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# Tolerance and sensitization to the locomotor-activating effects of cocaine are mediated via independent mechanisms

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**Abstract**

Tolerance or sensitization to the locomotor-activating effects of cocaine occurs depending upon the treatment regimen that is used. When cocaine is injected on a daily basis, sensitization occurs, whereas continuously infused cocaine leads to tolerance. Male Sprague–Dawley rats were treated for 7 days with continuous cocaine (50 mg/kg/day) via subcutaneously implanted osmotic minipumps, after which the pumps were removed. Locomotor activity was measured for 1 h each day. Some rats were challenged with an injection of cocaine (7.5, 15 or 30 mg/kg) either 2 or 9 days after pump removal. Two days after the pumps were removed (Day 10), there were no significant differences between cocaine- or saline-treated rats in the amount of locomotor activity produced by the challenge injections. However, cocaine-treated rats challenged with cocaine 9 days after pumps were removed (Day 17) exhibited significant tolerance, as evidenced by a shift downward of the cocaine curve, as compared to saline controls. When the rats were injected again on the next day (Day 18), the activity levels of both groups increased, as compared to the effects observed on Day 17. Thus, although the cocaine-treated rats were still tolerant compared to the saline-treated rats, they were sensitized compared to their previous response to a challenge injection. These findings indicate that tolerance and sensitization to the locomotor-activating effects of cocaine can exist simultaneously, which suggests that they are mediated by separate mechanisms.


*Keywords:* Cocaine; Locomotor activity; Behavior; Chronic treatment

## 1. Introduction

Both tolerance and sensitization to the locomotor-activating effects of cocaine have been reported after chronic administration. The development of either tolerance (a decreased response to a drug) or sensitization (an increased response to a drug) to locomotor-activating effects seems to depend upon the paradigm by which cocaine is administered. In general, in rodents, intermittent administration of cocaine has been shown to produce sensitization while continuous infusions of cocaine lead to tolerance (Inada et al., 1992; Izenwasser et al., 1999; Kalivas et al., 1988; King et al., 1992; Kunko et al., 1998; Reith et al., 1987).

Frequently, the studies in which tolerance and sensitization have been measured differ from one another in the length of treatment, doses of cocaine administered and time since the last drug administration, suggesting that these factors might play a role in determining the behavioral and neurochemical consequences of chronic cocaine administration. Because of these differences between studies, there have been variable results reported on multiple neurochemical measures associated with chronic cocaine administration (e.g., Baumann and Rothman, 1993; Izenwasser and Cox, 1990, 1992; Pettit et al., 1990; Pilotte et al., 1991; Reith et al., 1987; Zeigler et al., 1991).

In general, it has been thought that tolerance is an opposing process to sensitization, which is often called reverse tolerance (e.g., Kilbey and Ellinwood, 1977; Post and Kopanda, 1975). The development of sensitization in a tolerant animal has not, however, been studied. The present study was conducted to look at the locomotor-

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activating effects of cocaine injections in rats that have become tolerant as a result of being treated with and withdrawn from a continuous cocaine infusion. In essence, the development of sensitization was measured in rats that were made tolerant and that remained tolerant to cocaine.

## 2. Methods

### 2.1. Treatments

Male Sprague–Dawley rats (200–250 g, Taconic, Germantown, NY) were kept on a 12-h light/dark cycle (lights on at 7:00 a.m.) with food and water available ad libitum. Animals were anesthetized with halothane and Alzet osmotic minipumps model 2001 (Alza, Palo Alto, CA) delivering approximately 1 μl/h for 7 days were implanted subcutaneously between the scapulae, as described previously (Izenwasser and Cox, 1992; Izenwasser et al., 1999; Kunko et al., 1997). The pumps contained either saline (0.9% sodium chloride; 24 μl/day) or a concentration of drug resulting in delivery of approximately 30, 50 or 100 mg/kg/day of cocaine, expressed as free base. The minipumps were filled and soaked in saline at 37 °C for at least 4 h prior to surgery, so as to reach a constant pumping volume before being implanted into the animals. The dose of drug delivered was determined by the pumping rate and average body weight of the animals in each individual experiment. Seven days after the pumps were implanted (Day 8), the animals were anesthetized and the pumps were removed.

Every day during and after the continuous infusions, each animal received an intraperitoneal injection of saline immediately prior to the testing session with the following exceptions: Two days after pump removal (Day 10), some animals received a challenge injection of 7.5, 15 or 30 mg/kg cocaine ip immediately prior to locomotor activity testing. Nine days after pump removal (Day 17), separate groups of animals were challenged with either 7.5, 15 or 30 mg/kg cocaine. Each animal received only a single dose. The next day (Day 18), each animal received the same dose as the day before.

### 2.2. Locomotor activity

Locomotor activity was measured for 1 h each day starting 4 h after pump implantation and again every 24 h for 18 days. Acrylic chambers were placed inside Digiscan activity monitors (Omnitech Electronics, Columbus, OH) that were equipped with infrared light sensitive detectors mounted 2.5 cm apart along two perpendicular walls. Mounted along the opposing walls were infrared light beams that were directed at the detectors. One count of horizontal activity was registered each time the rat interrupted a beam.

## 3. Results

Continuous cocaine administration produced significant alterations in locomotor activity, in a dose-dependent manner, as we have shown previously (Izenwasser et al., 1999; Kunko et al., 1998). These changes were characterized by an increase in activity, as compared to saline, 4 h after the pumps were implanted, in the rats that received 50 or 100 mg/kg/day of cocaine (Fig. 1). In the group that was treated with the lowest dose of cocaine (30 mg/kg/day), activity on Day 1 was not significantly different from saline. In all three cocaine groups, activity levels were higher 24 h later (treatment Day 2), after which activity levels decreased slowly, and by the last days of treatment reached a plateau that was not significantly different from that observed on Day 1.

Within 4 h of pump removal on Day 8, activity of the cocaine-treated animals dropped to saline levels regardless of the dose tested (Fig. 1), suggesting that the increase in activity is not a conditioned response. When the rats were treated with saline injections for 10 days following the removal of the pumps, activity of the group that had been treated with the cocaine pumps was not significantly different from those in the saline-treated group. The intermediate dose of 50 mg/kg/day was selected for the remainder of the studies. This dose was chosen because the amount of

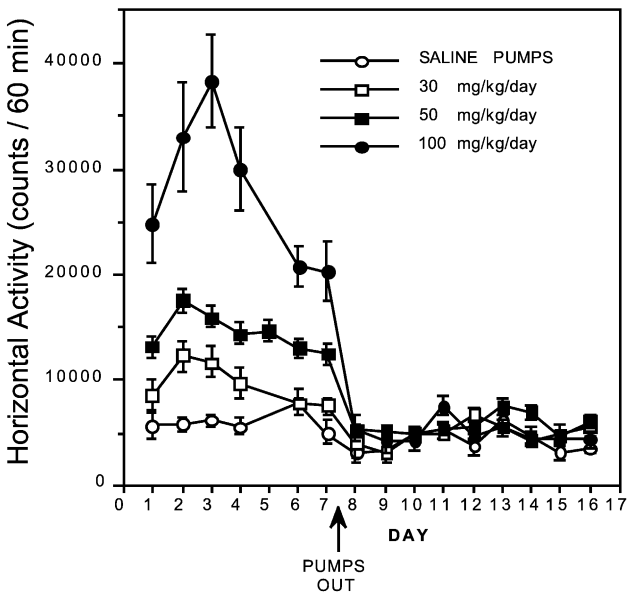


Fig. 1. Tolerance to the locomotor-activating effects of continuously infused cocaine. Locomotor activity measured for 1 h periods every 24 h starting 4 h after pump implantation in animals with saline pumps ($n=36$) or cocaine pumps (30 mg/kg/day, $n=6$; 50 mg/kg/day, $n=36$; 100 mg/kg/day, $n=6$). Maximal activity with each dose of cocaine occurred 24 h after pump implantation and partial tolerance developed over the course of 4 days. The pumps were removed approximately 4 h before testing on Day 8 of treatment. Each animal received an intraperitoneal saline injection immediately prior to each testing session. Each point represents mean ± S.E.M. for $n$ rats. The data for Day 5 in the saline, 30 mg/kg and 100 mg/kg cocaine group were lost due to a computer malfunction on that day.

activity produced over the course of the study was high enough to allow measurement of decreases in activity, but low enough that there was not a ceiling effect.

Two days after pump removal (Day 10), neither tolerance nor sensitization to challenge doses (7.5, 15 or 30 mg/kg) of cocaine was evident in the group that was pretreated with a continuous infusion of 50 mg/kg/day cocaine (cocaine pumps), as compared to the saline-treated rats (saline pumps) (Fig. 2). In this study, each of the rats was challenged with a single dose of cocaine on Day 10. There were no significant differences in the dose–effect curves for the cocaine challenge between these two groups of rats.

In contrast, 9 days after cessation of treatment (Day 17), the cocaine pretreated animals were tolerant to cocaine, as compared to the animals that had initially been treated with saline (Fig. 3A). This was evidenced by a significant [$F(1,64) = 11.52$, $P < .001$] difference between the dose–effect curves for the cocaine challenge in the cocaine-treated animals, as compared to the saline pretreated animals. When the same animals received an additional cocaine challenge on the next day (10 days after pump removal) the animals pretreated with cocaine were still significantly [$F(1,64) = 10.754$, $P < .002$] tolerant compared to the saline pretreated animals (Fig. 3B). There was not a significant Treatment × Dose interaction on either day. Even though the cocaine-treated rats remained tolerant to the cocaine challenge injection compared to the saline-treated rats, both groups were sensitized compared to their responses of the day before (Fig. 4A,B). Fig. 4 shows a comparison of the responses to a cocaine challenge on Day 18 in the same animals that were challenged with cocaine on Day 17. In both the (A) saline-treated [$F(1,62) = 10.55$, $P < .002$] and (B) cocaine-treated [$F(1,66) = 7.762$, $P < .007$] rats, the curves were shifted to the left and upward after the second injection of cocaine. Each animal received the same dose on Day 18 as on Day 17 and each animal was injected with a single dose of cocaine. There was not a significant Day × Dose interaction in either group, which is supported by the parallel curves in each graph. In addition, a three-way interaction comparing Treatment × Dose × Day was not significant.

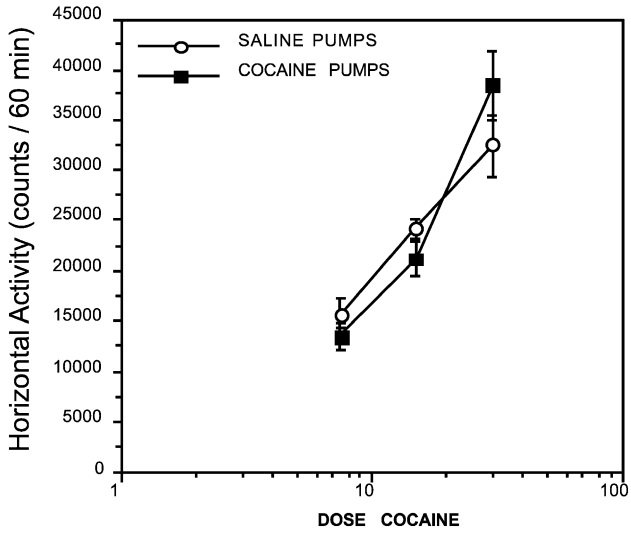


Fig. 2. Lack of tolerance to a cocaine challenge injection 48 h after cessation of continuous infusions. Effect of 7.5, 15 or 30 mg/kg cocaine injection on locomotor activity, 48 h after removal of either saline pumps or cocaine pumps (50 mg/kg/day). Animals were tested for 1 h immediately after the cocaine injection. For the 7.5 and 30 mg/kg doses, $n = 6$/group; for the 15 mg/kg dose, $n = 24$/group. Each point represents mean ± S.E.M. for $n$ rats.

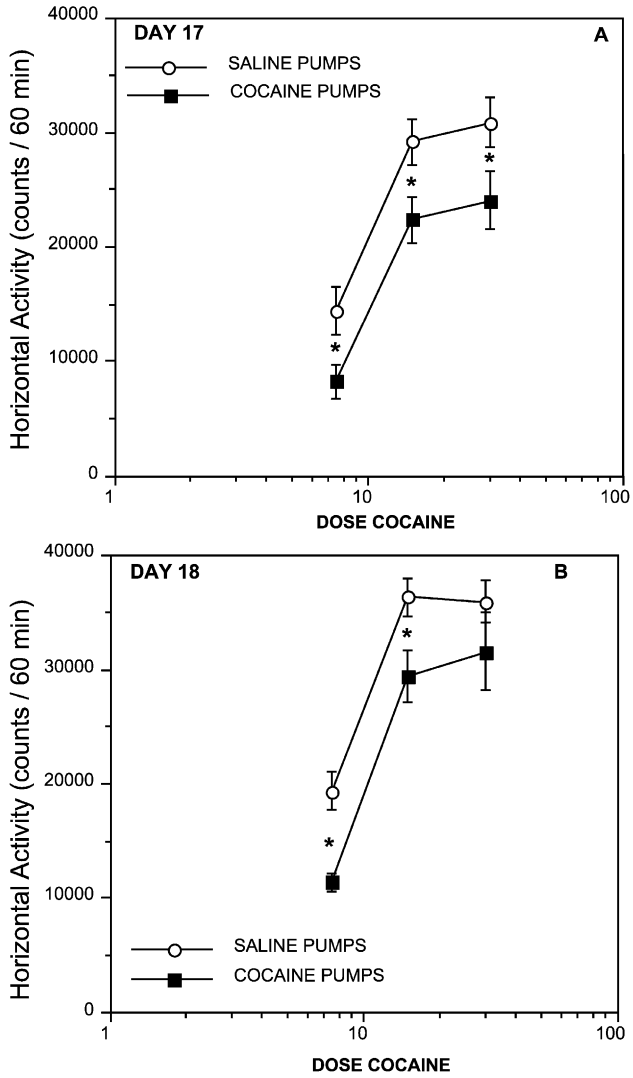


Fig. 3. Tolerance to cocaine challenge injections 9 and 10 days after cessation of continuous infusions. Locomotor activity measured for a 1-h period immediately after injection with cocaine on (A) Day 17 [9 days after cessation of continuous infusions of saline or cocaine (50 mg/kg/day)] and (B) Day 18 (10 days after cessation of continuous infusions of saline or cocaine (50 mg/kg/day). On both days, the group pretreated with cocaine exhibited significantly less activity than the saline-pretreated group (tolerance) in response to a challenge injection of cocaine. For the 7.5 and 30 mg/kg doses, $n = 8$/group; for the 15 mg/kg dose, $n = 20$/group. Individual animals received only a single dose of cocaine, on each of 2 consecutive days. Each point represents mean ± S.E.M. for $n$ rats.

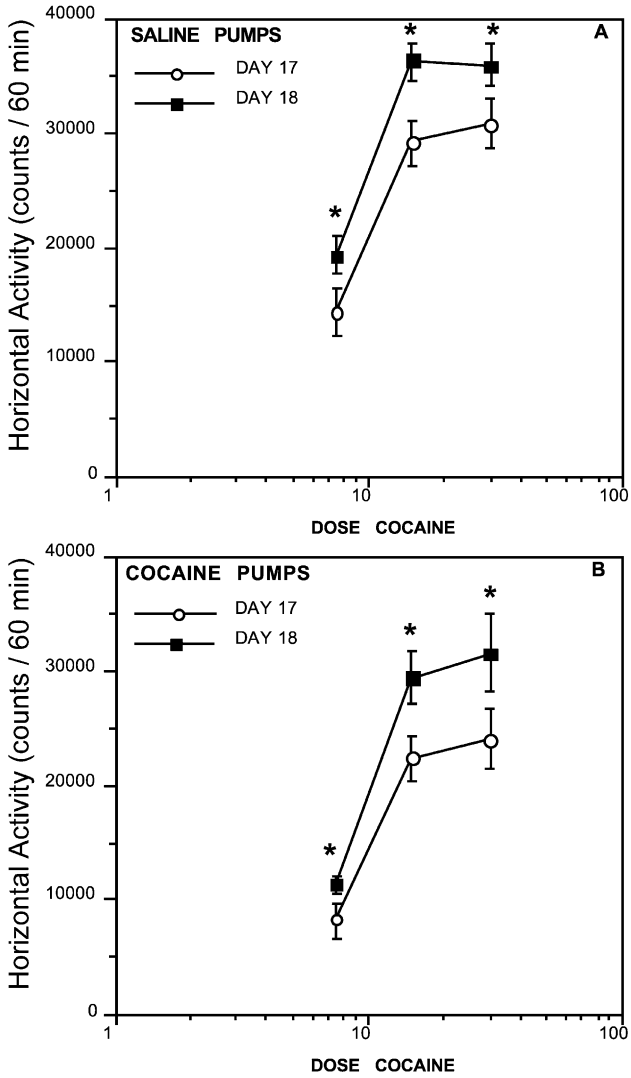


Fig. 4. Sensitization to the second of two cocaine challenge injections 10 days after cessation of continuous infusions of either saline or cocaine. Locomotor activity measured for a 1-h period immediately after injection with cocaine on Days 17 and 18, 9 and 10 days after cessation of continuous infusions of (A) saline or (B) cocaine (50 mg/kg/day). Both groups exhibited significantly higher levels of activity (sensitization) following their second cocaine injection (Day 18) as compared to the first injection (Day 17). For the 7.5 and 30 mg/kg doses, $n = 8$/group; for the 15 mg/kg dose, $n = 20$/group. Individual animals received only a single dose of cocaine, on each of 2 consecutive days. Each point represents mean ± S.E.M. for $n$ rats.

An analysis of $ED_{50}$ values for all four curves showed that the $ED_{50}$ did not change across groups or days. In all cases, the $ED_{50}$ for cocaine to stimulate locomotor activity was approximately 14 mg/kg.

## 4. Discussion

Cocaine produced a unique pattern of activity during the 7-day period of continuous infusion, whereby activity was significantly increased on Day 1, with an even greater effect on Day 2. Over the next several days, activity decreased to a plateau that was not significantly different from that observed on Day 1. As discussed previously, it is not clear whether this pattern represents an initial early sensitization to the locomotor-activating effects (Day 2) and a loss thereof, or a gradual increase in effect, to which tolerance develops (Izenwasser et al., 1999; Kunko et al., 1998). What we do know is that there are no significant differences in the amount of cocaine or its metabolites in either plasma or brain across days during the 7-day treatment period (Kunko et al., 1998). Since cocaine levels in the brain are not significantly different between Days 1 and 2, it is more likely that the increase in activity on Day 2 represents a sensitized response, compared to Day 1. If this is so, then the slow decrease back to the initial level of activity (as seen on Day 1) represents a loss of this sensitization. This idea is further supported by the fact that, although partial tolerance to the continuous infusion of cocaine occurred, the cocaine-treated animals were not tolerant to a cocaine challenge injection during the period of early withdrawal from the infusions (2 days after pump removal), compared to the saline pump rats challenged with cocaine. These data are consistent with previous studies showing that tolerance to a challenge injection of cocaine was not evident 1 day after the end of the continuous infusion, but could be seen on the seventh day of withdrawal (Inada et al., 1992; King et al., 1992; Reith et al., 1987).

After 9 days of withdrawal (Day 17), tolerance had developed to a challenge injection of cocaine. These data suggest that the development of tolerance is a compensatory mechanism that occurs during the absence of the drug (withdrawal) subsequent to a continuous infusion. The tolerance to cocaine in the cocaine-pretreated rats is not likely to be a conditioned response for two reasons. First, the level of activity exhibited by this group of animals was greater than the saline-pretreated rats during all 7 days of the infusion period. Second, the cocaine was continuously infused for 7 days, and was not, therefore, solely associated with the locomotor activity chamber.

The tolerance to cocaine is in contrast to what is seen traditionally with tolerance/dependence producing drugs like opiate agonists or ethanol, where tolerance develops during drug use, and diminishes subsequent to stopping use. The tolerance that is seen here to cocaine also differs from opioid tolerance, in that there is a shift downward, but not to the right of the dose effect curve. Tolerance to opioids is characterized by a shift to the right in the dose–effect curve, followed by a shift downward. This shift to the right with opiates is thought to be due to a receptor reserve (spare receptors) (Chavkin and Goldstein, 1982). Thus, it is interesting that although with cocaine there is a shift downward, there is no shift to the right. This suggests that the mechanism by which this tolerance occurs may not be explained in the same manner as that of opiate tolerance and that perhaps there are not spare receptors mediating this effect. Since the effects of cocaine on behavior are mediated

directly by its blockade of multiple monoamine transporters and the indirect activation of multiple receptors, the alterations in behavior seen following chronic administration may not be as easily explained as that of tolerance to opiate agonists.

When the rats were tested the next day (Day 18) with another cocaine injection, they were still tolerant to a challenge injection of cocaine, compared to the response of the saline pump treated rats. Compared to their responses on Day 17, however, the cocaine-treated rats were sensitized to the cocaine injections (i.e., the same dose of cocaine produced significantly greater activity compared to the day before). Thus, the cocaine-treated animals exhibited both tolerance and sensitization to the same cocaine injection, depending upon which comparison is made. These data show that sensitization to cocaine can occur in rats that are tolerant to cocaine.

It could be argued that the increased activity on Day 18 vs. Day 17 in the cocaine-treated rats was merely a return to normal; however, the parallel increase in the saline pump rats suggests that this is not true. It is possible that the cocaine-treated rats would eventually equal the saline pump rats with continued repeated cocaine administration, but it would be difficult to know whether this was due to a ceiling effect as both groups become increasingly sensitized, or whether the tolerance was still present.

The neurochemical mechanisms that underlie the development of tolerance or sensitization to cocaine are not well understood. What is known is that while the behavioral response to cocaine varies, cocaine levels in the brain are not altered either during the 7-day period of continuous infusion (Kunko et al., 1998) or following a challenge injection 1 week after the treatment period (Reith et al., 1987). Thus, the tolerance observed to both the continuous infusion itself and to a challenge injection after this pretreatment is not due to differences in the amount of cocaine in the brains of these animals. Continuous infusion of cocaine has no effects on dopamine transporter binding, whether measured after 1 (Izenwasser and Cox, 1992) or 7 (Hitri et al., 1996) days of withdrawal. In contrast, while daily administration of cocaine has been shown to have no effect on binding to dopamine transporters (Kula and Baldessarini, 1991), a decrease in dopamine transporter binding in the rat nucleus accumbens after withdrawal from repeated administration of cocaine has been reported (Sharpe et al., 1991).

Seven days after continuous infusion of cocaine, there is no change in basal dopamine uptake or release as measured by in vitro voltammetry (Jones et al., 1996), while electrically stimulated dopamine release is decreased after withdrawal from either intermittent or continuous cocaine administration (King et al., 1994). It has also been shown that there is tolerance to the ability of cocaine to increase extracellular dopamine after withdrawal from repeated intermittent cocaine (Kalivas and Duffy, 1993; Meil et al., 1995; Segal and Kuczenski, 1992), and tolerance to the inhibition of dopamine uptake by cocaine after continuous infusion (Izenwasser and Cox, 1992). Withdrawal from continuously infused cocaine also produces an increase in dopamine $D_2$ autoreceptor sensitivity (Gao et al., 1998; Jones et al., 1996; King et al., 1994; King et al., 1999), with no change in dopamine $D_2$ receptor number or mRNA level (King et al., 1994). The present data suggest that this change in autoreceptor function does not interfere with the development of sensitization. Thus, there have been many reports of differential alterations in dopaminergic markers, and sometimes alterations in the same direction are seen in both tolerant and sensitized animals. This has often led to confusion because of the hypothesis that tolerance and sensitization are opposite effects, and that any neurochemical changes should occur in opposite directions to match the behavioral changes. The present data indicate that tolerance and sensitization to the locomotor-activating effects of cocaine can exist simultaneously, and further suggest that they may be mediated via separate mechanisms. Certainly, these data suggest that sensitization can develop during a time period when the rats are tolerant to cocaine, compared to rats that have no prior experience with cocaine.

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