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Environmental and pharmacological sensitization: effects of repeated administration of systemic or intra-nucleus accumbens cocaine

M. Stacy Hooks*, Graham H. Jones, Scott E. Hemby***, and Joseph B. Justice Jr**

Department of Chemistry, Emory University, 1515 Pierce Drive, Atlanta, GA 30322, USA

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Abstract. The effects of repeated systemic or intra-nucleus accumbens cocaine administration on locomotor activity were examined for environmental dependence. Repeated IP administration of cocaine (15 mg/kg) for 5 days in the context of a given environment increased the locomotor response to a subsequent IP cocaine challenge in that environment. However, there were no differences in the locomotor response to a subsequent IP cocaine challenge in the test chamber in subjects which had received prior repeated IP administration of cocaine in the home-cage. In a second experiment, cocaine (100 μg/side) was infused into the nucleus accumbens (NACC) daily for 5 days. This repeated administration produced increases in locomotor activity to subsequent intra-NACC cocaine infusions that were environmentally independent. In contrast to the effects of repeated IP cocaine administration, subjects which received administration of vehicle, acute cocaine, or repeated cocaine in the NACC did not differ following an IP cocaine challenge. The results from these experiments indicate that increases in the response to IP cocaine following repeated IP administration are in part environmentally dependent. Moreover, repeated intra-NACC cocaine infusions increase the responsiveness of the NACC to subsequent intra-NACC cocaine. However, local activation of the NACC alone does not appear to be adequate to produce sensitization to systemically administered cocaine.

Key words: Cocaine – Nucleus accumbens – Sensitization – Locomotor activity – Conditioning – Rat

* *Present address:* Department of VCAPP, Washington State University, Pullman, WA 99164-6520, USA

** *Present address:* Department of Neuropsychopharmacology, Schering AG, Postfach 650311, W-1000 Berlin 65, Federal Republic of Germany

*** *Present address:* Center for the Investigation of the Neurobiology of Drug Abuse, Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University 300 S Hawthorne Road, Winston-Salem, NC 27103, USA

Correspondence to: J. B. Justice, Jr

Systemic cocaine administration produces pronounced increases in locomotor activity which are thought to be mediated via dopaminergic mechanisms of the nucleus accumbens (NACC; Kelly and Iversen 1976). Cocaine is a potent dopamine uptake inhibitor (Reith et al. 1986) and thus increases extracellular concentrations of dopamine in the NACC (Kalivas and Duffy 1990; Pettit et al. 1990 Hooks et al. 1991a). Infusions of cocaine directly into the NACC also increase locomotor activity (Delfs et al. 1990; Hemby et al. 1992) which can be blocked by neuroleptics (Delfs et al. 1990), while depletion of dopamine in the NACC by infusions of the neurotoxin 6-hydroxydopamine blocks the locomotor stimulating properties of cocaine (Kelly and Iversen 1976).

Repeated administration of cocaine is frequently associated with a progressive increase in the locomotor response to subsequent cocaine challenges (Post and Rose 1976; Roy et al. 1978). This potentiation of locomotor activity is environmentally dependent, particularly following low to moderate doses of cocaine (20 mg/kg or less; Post et al. 1987; Weiss et al. 1989). However, environmentally independent increases in locomotor activity can be observed following repeated cocaine treatment if much higher doses of cocaine are used (e.g. 40 mg/kg IP or greater; Gale et al. 1984; Post et al. 1988). These higher doses of cocaine are also known to induce pharmacological kindling (Post et al. 1988; Post and Weiss 1989; Weiss et al. 1990). The associations between cocaine-induced locomotor activity and environmental stimuli are sufficient that a conditioned locomotor response can be observed even if habituation to the test environment is allowed before the drug is administered during conditioning sessions (Post et al. 1988).

The NACC plays a role in the conditioned locomotor responses to cocaine (Post et al. 1988; Hemby et al. 1992). Repeated administration of IP cocaine (10 mg/kg for 10 days) in the test environment enhances the subsequent locomotor response to intra-NACC challenge infusions of both amphetamine and saline (Post et al. 1987). No increase in the locomotor response is observed following infusions into the caudate nucleus in rats pretreated with cocaine (Post et al. 1987, 1988). The dopaminergic projec-

tion to the NACC has been implicated in environmental sensitization by the observation that partial dopaminergic-depleting lesions of the NACC do not block the locomotor response to IP cocaine but do block the environmentally conditioned locomotor activity (Post et al. 1988). In addition, intra-accumbens cocaine infusions paired with distinct environmental cues can elicit a conditioned locomotor response (Hemby et al. 1992).

In man there appears to be a high degree of environmental conditioning to cocaine's reinforcing effects (Gawin et al. 1986; Dackis and Gold 1990). For example, cravings and withdrawal symptoms can be observed following the presentation of cocaine paraphernalia in humans even after months of abstinence (Childress et al. 1987).

The present series of experiments were designed to further investigate environmentally and pharmacologically induced changes in locomotor activity resulting from repeated systemic cocaine administration and from repeated infusions of cocaine into the NACC.

Materials and methods

Subjects. Subjects were 77 male Wistar rats (Harlan) weighing 320–400 g which were housed 4 per cage on a 12 h light-dark cycle (lights on from 07:00 to 19:00 hours). Subjects had free access to food and water in their home-cage. Testing was conducted between 08:00 and 17:00 hours.

Apparatus. Locomotor activity was measured in Plexiglas photocell cages (39 × 25 × 24 cm high). Each cage was equipped with two parallel, horizontal, infrared beams, 2 cm above the floor, spaced equally along the long axis of the cage. Interruption of alternate beams resulted in a locomotor count that was registered by an IBM computer. Illumination was provided by a light on the roof of each photocell cage. White noise was provided in each cage to prevent disturbances from the outside environment.

Drugs. Cocaine-HCl (15 mg/ml) was dissolved in 0.9% saline and injected in a volume of 0.1 ml/100 g for IP administration. For intra-NACC administration, cocaine-HCl (100 μg/0.5 μl) was dissolved in artificial cerebrospinal fluid (CSF) and infused in a volume of 0.5 μl/side. CSF was composed of 0.13 M sodium chloride, 0.98 mM magnesium chloride, 2.65 mM potassium chloride, 1.2 mM calcium chloride, 0.25 mM ascorbic acid, and 10 mM glucose at a pH of 7.2–7.4.

Experiment 1

The day before the initial drug treatment, subjects ($n = 32$) were placed in individual photocell cages for a 2 h habituation period. Subjects were weighed after the habituation period and assigned to one of four treatment groups, counter-balanced by the locomotor response during habituation and by body weight, to receive either 15.0 mg/kg cocaine in the test-cage (Coc-Test), 15.0 mg/kg cocaine in the home-cage (Coc-Home), saline in the test-cage (Veh-Test) or saline in the home-cage (Veh-Home). The sequence of testing for the four groups is shown in Table 1. The Coc-Test group was given repeated IP cocaine administration in the test-cage to allow possible conditioning effects of cocaine to be observed. The Coc-Home group was treated with IP cocaine in the home-cage for the first 4 days and with IP cocaine in the test-cage on day 5. The purpose of this group was to determine whether pharmacological sensitization to this dose of cocaine occurred irrespective of any environmental cues associated with previous exposure to the test chamber. The Veh-Test and Veh-Home served as controls to ensure that the testing procedure did not alter a subject's response to subsequent cocaine administration.

Table 1. The dose sequence for rats receiving IP cocaine. C = cocaine (15 mg/kg), V = vehicle (0.9% saline), T = test-cage (subject received drug in the test-cage), H = home-cage (subject received drug in the home-cage)

Group	Days					
	1	2	3	4	5	6
Coc-Test	CT	CT	CT	CT	CT	CT
Veh-Test	VT	VT	VT	VT	VT	CT
Coc-Home	CH	CH	CH	CH	CT	CT
Veh-Home	VH	VH	VH	VH	VT	CT

On test days subjects either received the appropriate drug in the home-cage or were placed in the photocell cages for a 1 h habituation period, administered the appropriate drug, and locomotor activity monitored for 1 h. On day 6 all subjects received IP cocaine (15 mg/kg) in the test-cage to determine the effects of the four different treatment procedures on an acute cocaine challenge.

Data analysis. Locomotor activity counts were subjected to analysis of variance (ANOVA). The totals for days 1–5 were subjected to a two-way ANOVA with one between-subjects factor, Drug treatment group, and one within-subjects factor, Days. The time courses for days 5 and 6 were analyzed with ANOVA with two between-subjects factors, Drug treatment and Environment, and one within-subjects factor, Time. In addition, simple main effects analyses were used to analyze the data from days 5 and 6. Where appropriate, post-hoc comparisons were made using Newman-Keuls analysis.

Experiment 2

Surgical procedures. Subjects ($n = 55$) were anesthetized with a 50 mg/kg IP injection of sodium pentobarbital (Nembutal) and placed in a stereotaxic frame. Bilateral stainless-steel guide cannulae (22 g) were implanted to access the NACC, AP + 3.4 from Bregma, Lat ± 1.7, Vert − 6.5 from dura with the incisor bar set at + 5 mm (Pellegrino et al. 1979). The guide cannulae were secured in place with the use of skull screws and dental cement. Removable stylets (31 g) were placed in the guide cannulae. Intramuscular penicillin (60 000 units) was administered immediately following surgery and a recovery period of 7–8 days was allowed before the initial exposure to the test-cage.

Intracerebral infusions were made bilaterally via 30 g infusion cannulae which protruded 1 mm below the guide cannulae. The infusion cannulae were attached via plastic (PE-10) tubing to 10 μl syringes mounted on a Razel infusion pump (Model A). The 0.5 μl/NACC infusions were delivered simultaneously over a 45 s period with an additional 1 min diffusion period allowed to elapse before withdrawing the infusion cannulae. The subjects were held lightly in a towel during the infusions.

Behavioral procedures. The day before the initial drug treatment, subjects were placed in the test-cages for a 2 h habituation period as in experiment 1. After the habituation period all subjects were removed and given a bilateral infusion of CSF following the described procedure. The purpose of this initial infusion was to reduce the non-specific consequences of infusion damage on the test days. Subjects were then weighed and assigned to one of five groups to receive either cocaine in the test-cage (Coc-Test), cocaine in the home-cage (Coc-Home), CSF in the test-cage (Veh-Test), CSF in the home-cage (Veh-Home), or CSF in the home-cage followed by cocaine in the test-cage on day 5 (Acute-Coc). The dose sequence and environmental location for the five groups are described in

Table 2. The dose sequence for rats receiving intra-NACC cocaine. C = cocaine (100 μg/side in 0.5 μl), V = vehicle (0.5 μl of CSF per side), T = test-cage (subject received drug in the test-cage), H = home-cage (subject received drug in the home-cage), IP = IP cocaine (15 mg/kg IP cocaine in the test-cage)

Group	Days					
	1	2	3	4	5	6
Coc-Test	CT	CT	CT	CT	CT	IPT
Veh-Test	VT	VT	VT	VT	VT	IPT
Coc-Home	CH	CH	CH	CH	CT	IPT
Veh-Home	VH	VH	VH	VH	VT	IPT
Acute-Home	VH	VH	VH	VH	CT	IPT

Table 2. Groups were counter-balanced for the locomotor score during habituation and for body weight.

The Coc-Test group was given daily infusions of cocaine (100 μg/side) in the test-cage to examine the influence of prior exposure to stimuli associated with cocaine on sensitization. Subjects in the Coc-Home group were given cocaine infusions (100 μg/side) on test days 1–4 in the home-cage as a control for any environmentally cued locomotor activity and to investigate pharmacologically induced changes in locomotor activity on test day 5. On test day 5, rats in this group were infused in the test-cage with cocaine. The Veh-Home and Veh-Test groups served as controls for the Coc-Home and Coc-Test groups, respectively, to assess any change in locomotor activity which may be due to non-specific damage as a result of the infusion procedure. An additional group (Acute-Coc) received saline on days 1–4 in the home cage and intra-NACC cocaine on day 5. This group was used to assess the acute effects of intra-NACC cocaine and to determine the effects of acute treatment with intra-NACC cocaine on subsequent response to IP cocaine.

On day 5, all groups were placed in the test-cage for 1 h to assess differences in locomotor activity during habituation. Following this hour, subjects were removed from the chamber, infused with the appropriate solution and returned to the test chamber for an additional hour. On day 6, subjects were habituated in the manner described previously and all were injected with IP cocaine (15 mg kg) and returned to the test-cage.

On test days 1 through 4, subjects were placed in the test-cage for a 1 h habituation period, infused, and locomotor activity monitored 1 h after each infusion. On day 5 all groups were infused in the test-cage and monitored as on days 1–4. On test day 6 all subjects received IP cocaine (15 mg/kg) in the test-cage and were monitored as before.

Histology. At the completion of testing subjects were anesthetized with 400 mg choral hydrate and perfused with 50 ml of saline followed by 50 ml formalin (10%). Following fixation, coronal sections (75 μm) were cut on a freezing microtome and each section through the NACC and associated structures were mounted on a glass slide and stained with thionin blue for determination of cannulae placements by a researcher unaware of experimental conditions.

Data analysis. Locomotor activity counts were subjected to ANOVA. The totals for days 1–5 were subjected to a two-way ANOVA with one between-subjects factor, Drug treatment, and one within-subjects factor, Days. The time courses for days 5 and 6 were analyzed with ANOVA with two between-subjects factors, Drug treatment and Environment, and one within-subjects factor, Time. In addition, simple main effects analyses were used to analyze the data from Days 5 and 6. Where appropriate, post-hoc comparisons were made using Newman-Keuls analysis. Least-squares linear regression was conducted to examine the relationship between locomotor activity in a novel environment and cocaine induced locomotor activity.

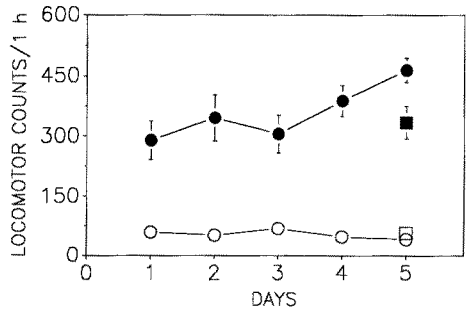

Fig. 1. Locomotor counts (mean ± SEM) following IP cocaine administration (15 mg/kg; $n = 8$ per group) for days 1–5. Locomotor activity increased following repeated cocaine administration in the test environment, $P < 0.025$. Rats treated with cocaine in the test-cage demonstrated greater locomotor activity than those treated with cocaine in the home-cage, $P < 0.05$. (●) Coc-Test; (○) Veh-Test; (■) Coc-Home; (□) Veh-Home

Results

Experiment 1: repeated IP cocaine administration

Locomotor response to IP cocaine on days 1–5 Figure 1 shows the total locomotor activity counts following treatment (either cocaine or saline) for test days 1–5. As expected, in those subjects repeatedly treated in the test-cage, cocaine administration significantly elevated locomotor activity compared with saline administration [$F(1, 14) = 143.90$, $P < 0.0001$] (Coc-Test versus Veh-Test groups). Furthermore, repeated cocaine treatment in the test-cage produced a gradual potentiation of this locomotor response as indicated by a Drug × Days interaction [$F(4, 56) = 3.29$, $P < 0.025$]. Also evident from Fig. 1 is that the potentiation of the locomotor response to cocaine following repeated administration is dependent upon the treatment environment. The Coc-Home group, which received the same exposure to cocaine as the Coc-Test group, but in the home-cage, showed significantly less activity on day 5 [$F(1, 28) = 31.95$, $P < 0.0001$].

A more detailed analysis of these results was conducted and the locomotor activity results from day 5 alone are depicted in Fig. 2A. As can be seen, both cocaine treated groups show elevated locomotor activity for the entire 60 min period compared with saline-treated subjects as indicated by a main effect of Drug, [$F(1, 28) = 176.89$, $P < 0.0001$], and a Drug × Time interaction [$F(11, 308) = 10.48$, $P < 0.0001$]. There was also a significant Drug × Environment interaction [$F(1, 28) = 7.66$, $P < 0.01$]. Simple main effects analysis indicates that subjects previously treated in the test-cage with cocaine showed significantly greater locomotor activity than those treated in the home-cage [$F(1, 28) = 31.95$, $P < 0.0001$]. However, there were no differences between the two saline-treated groups [$F(1, 28) = 0.46$, n.s.] (Veh-Home versus Veh-Test groups). Analysis of body weights showed no significant effect of cocaine treatment (no main effect of Drug [$F(1, 28) = 1.87$, n.s.], or a Drug × Environment interaction [$F(1, 28) = 2.03$, n.s.]).

Behavioral response to IP cocaine on day 6. Figure 2B depicts the results of cocaine administration on day 6.

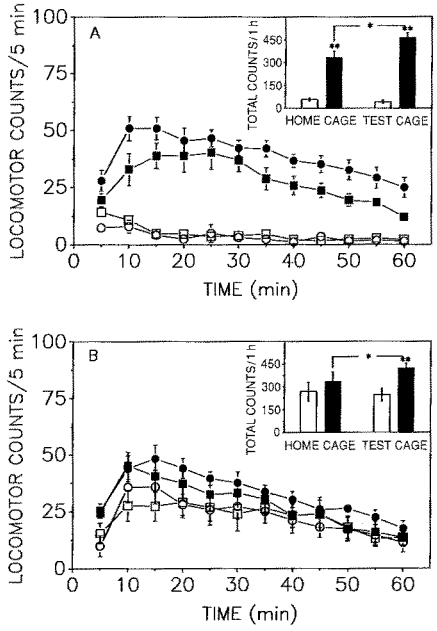

Fig. 2. Panel **A** represents the locomotor counts ± SEM for day 5 of IP drug treatment ($n = 8$ per group). Rats pre-treated in the test-cage with cocaine (15 mg/kg) exhibited a greater locomotor response than those pre-treated in the home-cage with cocaine. No differences were observed between the two saline groups. Panel **B** represents the locomotor counts ± SEM for day 6 of IP drug treatment on which all subjects received cocaine. There was a difference between cocaine and saline pretreatment groups in the test-cage, but not between pretreatment groups in the home-cage. $^{*}P < 0.05$, $^{**}P < 0.01$. (●) Coc-Test; (○) Veh-Test; (■) Coc-Home; (□) Veh-Home

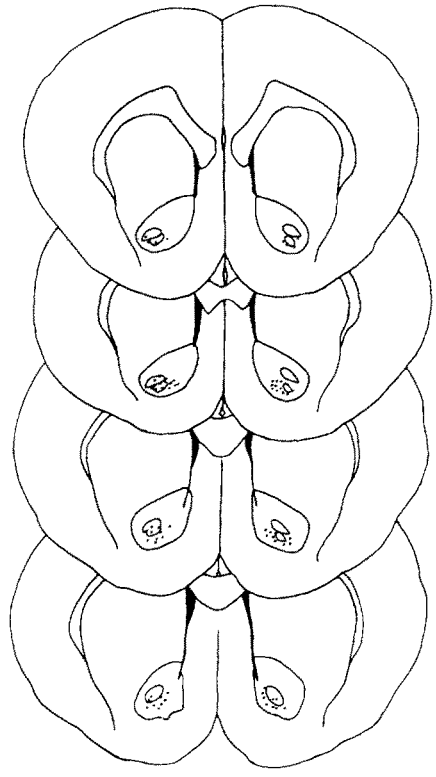

Fig. 3. Placements of the most ventral position of the injection cannulae of subjects included in the statistical analysis are represented by *filled circles*. Brain sections are modified from Paxinos and Watson (1986) and are from + 1.7 to + 1.0 A-P anterior to bregma

There was a significant difference in locomotor activity between subjects previously treated with cocaine and those previously treated with saline [$F(1, 28) = 5.78$, $P < 0.025$], and a Drug × Environment interaction [$F(1, 28) = 4.42$, $P < 0.05$]. There were no significant Environment × Time [$F(11, 352) = 0.83$], Drug × Time [$F(11, 352) = 1.63$] or Environment × Drug × Time [$F(11, 352) = 0.76$] interactions.

Simple main effects analyses showed there was a significant difference between subjects pretreated with either cocaine or saline in the test-cage to a subsequent cocaine challenge [$F(1, 28) = 22.40$, $P < 0.0001$]. There was only a trend for a difference between those subjects pretreated with either cocaine or saline in the home-cage to cocaine challenge [$F(1, 28) = 3.36$, $P < 0.08$] (see inset, Fig. 2B). Subjects previously treated in the test-cage with cocaine showed significantly greater locomotor activity than those treated in the home-cage with cocaine [$F(1, 28) = 5.36$, $P < 0.05$]. However, there were no differences between the two saline-treated groups [$F(1, 28) = 0.34$, n.s.] (Veh-Home versus Veh-Test groups). Cocaine treatment did not alter body weight [$F(1, 28) = 1.94$, n.s.].

Behavioral response during the habituation period. Repeated pairing of cocaine administration with the stimuli of the test-cages resulted in a significantly increased locomotor response which was evident during the habituation periods prior to drug testing. For example, there were no differences between the Coc-Test and Veh-Test groups during the 1 h habituation period on day 1 [$F(1, 14) = 1.11$, n.s.]. However, the Coc-Test group exhibited greater locomotor activity than the Veh-Test group during the habituation periods between days 2–6, [$F(1, 14) = 7.81$, $P < 0.02$].

Experiment 2: repeated intra-NACC cocaine

Locomotor response following intra-NACC infusions days 1–5. The cannula placements for the subjects included in the statistical analysis are depicted in Fig. 3. Ten subjects were excluded from the statistical analysis due to improper cannula placements. The total locomotor activity counts following drug administration for days 1–5 are depicted in Fig. 4. As can be seen, for those subjects treated in the test-cage (Coc-Test and Veh-Test groups) intra-NACC cocaine infusions significantly increased locomotor activity compared to CSF infusions [$F(1, 17) = 7.98$, $P < 0.02$]. The repeated administration of cocaine resulted in a gradual potentiation of the drug's effects on locomotor activity, as indicated by a Drug × Days interaction [$F(4, 68) = 6.65$, $P < 0.0001$].

The time course for locomotor activity on day 5 is shown in Fig. 5A. Intra-NACC cocaine infusions significantly elevated locomotor activity throughout the 60 min time period [$F(1, 33) = 32.85$, $P < 0.0001$]. However, there were no differences between those subjects pretreated in the test-cage and those pretreated in the home-

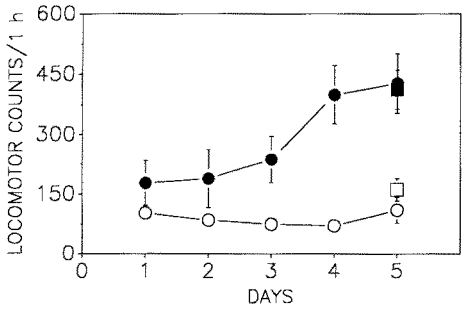

Fig. 4. Locomotor counts (mean ± SEM) following bilateral intra-NACC cocaine infusions (Coc-Test, $n = 9$; Coc-Home, $n = 10$; CSF-Test, $n = 10$; CSF-Home, $n = 8$) for days 1–5. An increase in locomotor activity following repeated administration of intra-NACC cocaine was observed irrespective of the pretreatment environment, $P < 0.0001$. (●) Coc-Test; (○) Veh-Test; (■) Coc-Home; (□) Veh-Home

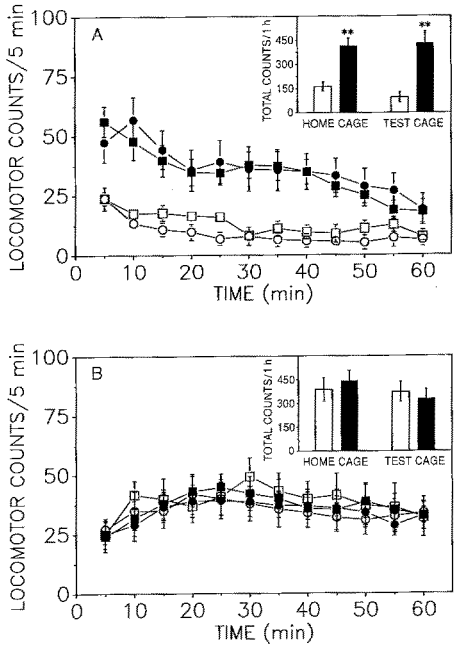

Fig. 5. Panel **A** represents the locomotor counts ± SEM for day 5 of intra-NACC drug treatment. Rats pre-treated with cocaine ($n = 9$ test, $n = 10$ home) exhibited a greater locomotor response than those pre-treated with CSF ($n = 10$ test, $n = 8$ home), $P < 0.0001$. Panel **B** represents the locomotor counts ± SEM for day 6 on which all subjects received IP cocaine (15 mg/kg). There were no differences between the cocaine and CSF pretreatment groups following the IP challenge injection. $^{**}P < 0.01$. (●) Coc-Test; (○) Veh-Test; (■) Coc-Home; (□) Veh-Home

cage [$F(1, 33) = 0.50$, n.s.], indicating a lack of environmental sensitization. Cocaine treatment did not alter body weight [$F(1, 33) = 0.72$, n.s.].

Behavioral response to IP cocaine on day 6. As shown in Fig. 5B all four groups showed similar levels of locomotor activity. No differences between the groups pretreated in the test-cage and those pretreated in the home-cage [$F(1, 33) = 0.97$, n.s.] were observed. There were also no differences between those groups pretreated with cocaine or saline [$F(1, 33) = 0.03$, n.s.]. The fact that there is no Drug × Environment interaction [$F(1, 33) = 0.56$, n.s.], further indicates that there is no difference between the four groups to IP challenge. Cocaine treatment did not alter body weight [$F(1, 33) = 0.72$, n.s.].

Behavioral response during the habituation period for NACC infused rats. The lack of environmental conditioning is further evident when the 1 h habituation period is considered. There were no differences in the locomotor activity between the Coc-Test and Veh-Test groups during the 1 h habituation period on day 1 [$F(1, 17) = 1.01$, n.s.], or for days 2–6 [$F(1, 17) = 0.15$, n.s.] (data not shown).

Pharmacological sensitization to intra-accumbens cocaine infusions. For further analysis, the two cocaine pretreated groups (Coc-Test and Coc-Home) were combined into one group, and referred to as Chronic-Coc, as they did not significantly differ. The two vehicle pretreated groups (Veh-Test and Veh-Home) were also combined into one group, Chronic-CSF. Figure 6A depicts the results for day 5. ANOVA indicated a significant difference between the Chronic-Coc, Acute-Coc, and Chronic-CSF groups [$F(2, 42) = 18.85$, $P < 0.0001$]. There was also a significant Group × Time interaction [$F(22, 242) = 2.66$, $P < 0.0001$]. Post-hoc comparisons revealed a greater locomotor response in the Chronic-Coc group than in

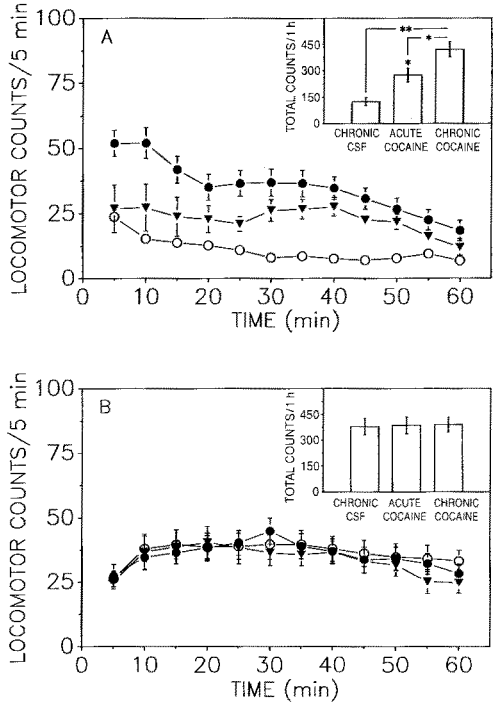

Fig. 6. Panel **A** represents the locomotor counts ± SEM for day 5 of intra-NACC drug treatment. Rats pre-treated with chronic cocaine ($n = 19$) exhibited a greater locomotor response than those pre-treated with acute cocaine ($n = 8$), or CSF ($n = 18$). Panel **B** represents the locomotor counts ± SEM for day 6 on which all subjects received IP cocaine (15 mg/kg). There were no differences between the chronic cocaine, acute cocaine and CSF pretreatment groups following the IP challenge injection. (●) Chronic-coc; (▼) acute-coc; (○) chronic-CSF

either the Acute-Coc group ($P < 0.05$) or the Chronic-CSF group ($P < 0.01$). The Acute-Coc group also had a higher locomotor response than the Chronic-CSF group ($P < 0.05$). Differences between the groups are unlikely to be due to the effects of non-specific damage following NACC infusions. For example, there were no significant differences between subjects that had either one or five previous infusions of CSF before cocaine infusion [$F(1, 17) = 1.01$, n.s.] (data not shown).

As shown in Fig. 6B, all three groups showed almost identical patterns of locomotor activity following IP cocaine administration on day 6 [$F(2, 42) = 0.19$, n.s.]. This figure shows the lack of effect of intra-NACC cocaine infusions on the subsequent locomotor response to IP cocaine.

Locomotor response to novelty and the response to intra-NACC injections. The locomotor response to novelty predicted a subject's locomotor responses to intra-NACC cocaine. When subjects were divided into two groups based on their locomotor score for the first hour of their initial exposure to the test-cage (Hooks et al. 1991b), high responding rats (HR) showed a greater locomotor response to cocaine on day 5 than low responding rats (LR) [$F(1, 33) = 12.66$, $P < 0.005$]. Moreover, locomotor response to novelty correlated with the locomotor response to intra-NACC cocaine on day 5 ($r = 0.60$, $P < 0.01$).

Discussion

The results of the present experiments suggest that there are significant differences between the effects of repeated IP and repeated intra-NACC cocaine. In agreement with previous literature (Post et al. 1988), repeated IP cocaine in the context of a given environment produces increases in the locomotor response to subsequent IP cocaine challenges. In contrast, repeated cocaine administration in the NACC elicited increases in locomotor activity to subsequent intra-NACC challenges irrespective of pre-treatment environment. However, prior exposure to repeated intra-NACC cocaine did not augment the locomotor response to an IP cocaine challenge.

In agreement with previous studies (e.g. Post et al. 1987), repeated IP administration of cocaine in the test-cage gradually enhanced the response to subsequent IP injections of cocaine in the same environment. This is indicated by the greater locomotor activity shown by the Coc-Test group compared to the Coc-Home group on day 5 (Fig. 1). Repeated administration of the same dose of cocaine outside the test-cage did not significantly enhance the locomotor response to a subsequent cocaine challenge when compared to subjects repeatedly treated with vehicle. This difference between the effects of repeated cocaine in the test-cage and in the home-cage could be due to differences in the amount of exposure to the test-cages, as the Coc-Home group received less exposure to the test cage than the Coc-Test group. However, this seems unlikely to have influenced the results as novelty usually increases locomotor activity in these situations and both groups were well habituated to the test-cages prior to testing on day 5. Also, there were no differences between the vehicle groups on days 5 or 6. The association between environmental stimuli and the effects of cocaine is further emphasized by an increase in the locomotor activity scores during the initial habituation period in the subjects treated with cocaine in the test-cages.

The findings from the current experiments are in agreement with previous results (Post et al. 1987; Hooks et al. 1991b) that indicate the augmentation of locomotor activity following repeated systemic cocaine treatment is at least partially environmentally dependent. A relatively robust association between the cocaine-induced locomotor activity and environmental stimuli is indicated by the inability of a 1 h habituation period to the test-cage prior to drug administration to prevent the environmentally associated effects. Repeated daily treatment over a 10 day period with lower doses of the drug (10 mg/kg/day) also produces increases in locomotor activity which have been demonstrated to be environmentally dependent (Post et al. 1988; Weiss et al. 1989, 1990). The present results have confirmed and extended these previous studies using a higher dose of cocaine and fewer pairings.

In the current experiments statistically significant pharmacological sensitization to IP administered cocaine was not observed. However, analysis of day 6 data indicates there is a trend toward sensitization to IP cocaine in the Coc-Home group after cocaine administration ($P < 0.08$). This trend toward sensitization on day 6 could be either pharmacological, from the five prior injections, or environmental from the single prior exposure to cocaine in the test-cage, or both. Whether pharmacological sensitization occurs is clearly dependent upon the dose of cocaine used and the length of treatment. For example, pharmacological changes can be demonstrated following very high doses of the drug, e.g. 40 mg/kg or higher (Post et al. 1987; Weiss et al. 1989). In addition, longer term treatment (10 or 30 days) with higher doses (30 mg/kg) has also been shown to alter the pharmacokinetics of cocaine (Pettit et al. 1990, Pan et al. 1991). These factors may have contributed to the trend towards pharmacological sensitization.

Previous studies have suggested that the NACC may play an important role in the increases in locomotor activity following repeated IP cocaine (Post et al. 1988). Post et al. demonstrated that rats which had been repeatedly treated with cocaine in the test-cage (10 mg/kg IP for 10 days) showed a greater response to intra-NACC amphetamine than those subjects that received cocaine in the home-cage. There was, however, no difference between the two groups following a challenge infusion of amphetamine into the caudate nucleus. Other studies have indicated that there are not only enhanced levels of locomotor activity, but also increased extracellular dopamine levels in the NACC following repeated IP cocaine (Kalivas and Duffy 1990; Pettit et al. 1990). In the latter study this greater increase in NACC dopamine concentration could be accounted for in part by the accompanying increases in brain cocaine levels (Pettit et al. 1990; Pan et al. 1991). The NACC is also implicated in sensitization by 6-hydroxydopamine lesions of this structure. Lesions which partially deplete (less than 60%) dopamine in the NACC do not block the locomotor activity induced by a high dose of cocaine (40 mg/kg) but do prevent sensitization produced

by administration of this dose of the drug (Post et al. 1988). More extensive lesions of the NACC (90% or greater) have been shown to block both the unconditioned (Kelly et al. 1976) and the conditioned (Gold et al. 1988) locomotor responses to psychomotor stimulants.

In the present experiments infusions of cocaine directly into the NACC produced a pronounced increase in locomotor activity, confirming the results of two previous studies (Delfs et al. 1990; Hemby et al. 1992). However, in contrast to the effects of systemic cocaine administration in expt 1, repeated infusions of cocaine directly into the NACC produced an increase in locomotor activity across days which was environmentally independent. This was demonstrated by the increase in locomotor activity across days in the Coc-Test group and by the lack of difference in Coc-Home and Coc-Test group following intra-NACC cocaine on day 5 (Fig. 4), while only the Coc-Test group increased following IP cocaine (Fig. 1). This marked difference in effect between the two routes of drug administration is not due to the absolute levels of locomotor activity achieved as both IP and intra-NACC cocaine elicited approximately equivalent levels of activity. One possible reason that environmentally-independent locomotor sensitization is observed following NACC infusions is that the NACC was exposed to much higher cocaine concentrations than with IP injections. As intra-NACC infusions of cocaine produced sensitization in the Coc-Home group while IP administration did not produce sensitization in the Coc-Home group, it is possible that pharmacological sensitization is favored by a high local concentration in the NACC compared to more widespread distribution of a lower concentration.

Another possible reason for the difference between the intracranial and IP cocaine results is that even though much higher concentrations of cocaine in the NACC resulted from the intracranial infusions, only 4% as much cocaine was administered intracranially compared to the IP dose. Therefore it is unlikely that intra-NACC cocaine administration produces the same profile of peripheral effects that are observed following systemic cocaine administration, such as increases in heart rate, body temperature, and activation of the sympathetic nervous system (Ritchie and Greene 1980). These and other peripheral effects may be important as stimuli in developing an environmental association, as seen in the IP Coc-Test group. The differences in the stimulus properties of intracranial and IP cocaine that may influence locomotor sensitization should be the focus of future studies.

As shown in Fig. 5B, repeated infusions of cocaine into the NACC irrespective of pre-treatment environment did not augment the locomotor response to a subsequent IP cocaine challenge. This indicates that, although the intra-NACC cocaine infusions produce changes which increase the response to locally applied cocaine, these changes are not sufficient to alter the response to IP cocaine. There are several possible explanations for this. One is that the infusion process causes nonspecific damage which prevents sensitization from being observed. Evidence for this possible confound is that the temporal profiles of the IP data are altered in the NACC cannulated rats (compare Fig. 2B versus Fig. 5B). This is apparent in the flattened and prolonged temporal profile following IP cocaine challenge in the NACC-cannulated rats compared to the non-cannulated subjects. In previous experiments (Post et al. 1988) lesions of the NACC blocked sensitization to IP cocaine. However, in the current experiment non-specific damage does not seem to block the responsiveness of the NACC since the locomotor response to intra-accumbens cocaine is augmented with repeated NACC administration and subjects display normal overall levels of locomotor activity in response to cocaine administered IP. Also, previous studies show that repeated infusions into the NACC of amphetamine, which can be neurotoxic at high doses, do not block the effects of psychomotor stimulants (Kalivas and Weber 1988; Hooks et al. 1992). Nevertheless, one cannot rule out that the cause of the altered profile may also have masked development of sensitization to IP cocaine.

The present findings are consistent with previous studies on psychomotor stimulant drugs which showed repeated infusions of amphetamine into the NACC did not potentiate the locomotor response to systemic administration of either amphetamine (Kalivas and Weber 1988; Hooks et al. 1992), cocaine (Kalivas and Weber 1988; Hooks et al. 1992), or morphine (Vezina and Stewart 1990). However, repeated infusions of amphetamine into the A9 or A10 regions of the ventral mesencephalon do increase the locomotor response to systemic challenge injections of either amphetamine (Kalivas and Weber 1988; Hooks et al. 1992), cocaine (Kalivas and Weber 1988; Hooks et al. 1992), or morphine (Vezina and Stewart 1990). It therefore appears that changes within the NACC alone are not responsible for the sensitization to IP cocaine.

In summary, the present experiments demonstrate differences between repeated IP and repeated intra-NACC cocaine administration in environmentally and pharmacologically dependent sensitization. The results indicate that repeated IP administration of cocaine produces increases in locomotor activity to an IP cocaine challenge which are environmentally dependent and which may also be pharmacologically dependent. In contrast, while repeated intra-NACC infusions increased the locomotor response to subsequent intra-NACC cocaine administration, they had no effect on the locomotor response to IP cocaine. Thus, although the NACC is apparently an essential structure for the expression of the locomotor response to systemically administered psychomotor stimulants, activation of the NACC alone does not appear to be sufficient to produce sensitization to IP cocaine. However, this structure is apparently sufficient for development of pharmacological sensitization to intra-NACC cocaine.

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References

Childress AR, McLellan AT, Ehrman RN, O'Brein CP (1987) Extinction of conditioned responses in abstinent cocaine or

opioid users. In: NIDA Research Monograph Series 76: Problems of drug dependence. US Government Print Office, Washington DC
Dackis CA, Gold MS (1990) Addictiveness of central stimulants. Adv Alcohol Subst Abuse 9:9–26
Delfs JM, Schreiber L, Kelley AE (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J Neurosci 10:303–310
Gale K (1984) Catecholamine-independent behavioral and neurochemical effects of cocaine in rats. In: Sharp CW (ed) NIDA Research Monograph Series 54: Mechanisms of tolerance and dependence. US Government Print Office, Washington DC pp 323–332
Gawin FH, Byck R, Kleber HD (1986) Desipramine augmentation of cocaine abstinence: initial results. Clin Neuropharmacol 9 [Suppl 4]:202–204
Gold LH, Swerdlow NR, Koob GF (1988) The role of mesolimbic dopamine in conditioned locomotion produced by amphetamine. Behav Neurosci 102:544–552
Hemby SE, Jones GH, Justice JB, Neill DB (1992) Conditioned locomotor activity but not conditioned place preference following intra-accumbens infusions of cocaine. Psychopharmacology 106:330–336
Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr (1991a) Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse 9:121–128
Hooks MS, Jones GH, Smith AD, Neill DB, Justice JB, Jr (1991b) Individual differences in locomotor activity and sensitization. Pharmacol Biochem Behav 38:467–470
Hooks MS, Jones GH, Liem BJ, Justice JB, Jr (1992) Sensitization and individual differences to IP amphetamine, cocaine, or caffeine following repeated intra-cranial amphetamine infusions. Pharmacol Biochem Behav 43:815–823
Kalivas PW, Duffy P (1990) Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48–58
Kalivas PW, Weber B (1988) Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J Pharmacol Exp Ther 245:1095–1102
Kelly PH, Iversen SD (1976) Selective 6-OHDA induced destruction of mesolimbic dopamine neurons: abolition of psychostimulant induced locomotor activity in rats. Eur J Pharmacol 40:45–56
Pan HT, Menacherry S, Justice JB, Jr (1991) Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. J Neurochem 56:1299–1306
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. Academic Press, New York
Pellegrino LJ, Pellegrino AS, Cushman AJ (1979) A stereotaxic atlas of the rat brain. Plenum Press, New York
Pettit HO, Pan HT, Parsons LH, Justice JB, Jr (1990) Extracellular concentrations of cocaine and dopamine are enhanced during chronic cocaine administration. J Neurochem 55:798–804
Post RM, Rose H (1976) Increasing effects of repetitive cocaine administration in the rat. Nature 260:731–732
Post RM, Weiss SRB (1989) Sensitization, kindling, and anticonvulsants in mania. J Clin Psychiatry 50:23–30
Post RM, Weiss SRB, Pert A (1987) The role of context and conditioning in behavioral sensitization to cocaine. Psychopharmacol Bull 23:423–429
Post RM, Weiss SRB, Pert A (1988) Cocaine-induced behavioral sensitization and kindling: implications for the emergence of psychopathology and seizures. In: Kalivas PW, Nemeroff, CB (eds) The mesocorticolimbic dopamine system. New York Academy of Sciences, New York, vol 537, pp 292–308
Reith MA, Meisler BE, Sershen H, Lajtha A (1986) Structural requirements for cocaine congers to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem Pharmacol 35:1123–1129
Ritchie JM, Greene NM (1980) Local anesthetics. In: Gilman AG, Goodman LS, Gilman A (eds) Goodman and Gilman's The pharmacological basis of therapeutics. Macmillan New York, pp 307–308
Roy SN, Bhattacharyya AK, Pradhan S, Pradhan SN (1978) Behavioral and neurochemical effects of repeated administration of cocaine in rats. Neuropharmacology 17:559–564
Vezina P, Stewart J (1990) Amphetamine administered to the ventral tegmental area but not to the nucleus accumbens sensitizes rats to systemic morphine: lack of conditioned effects. Brain Res 516:99–106
Weiss SRB, Post RM, Pert A, Woodward R, Murman D (1989) Context-dependent cocaine sensitization: differential effects of haloperidol on development versus expression. Pharmacol Biochem Behav 34:655–661
Weiss SRB, Post RM, Costello M, Nutt DJ, Tandesiarz S (1990) Carbamazepine retards the development of cocaine-kindled seizures but not sensitization to cocaine-induced hyperactivity. Neuropsychopharmacology 3:273–281