rearing on this test day relative to the first injection day that was significant at doses of 10 mg/kg, IP and 3.0 and 4.2 mg/kg, IV (Wilcoxon signed-ranks test, $P<0.05$).

Dose-response curves for locomotion and headbobbing

Following acute administration of cocaine, there was a great amount of variability in locomotor activity and stereotypic behaviors were not reliably observed, thus making it difficult to compare dose-response curves across routes of administration. Following repeated administration, however, differences in the dose-response curves for these behaviors across routes of administration were more apparent due to lower variability. Following the last injection (i.e., test for context-independent sensitization), cocaine administered IP produced an inverted U-shaped dose-response curve for locomotion [$F(3, 31)=5.38$, $P<0.01$]. Doses of 10 and 20 mg/kg, IP produced more locomotor activity relative to the dose of 40 mg/kg, IP (Fisher LSD tests, $P<0.05$). In contrast, cocaine administered IV produced a monotonic increase in locomotor activity following the last injection [$F(5, 36)=5.97$, $P<0.001$]. The dose of 5.6 mg/kg, IV produced an increase in locomotor activity relative to all other groups (Fisher LSD tests, $P<0.05$). The opposite pattern was observed for changes in headbobbing across routes of administration. Following the last injection, cocaine administered IP produced a monotonic increase in headbobbing ($H=20.8$, $P<0.001$). The dose of 20 mg/kg, IP produced an increase in headbobbing relative to 10 mg/kg, IP; and 40 mg/kg, IP produced an increase in headbobbing relative to 20 mg/kg, IP (Mann-Whitney U-tests, $P<0.05$). In contrast, following the last injection, cocaine administered IV produced an inverted U-shaped dose-response curve for headbobbing ($H=26.8$, $P<0.001$). The dose of 4.2 mg/kg, IV produced an increase in headbob-

bing relative to both 3.0 and 5.6 mg/kg, IV (Mann-Whitney U-tests, $P<0.05$).

Discussion

The present study provides the first comparison of the stimulant properties of cocaine with IP and IV routes of administration. Following the last injection (i.e., test for context-independent sensitization), cocaine administered IP produced an inverted U-shaped dose-response curve for locomotion. In contrast, cocaine administered IV produced a monotonic ascending dose-response curve for locomotion following the last injection. The descending limb of the dose-response curve for locomotion following the last injection of cocaine administered IP may have been due to the high frequency of headbobbing at higher doses interfering with locomotion. It is possible that higher doses of cocaine administered IV may have resulted in a descending limb of the dose-response curve for locomotion. This may not be the case, however, since it appeared that stereotypic headbobbing had reached asymptote within the range of doses administered IV. Furthermore, we have administered 8.0 mg/kg, IV to rats on 2 consecutive days and found they exhibited convulsions continuously for 4–5 min and 33% died (unpublished observation). In any case, the pattern of behaviors produced by the highest, and marginally toxic, doses used in the present study appears to differ depending on the route of administration.

Repeated administration of cocaine produced sensitization of stereotypy with both routes of administration, although the behaviors that became sensitized and the degree of sensitization varied. Both routes of administration produced sensitization of headbobbing, consistent with previous research (Post and Rose 1976; Orona et al. 1994). Sensitization of headbobbing was more robust following IP administration than it was following IV administration (i.e., a maximal increase of 16, 600% at 40 mg/kg, IP versus a maximal increase of 915% at 4.2 mg/kg, IV following the last injection relative to the first). The higher frequency of headbobbing obtained following repeated IP administration relative to repeated IV administration may be due to pharmacokinetics, such as an increase in the amount of time drug is present in the bloodstream following IP administration relative to IV administration (McKim 1991). Sensitization of head-down sniffing was evident only in animals given IV cocaine and 40 min exposures, and this sensitized response was less robust (i.e., a 90% increase) relative to the sensitized headbobbing response. Repeated administration of cocaine did not produce sensitization of rearing. In contrast, Orona et al. (1994) have previously reported sensitization of rearing following IV cocaine administration. Procedural differences which may account for this discrepancy include differences in habituation to the test environment and number of injections administered.

There was no sensitization of locomotor activity with either route of administration. Sensitization of locomotor activity has been reported previously following both repeated IP and IV administration of cocaine (Post and Rose 1976; Roy et al. 1978; Kalivas et al. 1988; Hooks et al. 1990; Zahniser and Peris 1991; Tella 1994). The reason for the lack of sensitization of locomotor activity in the present study is unclear, but several explanations can be offered. First, it is possible that sensitization of stereotypic behaviors may have interfered with locomotor activity. Many studies that report sensitization of locomotor activity utilize regimens involving only one to five injections (Lin-Chu et al. 1985; Kalivas et al. 1988; Weiss et al. 1989). The results from earlier injections in the present study revealed increases in locomotor activity that were no longer evident by the sixth injection, perhaps due to sensitization of stereotypy. Second, many studies that report behavioral sensitization habituate animals to the test environment prior to the cocaine injection. In the present study, however, the animals were immediately placed in the test environment following cocaine administration in order to maximize CS-US overlap and to avoid latent inhibition from CS pre-exposure. It seems intuitive that cocaine-induced locomotor activity may appear more robust in habituated animals relative to nonhabituated animals, and this may account for the lack of sensitization of locomotion in the present study. A problem with this explanation, however, is that direct comparison of these procedures has indicated greater sensitization in nonhabituated animals relative to habituated animals (Kiyatkin 1992). Third, many researchers assess cocaine-induced locomotor activity using automated photocell systems that have detectors positioned close to each other at various planes in the apparatus. These systems detect small movements of the animal's body and may be more sensitive to detecting sensitization effects. It seems possible, however, that these systems may be recording an increase in stereotypic movements rather than the reported increase in locomotor activity. Indeed, Petry et al. (1992) found that rats registered high photocell counts while engaged in stationary activities such as grooming and stereotypy. Collectively, our studies suggest that totaling the number of beam breaks in an activity box with multiple photocells may not necessarily reflect changes in locomotion, and demonstrate the importance of measuring stereotypy, as well as locomotor activity.

Sensitization of headbobbing and head-down sniffing was evident in rats receiving cocaine in the noninjection compartment, indicating a context-independent component. Thus, these sensitized responses appear to involve changes that are independent of conditioning factors. Furthermore, the sensitized responses produced by both low and high doses of cocaine involved a context-independent component. Post et al. (1992) suggested that administering low doses of cocaine may produce sensitization that is context-dependent, whereas administering high doses and/or increasing the number of injections may produce sensitization that is context-independent. The reason sensitized responses observed at low doses in the present study involved a context-independent

component may be due to the high number of injections given.

The present study did not directly assess the role of conditioning in sensitized responses (i.e., context-dependent sensitization) since there was no control group challenged with cocaine in their injection compartment for comparison to those challenged in their noninjection compartment. Greater responses in the former group relative to the latter would suggest that the response also involved a context-dependent component. The present study did, however, assess whether animals exhibited CA by challenging the animals with saline in their injection environment (i.e., exposure to CS alone). With both routes of administration, some of the cocaine dosage groups exhibited CA on this test day.

Cocaine produced a stepwise dose-response curve for CPP with both routes of administration, consistent with the results from a recent meta-analysis (Bardo et al. 1995). Nomikos and Spyraki (1988), however, have reported an inverted U-shaped dose-response curve for cocaine administered IV, in which CPP was evident within a narrow dose range (i.e., 0.5–2.5 mg/kg, IV) and conditioned place aversion (CPA) was demonstrated at higher doses (i.e., 5.0 and 10.0 mg/kg, IV). In the present study, CPP was evident at a dose (i.e., 5.6 mg/kg, IV) that produced CPA in the Nomikos and Spyraki study. Six exposures were conducted in the present study, whereas four exposures were conducted in the Nomikos and Spyraki study. It is possible that more drug-environment exposures are necessary to obtain CPP with high doses administered IV. Perhaps tolerance develops to aversive properties of high doses, such that CPP is established during later exposures. Alternatively, sensitization to the rewarding properties of cocaine may develop with repeated administration, as has been suggested previously (Lett 1989; Shippenberg and Heidbreder 1994).

The findings from the present study suggest that the optimal parameters for conditioning the rewarding and stimulant properties of cocaine may vary. For example, rats given the dose of 1.0 mg/kg, IV exhibited CPP when exposed to the compartment for 40 min, but not 20 min. In contrast, conditioned headbobbing and head-down sniffing were evident following 20-min exposures, but not following 40-min exposures. These findings suggest that longer exposure times may be optimal for CPP, whereas shorter exposure times may be optimal for conditioning the stimulant properties of cocaine. The optimal exposure time may vary due to differences in time course of the unconditioned effects of the drug. The stimulant effects are most robust in the first 20 min, whereas reinforcing effects may last longer. If this is the case, then shorter exposure times would maximize CS-US overlap for stimulant effects, whereas longer exposure times would maximize CS-US overlap for reinforcing properties.

In summary, sensitization of locomotor activity was not evident following either repeated IP or IV cocaine administration, however, there was sensitization of headbobbing and sniffing. Sensitization of headbobbing was more robust following IP administration than it was following IV administration, and in both cases, the response involved a context-independent component. These findings demonstrate the importance of measuring stereotypy, as well as locomotor activity, when assessing the stimulant properties of cocaine. Furthermore, the procedure used in the present study provides an efficient method for measuring the rewarding and unconditioned and conditioned stimulant properties of cocaine.

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