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ORIGINAL INVESTIGATION

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Modulation of morphine sensitization in the rat by contextual stimuli

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Abstract *Rationale:* The repeated administration of addictive drugs, such as amphetamine, cocaine, and morphine, produces a progressive enhancement (sensitization) of their psychomotor activating effects. We have previously shown that administration of amphetamine or cocaine in a distinct test environment promotes more robust psychomotor sensitization than if they are given at home. No information is available, however, on whether this environmental manipulation has a similar effect on sensitization to morphine, a drug that enhances dopamine (DA) release in the striatum indirectly by disinhibiting midbrain DA neurons. *Objectives:* The main goal of present study was to determine whether exposure to a distinct environmental context facilitates morphine sensitization. *Methods:* As an index of psychomotor activation, we used rotational behavior in rats with a unilateral 6-hydroxydopamine lesion of the mesostriatal DA system. There are inconsistencies in the literature regarding the ability of morphine to elicit rotational behavior. Therefore, in experiment 1 we determined the effect of 2.0, 3.0, 4.0, 6.0, and 8.0 mg/kg, IP, of morphine on rotational behavior. In experiment 2, we studied the effect of five consecutive IV infusions of saline or morphine (2.0 mg/kg) in rats treated either in their home cage or in a distinct and relatively novel test environment. After 5 days of withdrawal, all rats received an IV infusion of 2.0 mg/kg morphine (Morphine challenge). The following day all rats received an IV infusion of saline (Saline challenge). *Results:* Morphine produced a dose-dependent increase in rotational behavior. Environmental novelty enhanced both the acute psychomotor response to morphine and its ability to induce psychomotor sensitization. Furthermore, a conditioned rotational response was seen only in animals treated in the novel environment. *Conclusions:* Environmental novelty can facilitate the development of sensitization to the psychomotor activating effects of major addictive drugs, such as amphetamine, cocaine, and morphine.

Keywords Morphine · Dopamine · 6-OHDA · Mesostriatal dopamine system · Nucleus accumbens · Psychomotor activity · Rotational behavior · Sensitization · Environment · Novelty · Context · Associative learning · Conditioning · Stress

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Introduction

Most addictive drugs, including amphetamine, cocaine, and morphine, produce psychomotor activation, an effect attributed at least in part to their ability to enhance dopamine (DA) transmission in the striatal complex (Wise and Bozarth 1987; Di Chiara and Imperato 1988b). The psychomotor responses to these drugs progressively increase (i.e., sensitize) upon repeated administrations (for reviews, see Kalivas and Stewart 1991; Stewart and Badiani 1993), and there is some evidence that the rewarding properties of drugs also undergo sensitization (Woolverton et al. 1984; Piazza et al. 1989; Robinson and Berridge 1993). It has been suggested, therefore, that sensitization-related neuroadaptations may contribute to the development of drug addiction (see Robinson and Berridge 1993).

However, the ability of drugs to induce sensitization is not solely a function of their neuropharmacological properties. For example, we have reported that environmental context can modulate the development of psychomotor sensitization to amphetamine and cocaine, drugs that act on the DA transporter to release and/or block the reuptake of DA (Badiani et al. 1995a, 1995b, 1997; Robinson et al. 1998). In particular, we have found that the sensitization produced by amphetamine and cocaine is greatly attenuated if these drugs are administered IV

via a remotely controlled infusion apparatus, in the absence of environmental cues usually associated with drug treatment, such as the entry of the experimenter in the testing room, the handling of the animals, the needle jab, etc. (Crombag et al. 1996; Browman et al. 1998a, 1998b; Fraioli et al. 1999). The main goal of the present study was to determine whether the context can also modulate the development of sensitization to morphine, a drug that disinhibits midbrain DA neurons (Johnson and North 1992) and enhances both psychomotor activation and striatal DA concentrations (Pert and Sivit 1977; Di Chiara and Imperato 1988a).

In our previous studies, we used rotational behavior in rats with a unilateral 6-hydroxydopamine (6-OHDA) lesion of the mesostriatal DA system as an index of amphetamine- and cocaine-induced psychomotor activation (Ungerstedt and Arbuthnott 1970; Glick et al. 1983). The rationale for using this preparation has been discussed elsewhere (Badiani et al. 1995c; Crombag et al. 1999). Like amphetamine and cocaine, morphine should produce rotational behavior in animals with a unilateral 6-OHDA lesion, and such an effect has been reported in both mice (Ehsan and Åkerman 1997) and rats (Cowan and et al. 1975a, 1975b) with a 6-OHDA lesion. Other authors, however, have found little or no effect of morphine on rotational behavior in the rat (Hirschorn et al. 1983; Kimmel et al. 1995, 1998; Ehsan and Åkerman 1997; Kimmel and Holtzman 1997). It is possible that these discrepancies are due to dose-dependent effects of morphine on psychomotor activity, because low to medium doses of morphine increase locomotor activity, whereas higher doses induce locomotor activation only after an initial period of inhibition (Babbini and Davis 1972; Vasko and Domino 1978). Therefore, a preliminary experiment was conducted to study the effect of a range of low to intermediate doses of morphine on rotational behavior in rats with a unilateral 6-OHDA lesion.

Materials and methods

Subjects

Forty-seven male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc.), weighing 200–225 g upon arrival, were used in this study. Rats were individually housed in the main animal colony room with ad libitum access to food and water, under a 14:10 h light-dark cycle (lights on at 0700 hours). All procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (Washington, D.C.: National Academy Press, 1996).

6-OHDA lesion

As discussed in detail elsewhere (Badiani et al. 1995c; Crombag et al. 1999), there are two main reasons for studying rotational behavior in rats with a unilateral 6-OHDA lesion rather than locomotor activity in rats without a lesion. First, the dose-effect curve for rotational behavior induced by many psychomotor activating drugs (for example, amphetamine) is linear over a wide range of doses, whereas often this is not the case for locomotor activity in rats without a 6-OHDA lesion (see Crombag et al. 1999). Second, the progressive increase in drug effect seen during sensitization might result in a progressive increase in rotational behavior, but not of locomotor activity (see Crombag et al. 1999).

At least 1 week after their arrival, all rats received a unilateral 6-OHDA lesion of the mesostriatal DA projections using a procedure described previously (Robinson 1984). Briefly, the animals were anaesthetized with sodium pentobarbital, pretreated with 15 mg/kg desmethylipramine (to prevent uptake of 6-OHDA by noradrenergic terminals), and mounted on a stereotaxic apparatus. 8 μ g of 6-OHDA (dissolved in 4 µl of a saline-ascorbic acid solution) were infused into the medial forebrain bundle. The coordinates of the infusion cannula tip, relative to bregma, were: AP –3.0 mm, ML ±1.8 mm, DV –8.2 mm. In experiment 2, immediately after the 6-OHDA lesion a 15-mm stainless steel post (15-gauge tubing) and an L-shaped length of PE5 tubing were cemented to the skull. The former was used later to tether the rats to a liquid swivel for the IV infusions, and the latter to anchor the distal end of the catheter.

After a 10-day recovery period, all animals were injected SC with 0.05 mg/kg apomorphine, a direct DA agonist. Because denervation supersensitivity of postsynaptic DA receptors develops only after at least 90% of dopaminergic terminals have been destroyed, only animals with such a lesion will rotate contraversively in response to a low dose of apomorphine (Hefti et al. 1980a, 1980b). Animals that did not make at least eight full rotations during a 2-min observation period were excluded from the study.

Intravenous catheter

About 2 weeks after the 6-OHDA lesion, the rats used in experiment 2 received an IV catheter into their jugular vein using standard surgical techniques. The IV end of the catheter was made of silicone tubing (0.30 mm inside diameter, and 0.64 mm outside diameter), whereas the external end (which exited through the skin of the nape of the neck) was made of polyethylene tubing (0.38 mm inside diameter, and 1.09 mm outside diameter). The details concerning catheter construction and catheterization procedure have been described previously (Weeks 1972; Crombag et al. 1996). At the end of the surgery, the catheter was filled with gentamicin solution (50 mg/ml) to prevent infections. Every morning (0800–1000 hours) for the entire duration of the experiment the catheters were flushed with 50 µl of sterile heparin solution.

Procedures

Experiment 1

The aim of experiment 1 was to determine whether morphine could induce rotational behavior in rats with a unilateral 6-OHDA lesion of the mesostriatal DA system. During a 6-day testing phase, the rats (n=16) were transported once daily from the main animal colony room to cylindrical plastic cages (25 cm diameter, 36 cm height) and allowed to habituate to these cages for 15 min. On each test session, each animal received an IP injection of one of six doses of morphine: 0.0 (i.e., vehicle), 2.0, 3.0, 4.0, 6.0, or 8.0 mg/kg. The doses were administered in a random sequence. The animals were videotaped for 120 min following the injections and their behavior was scored later by an observer.

Experiment 2

The aim of experiment 2 was to determine whether environmental novelty enhances the psychomotor activating effects of morphine, and in particular the development of sensitization. The rats were subdivided into four groups: saline-home (n=7), morphine-home (n=9), saline-novelty (n=7), and morphine-novelty (n=8). The experiment lasted for a total of 19 days, including a 7-day period of habituation to housing conditions, a 5-day period of intermittent treatment, a 5-day period of withdrawal, a Morphine challenge, and a Saline challenge.

Habituation (days H1–H7). On day H1, the saline-home and the morphine-home groups were housed in opaque plastic cylindrical cages (25 cm diameter, 36 cm height), equipped with a drinking tube and with ground corncob bedding on the floor. From day H4 until the end of the experiment, the rats were tethered via a flexible stainless steel cable to a liquid swivel mounted on a counterbalanced arm located above the test cage, which allowed the animals to move freely within the cage. Thus, for these rats the test cages were also their "home." On days H4–H7 the rats received mock infusions to habituate them to the noise of the electronic pumps and to the infusion procedures. Immediately after catheter flushing, catheters were connected to infusion lines filled with heparin solution, which in turn were connected via the liquid swivel to a syringe mounted on an electronic pump. The distal portion of the infusion lines was filled with saline solution (a tiny air bubble separated the treatment solution from the heparin solution that filled the remainder of the infusion line). At about 1400 hours the pumps were activated by remote control, from outside the testing room, and the rats were given a mock infusion at a rate of 10 µl/min, consisting of 30 µl heparin solution (internal volume of the catheter), followed by 15 µl saline solution, and by 15 µl heparin solution, for a total of 60 µl over 6 min. At 1800 hours, the infusion lines were disconnected and the catheters flushed with heparin solution. During this phase the rats in the saline-novelty and morphine-novelty groups were left in the main animal colony room and their catheters were flushed twice a day with heparin, using the same schedule as for the home animals.

Intermittent treatment (days 1–5). On days 1–5, at 1400 hours, the rats were given an infusion of either saline (saline-home and saline-novelty groups) or 2.0 mg/kg morphine (morphine-home and morphine-novelty groups). The infusion procedures for the two home groups were similar to those used for the mock infusions (see above), except that the morphine-home group received 15 µl morphine solution instead of saline. Thus, although the rats were given a total infusion of 60 µl over 6 min, morphine was delivered over a period of 1.5 min. In contrast, rats in the novelty groups were transported each day from the animal colony to a testing room and placed in buckets identical to those in which home rats lived (including the presence of food and water). They were then tethered, and connected to an infusion line filled with either saline (saline-novelty) or morphine (morphine-novelty) and given an infusion, as for the home animals. Also in this case, the infusion consisted of a total of 60 µl over 6 min.

Behavior was recorded via a computerized rotometer system described previously (McFarlane et al. 1992), using videocameras and VCR equipment as a backup system.

Withdrawal (days 6–10). Following the last morphine or saline treatment all animals were left undisturbed for 5 days, except for the husbandry routine, weighing procedures, and catheter flushing procedures.

Morphine and Saline challenges (days 11 and 12). On day 11, at 1400 hours, all rats were administered an infusion of 2.0 mg/kg morphine (Morphine challenge) to test for the expression of morphine sensitization, using the same treatment procedures described above. On day 12, at 14:00 hours, all rats received an infusion of saline (Saline challenge) to test for the expression of a conditioned response to the infusion procedure.

Catheter patency. Catheter patency was assessed on days 5 and 11 by administering 0.2 mg/kg thiopental IV. The data from rats that did not become ataxic within 5 s were excluded from the study.

Drugs

Before surgery the animals received 0.2 mg/kg, IP, of atropine methyl nitrate (Sigma Chemical Company, St Louis, Mo., USA) dissolved in saline (0.5 mg/ml). Surgical anesthesia was induced with 52 mg/kg, IP, of pentobarbital sodium, dissolved (64.8 mg/ml) in a 10% ethanol solution (Nembutal, The Butler Company, Columbus, Ohio, USA), supplemented with methoxyflurane (Metofane ®, Mallinckrodt Veterinary, Mundelein, Ill., USA). 6-Hydroxydopamine (2,4,5-trihydroxyphenethylamine hydrobromide) and apomorphine hydrochloride (Sigma) were dissolved in a 0.9% saline-0.1% ascorbate solution (2 mg/ml and 0.1 mg/ml, respectively). Desipramine hydrochloride (Sigma) was dissolved in deionized water (0.1 mg/ml). Heparin (Sigma) was dissolved in saline (30 USP/ml). Thiopental sodium (Pentothal, Abbott Laboratories, Chicago, Ill., USA) was dissolved (20 mg/ml) in saline. Morphine sulfate (Sigma) was dissolved in saline. All drug weights refer to the weight of the salts. All solutions for IV administration were prepared with 0.9% saline buffered at pH 7.3.

Statistical analysis

Experiment 1

The data from experiment 1 were analyzed using an ANOVA with repeated measures on the factor dose (six levels). Paired *t*-tests were used to compare the effect of each dose to that of vehicle. Given that each rat received saline and the five doses of morphine in a random sequence according to a non-counterbalanced design, it was impossible to conduct an appropriate statistical analysis of the changes in drug responsiveness over test sessions.

Experiment 2

Time course data for rotational behavior for each session during repeated treatment, and for the Saline and Morphine challenge test days were analyzed using three-way ANOVAs with repeated measures on the factor time (treatment or pretreatment, two levels, saline and morphine; environment, two levels, home and novelty; time, 12 levels, one for each 15-min bin). Additional two-way ANOVAs were conducted at each time interval. Furthermore, given that on day 1 the psychomotor response to novelty subsided by the end of the first hour, the cumulative data for the 60- to 180-min period were analyzed using a two-way ANOVA (treatment, two levels; environment, two levels).

Total number of rotations during the intermittent treatment phase were analyzed using a three-way ANOVA with repeated measures on the factor time (treatment, two levels; environment, two levels; time, five levels, one for each day of treatment). Regression lines for rotational behavior across test sessions were calculated in individual animals to yield slope coefficients. These were analyzed using a two-way ANOVA (treatment, two levels; environment, two levels).

When the ANOVA indicated significant differences, Fisher PLSD tests were used for post-hoc comparisons.

To make the Results section as readable as possible, only the major results of the statistical analyses are reported in the text. The statistical details are reported in the figure legends.

Results

Experiment 1

Figure 1, left panel, illustrates the dose-effect curve for the effect of morphine on rotational behavior (dark circles). There was a significant effect of treatment ($P<0.0001$) and all doses of morphine produced a significant increase in the number of ipsiversive rotations when compared to saline (all P values<0.01), which itself produced little rotational behavior. However, the dose-effect curve had an inverted-U shape, indicating that at higher doses morphine suppressed rotational behavior, relative to that seen with maximally effective doses.

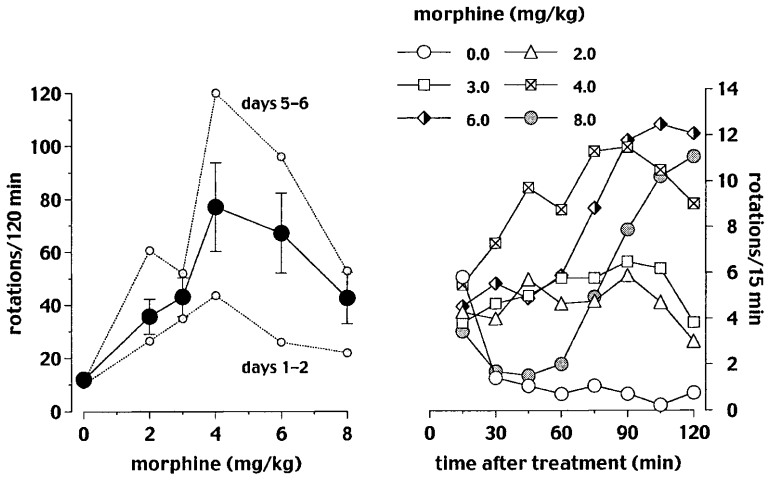

Fig. 1 *Left panel:* effects of vehicle and five doses of morphine on rotational behavior. *Large closed circles* represent the mean number of rotations (±SEM) made by 16 animals receiving each dose. Morphine produced dose-dependent effects on rotational behavior [$F(15,5)=7.55$, $P<0.0001$]. All doses produced a significant increase in rotational behavior over that produced by vehicle (all P values<0.01). *Small gray circles* represent the mean number of rotations (±SEM) made by animals receiving drug treatments during days 1–2 or days 5–6 of testing, respectively (these data are shown for illustrative purposes because the experimental design made it impossible to conduct an appropriate statistical analysis, see text). *Right panel:* time course of rotational behavior for the same animals whose behavior is illustrated in the *left panel*

Figure 1, left panel, also depicts the effects of the different doses of morphine on rotational behavior on the first two test sessions (days 1–2) and on the last two test sessions (days 5–6). All doses of morphine produced greater rotational behavior during later test sessions, compared to early test sessions. For example, rats that received 4.0 mg/kg morphine on days 1 and 2 made about 42 rotations in 120 min, whereas rats that received the same dose on days 5 and 6 made about 120 rotations. In contrast, the response to vehicle injection remained constant over the course of testing. This suggests that sensitization occurred across test sessions, but the design of the experiment made it impossible to conduct an appropriate statistical analysis of this effect (see Statistics).

Figure 1, right panel, illustrates the time course of rotational behavior for each dose of morphine. It can be seen that at doses of 2.0 and 3.0 mg/kg morphine produced rotation at a low, steady level for approximately 90 min, after which the response began to decrease. At the dose of 4.0 mg/kg morphine produced a larger effect, with a peak response occurring 90–115 after the injection, that is, in time interval 75–90 min. At 6.0 mg/kg, low levels of rotation were seen during the first hour, followed by a substantially larger response during the second hour. The highest dose tested, 8.0 mg/kg, produced a similar pattern as 6.0 mg/kg, but during the first hour the rate of rotational behavior was as low as that seen in vehicle-treated animals.

Experiment 2

Figure 2 illustrates the time course of rotational behavior produced by five consecutive IV infusions of saline or morphine (2.0 mg/kg) in rats tested either at home (saline-home and morphine-home groups) or in a distinct and relatively novel test environment (saline-novelty and morphine-novelty) conditions. Figure 3, left panel, illustrates the total number of rotations for each test session, and the lines of regression calculated across test sessions. Figure 3, right panel, illustrates the mean slope coefficients for the regression lines calculated in individual rats. Figure 4 illustrates the effect on rotational behavior produced by an IV challenge with 2.0 mg/kg morphine (Morphine challenge) or saline (Saline challenge) in animals pretreated with saline or morphine.

Acute morphine

As shown in Fig. 2 and Fig. 3, the administration of saline or morphine under the home condition had negligible effect on rotational behavior. In contrast, mere exposure to novelty produced an increase in rotational behavior ($P<0.0001$ versus the saline-home and the morphine-home groups) that was maximal during the first 15-min interval and then returned to baseline values within 75–90 min (Fig. 2, day 1). Animals given morphine in association with environmental novelty (morphine-novelty group) also exhibited an increase in rotational behavior ($P<0.0001$ versus the saline-home and the morphine-home groups) but with a different time course. Although the ANOVA showed no significant effect of treatment ($P=0.40$) or treatment×environment interaction ($P=0.997$), there was in fact a time×treatment×environment interaction ($P<0.0001$) with a simple time×treatment interaction for category novelty ($P<0.0001$) but not for category home ($P=0.998$). Post-hoc tests indicated that rotational behavior in the morphine-novelty group was smaller than in the saline-novelty group in intervals 0–15 and 15–30 min and greater than in the saline-novelty group in intervals 75–90, 90–105, 105–120, 120–135,

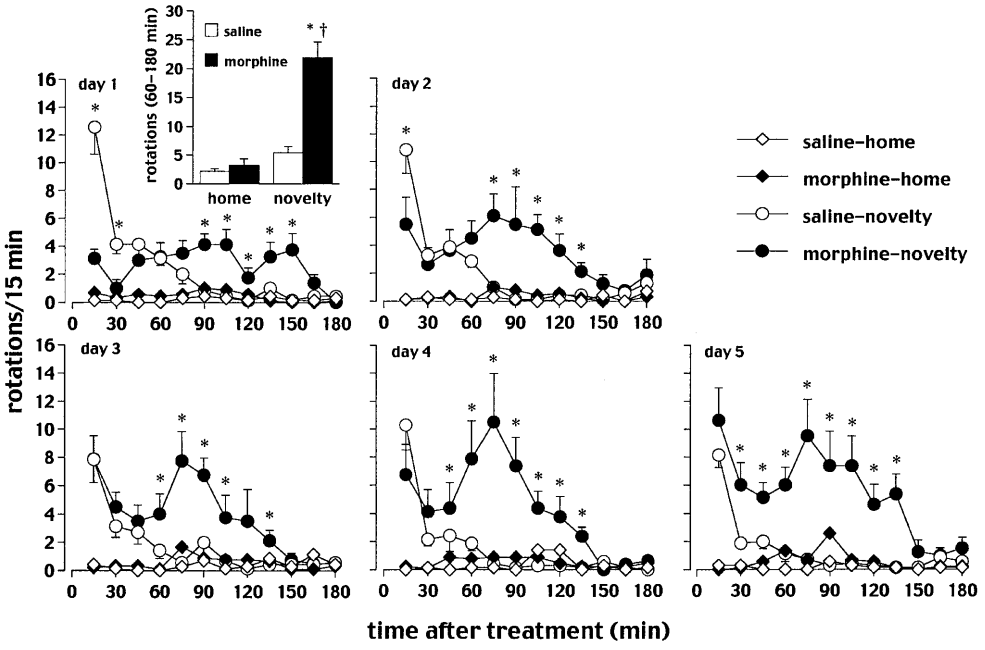

Fig. 2 Time course (15-min blocks) of rotational behavior (means±SEM) produced by five consecutive daily IV infusions of either saline or 2.0 mg/kg morphine administered either in a home (saline-home and morphine-home) or a novel environment (saline-novelty and morphine-novelty). The ANOVA indicated a significant effect of treatment on days 3, 4 and 5 [all $F(1,27)$ values >5.92; all P values <0.022], of environment on all days [all $F(1,27)$ values >16.90; all P values<0.001], and of time on all days [all $F(11,297)$ values >9.20; P<0.0001]. There was a treatment×environment interaction on days 3 and 5 [all $F(1,27)$ values >4.57; all P values<0.042]. All interactions involving the factor time were significant [all $F(11,297)$ values >2.476; all P values<0.005], except for the treatment×environment×time interaction on day 3. The *insert panel* refers to data averaged across intervals 60–180 min for day 1. The ANOVA indicated a significant effect of treatment [$F(1,27)$=25.35, P<0.0001] and environment [$F(1,27)$=39.72, P<0.0001], and a significant treatment×environment interaction [$F(15,5)$=7.55, P<0.0001]. *Morphine-novelty group versus saline-novelty group (P<0.05). †Morphine-novelty group versus morphine-home group (P<0.05)

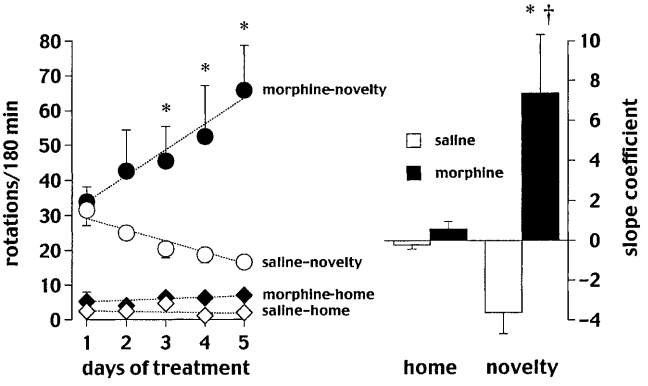

Fig. 3 *Left panel:* total number of rotations (means±SEM) for the same data illustrated in Fig. 2. The ANOVA indicated a significant effect of treatment [$F(1,27)$=8.22; P<0.01] and environment [$F(1,27)$=38.32; P<0.0001], but not of test session [$F(4,108)$=0.85; P=0.49]. There was a treatment×environment interaction [$F(1,27)$=4.96; P=0.034], a treatment×time interaction [$F(4,108)$=5.57; P<0.001], and a treatment×environment×time interaction [$F(4,108)$=4.28; P<0.01]. There was no environment×time interaction [$F(4,108)$=0.80; P=0.53]. Post-hoc Fisher PLSD tests indicated that, overall, the morphine-home group did not differ from the saline-home group (P=0.65), whereas the morphine-novelty group differed from all other groups (all P values=0.01). The *dotted lines* represent the lines of regression of rotational behavior across test sessions. *Right panel:* mean slope coefficients (±SEM) of the regression lines. Mean slope coefficients for the saline-home and morphine-home groups did not differ from zero (P=0.33 and P=0.19, respectively). Mean slope coefficients for the saline-novelty and morphine-novelty groups were significantly different from zero (P=0.014 and P=0.041, respectively). The ANOVA indicated a significant effect of treatment [$F(1,27)$=13.13, P=0.001], but not environment [$F(1,27)$=1.07, P<0.31], and a treatment×environment interaction [$F(1,27)$=9.83, P=0.004]. *Morphine-novelty group versus saline-novelty group (P<0.05). †Morphine-novelty group versus morphine-home group (P<0.05)

and 135–150 min. Because of the different time course of rotational behavior, there were no differences in the total number of rotations between the saline-novelty and the morphine-novelty group. However, when the ANOVA was limited to the 60- to 180-min period (as illustrated in the insert panel of Fig. 2), the morphine-novelty group was significantly different from all other groups (all P values<0.0001).

Repeated morphine

As illustrated in Fig. 3, there were no significant changes in rotational behavior across test sessions for the saline-home and morphine-home groups, and the mean slope coefficients over test sessions (calculated in individual rats) did not differ from zero in either group (–0.21±0.40, P=0.33, for the saline-home group; 0.58±0.40, P=0.19, for the morphine-home group). In contrast, in the saline-nov-

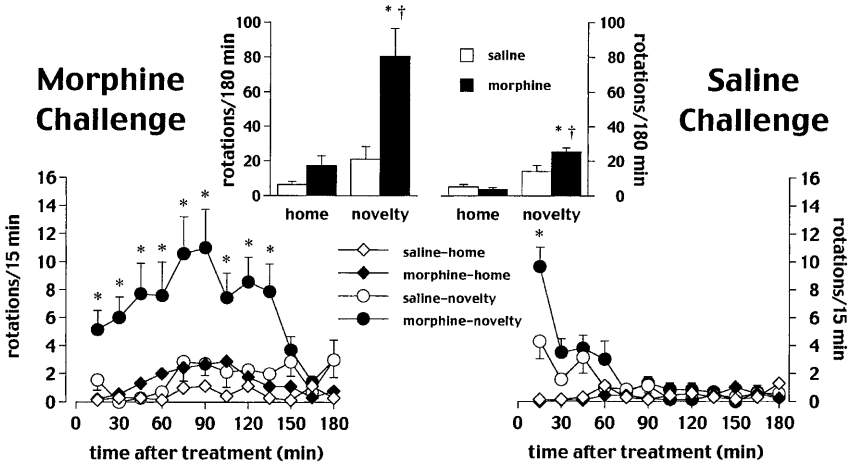

Fig. 4 *Left panel:* time-course (15-min blocks) of rotational behavior (means±SEM) produced by a challenge infusion of morphine (2 mg/kg, IV) in rats that had previously received five daily infusions of either saline or morphine (2 mg/kg, IV) under either home or novel conditions (see Fig. 2 and 3). The ANOVA indicated a significant effect of pretreatment [$F(1,26)=14.82$, $P<0.001$], of environment [$F(1,26)=18.34$, $P<0.001$], and of time [$F(11,286)=6.67$, $P<0.0001$]. There was a pretreatment×environment interaction [$F(1,26)=7.05$, $P=0.013$]. All interactions involving the factor time were significant [all $F(11,286)$ values >2.33; all P values<0.01]. The *insert panel* refers to the total number of rotations in the test session. *Right panel:* time-course (15-min blocks) of rotational behavior (means±SEM) produced by a challenge infusion of saline in rats that had previously received five daily infusions of either saline or morphine (2 mg/kg, IV) plus a morphine challenge under either home or novel conditions. The ANOVA indicated a significant effect of pretreatment [$F(1,25)=5.06$, $P=0.034$], of environment [$F(1,25)=48.93$, $P<0.0001$], and of time [$F(11,275)=14.39$, $P<0.0001$]. There was a pretreatment×environment interaction [$F(1,25)=8.26$, P<0.01]. All interactions involving the factor time were significant [all $F(11,275)$ values >2.62; all P values=0.003]. The *insert panel* refers to the total number of rotations in the test session. *Morphine-novelty group versus saline-novelty group ($P<0.05$). †Morphine-novelty group versus morphine-home group ($P<0.05$)

elty and morphine-novelty groups there were significant changes in rotational behavior across test sessions, but in opposite directions. Figure 3 shows that rotational behavior progressively decreased in the saline-novelty group, as indicated by a negative slope coefficient (–3.63±1.06, $P=0.014$), whereas rotational behavior progressively increased (i.e., sensitized) in the morphine-novelty group, as indicated by a positive slope coefficient (7.36±2.95, $P=0.041$). The enhanced responsiveness to morphine in the morphine-novelty group was evident throughout the duration of the test session. Indeed, by day 3 the inhibitory effect of morphine on novelty-induced activity was lost and by day 5 rotational behavior in the morphine-novelty group was greater than in the saline-novelty group for all time intervals except interval 0–15 min.

Morphine and Saline challenge

On the Morphine challenge test day, when all groups were challenged with an IV infusion of 2.0 mg/kg morphine, there was clear sensitization in the morphine-novelty group, but not in the morphine-home group treatment. Figure 4, left panel, shows that this dose of morphine produced very low levels of rotational behavior in the saline-home, saline-novelty, and morphine-home groups, which did not differ from each other (all P values>0.26), whereas it produced robust psychomotor activation in the morphine-novelty group (all P values≤0.0001 versus all other groups). The ANOVA indicated a significant effect of pretreatment ($P<0.001$) and of environment ($P<0.001$) and a pretreatment×environment interaction ($P=0.013$).

On the Saline challenge test day (Fig. 4, right panel), when all groups were challenged with an IV infusion of saline, only the morphine-novelty group showed a conditioned rotational response, as indicated by a pretreatment×environment interaction ($P<0.01$).

Discussion

We report two findings. First, morphine produced a dose-dependent enhancement of rotational behavior in rats with a unilateral lesion of the mesostriatal dopaminergic system. Second, repeated administrations of morphine produced sensitization to this effect when it was given in association with a distinct and relatively novel test environment, but not when given to animals tested in a physically identical environment in which they lived.

Morphine-induced rotational behavior

The present results are consistent with the reports by Cowan and colleagues (1975a, 1975b) and with the well-characterized effects of morphine on locomotor activity (Babbini and Davis 1972). In contrast, Kimmel and colleagues (Kimmel et al. 1995, 1998; Kimmel and Holtzman 1997), Hirschorn and colleagues (1983), and Ehsan and Åkerman (1997) all reported that morphine produces very little, if any, rotational behavior in the rat. It is most likely that the doses of morphine that failed to produce rotational behavior were either below some threshold for

producing rotation, or, as in the case of higher doses, produced motor depression as the predominant behavioral effect. Indeed, most doses previously examined fall outside the range used in the present study. It is worth noting that morphine appeared to be much less effective in eliciting rotational behavior than the indirect DA agonist amphetamine and that the maximal rate of rotation induced by morphine in the present study is well below that produced by amphetamine under similar experimental conditions (see Badiani et al. 1997).

Sensitization of morphine-induced rotational behavior

The repeated administration of morphine is known to produce sensitization to its locomotor activating effects (Babbini and Davis 1972; Shuster et al. 1975; Bartoletti et al. 1983; Vezina and Stewart 1984). In the present study we report that sensitization also develops to morphine-induced rotational behavior in rats with a unilateral lesion of the mesostriatal dopaminergic system. This finding is in agreement with a recent report by Volpicelli and colleagues (1999), whereas it appears to be at odds with the results of other authors who also examined the effects of chronic treatments on rotational behavior. For example, Watanabe and colleagues (1979) reported tolerance to the stimulant effect of morphine, and Kimmel and colleagues (1995) reported tolerance to the depressant, but not to the stimulant, effect of morphine. It must be emphasized, however, that these authors used drug regimens quite different from the intermittent treatment with low doses used in the present study. Watanabe and colleagues (1979) administered two daily SC injections of morphine hydrochloride at doses escalating progressively from 5 to 40 mg/kg. Kimmel and colleagues (1995) administered continuous SC infusion of morphine, via an osmotic minipump. These drug regimens result in sustained high blood levels of morphine which can produce changes in drug responsiveness quite different from, and sometimes opposite to, those produced by intermittent treatment (see Stewart and Badiani 1993).

Environmental modulation of morphine-induced rotational behavior

We have reported previously that the acute psychomotor response to amphetamine is greater in rats that receive treatments in association with environmental novelty relative to rats that receive the treatment in their home cages (Badiani et al. 1995a, 1997, 1998); especially when the drug is administered via a remotely controlled IV delivery system that minimizes treatment-associated stimuli (Crombag et al. 1996; Browman et al. 1998b; Fraioli et al. 1999). In the present study, we found that environmental novelty enhances the acute psychomotor activating effects of morphine as well. This was not due to the summation of the psychomotor activating effects of novelty and morphine, as indicated by the fact that morphine actually decreased novelty-induced activation and that rotational behavior in the morphine plus novelty group was maximal at a time when rotational behavior produced by either novelty or morphine alone was negligible (see Fig. 4, day 1).

The environmental conditions under which morphine was administered had a powerful effect not only on its acute effects, but also on the development of sensitization. As shown previously for amphetamine (Badiani et al. 1995a, 1997; Crombag et al. 1996; Browman et al. 1998b; Fraioli et al. 1999) and cocaine (Badiani et al. 1995b; Crombag et al. 1996; Browman et al. 1998a), the magnitude of morphine sensitization was greatly facilitated when the drug was administered in association with a distinct and relatively novel environment. Indeed, the dose of morphine used in the present experiment (2.0 mg/kg), failed to induce sensitization in the morphine-home group, but produced robust sensitization in the morphine-novelty group. Further studies are necessary to determine whether higher doses of morphine would produce sensitization regardless of environmental condition, as shown previously for amphetamine and cocaine (Browman et al. 1998a, 1998b).

It is possible that environmental novelty facilitated both the acute and the sensitized response to morphine because of its actions as a stressor. The ability of stressors to enhance sensitization, including morphine sensitization, is well documented (Antelman et al. 1980; Benedek and Szikszay 1985; Deroche et al. 1992) and it has been attributed to the activation of the hypothalamo-pituitary-adrenal axis, culminating in an increase in plasma corticosterone (Deroche et al. 1992). Exposure to a novel environment is known to increase plasma corticosterone (Hennessy and Levine 1977; Hennessy et al. 1977), an effect that does not necessarily habituate with repeated exposures (Hennessy 1991) even though the "novelty" of the environment necessarily decreases from a cognitive point of view. We have found, however, that adrenalectomy has no effect on the facilitation of amphetamine sensitization by novelty (Badiani et al. 1995c). It remains to be determined whether some other neuroendocrine response to stress, independent of the HPA axis, plays a role in this effect (for a discussion of this issue, see Badiani et al. 1995c).

Alternatively, it is possible that the environmental modulation of morphine sensitization described here is attributable to associative learning mechanisms. Drug-paired contexts, for example, can gate the expression of drug sensitization, a phenomenon known as context-dependent sensitization. That is, animals that receive repeated treatments in a specific test environment will exhibit sensitization only when they are tested in the same environment, but not when they are transferred to another test environment (Tilson and Rech 1973; Hinson and Poulos 1981; Vezina and Stewart 1984; Anagnostaras and Robinson 1996). In all our studies, however, all groups received their treatments in the same test environment, the only difference being that for one group of animals it was the home cage (home group), while for

the other group it was a distinct test environment (novelty group). Therefore the difference between these two groups is not an example of context-dependent sensitization.

Another possibility is that group differences in morphine sensitization were due to some other form of conditioning. Indeed, it has been suggested that the phenomenon of drug sensitization might represent a form of Pavlovian conditioning in which drug-paired CSs elicit a progressively larger conditioned response (CR) that adds to an unchanged unconditioned drug response (for a discussion of this issue, see Anagnostaras and Robinson 1996). As illustrated in Fig. 4, right panel, animals that had received morphine in association with a relatively novel environment did exhibit a psychomotor CR to this environment, whereas this was not the case for the animals that had received the same dose of morphine in their home cages. It is unlikely, however, that this CR can entirely account for the sensitized response to morphine. First, the CR illustrated in Fig. 4, right panel, was very small relative to the sensitized response illustrated in Fig. 4, left panel. Second, the CR almost completely subsided within 60 min (Fig. 4, right panel), whereas the peak in the response to morphine occurred 60–90 min after the treatment (Fig. 2 Fig. 4, left panel). Interestingly, in an earlier study with a similar design we observed a robust sensitized response to amphetamine when the treatment was administered after the CR had completely disappeared (Fraioli et al. 1999).

On the other hand, associative learning processes might have endowed the test environment with the ability to *modify* the psychomotor response to amphetamine independently of its ability to elicit a CR (see Stewart and Badiani 1993; Anagnostaras and Robinson 1996). Thus, the possible contribution of associative learning to the phenomenon described here cannot be easily discounted. It should be noted, however, that exposure to a distinct environment produces effects that are not mimicked by other types of conditioned stimuli. In a recent series of experiments we found that pairing a variety of discrete cues with repeated amphetamine treatments given in the home cage fails to facilitate amphetamine sensitization to the same extent as placement in a distinct test environment (Crombag et al., unpublished data). Finally, it must be emphasized that no explanation in terms of associative learning can account for the ability of environmental novelty to enhance acute drug effects.

The neural mechanisms responsible for the interaction between novelty and morphine might be similar to those implicated in the interaction between novelty and amphetamine. The psychomotor activating effects of both morphine and amphetamine are thought to depend primarily on the facilitation of dopaminergic transmission, although via different mechanisms of action (Fischer and Cho 1977; Pert and Sivit 1977; Di Chiara and Imperato 1988a; Johnson and North 1992). However, we have found that environmental novelty does not potentiate the acute psychomotor activating effect of amphetamine by modulating amphetamine-induced DA overflow in the striatal complex (Badiani et al. 1998, 2000). In contrast, environmental novelty greatly enhances the ability of amphetamine to induce the expression of the immediate early gene c-*fos* in striatal neurons expressing either D_1 or D_2 mRNA (Badiani et al. 1998, 1999). Thus, it appears that amphetamine in association with environmental novelty might engage different neural circuitry than either amphetamine or novelty alone. Experiments are underway to determine whether a similar interaction between novelty and morphine takes place at the level of the striatum and/or other brain areas.

Finally, although the animals used in the present study had a unilateral 6-OHDA lesion of the mesostriatal DA system, it is unlikely that these results are unique to animals with a lesion. We have shown in fact that even in neurologically intact animals, amphetamine and cocaine elicit a greater psychomotor sensitization when administered in association with a relatively novel environment (Fraioli et al. 1999; Uslaner et al. 1999). Furthermore, we found that environmental novelty produces similar effects on amphetamine- and cocaine-induced c-*fos* expression in animals with or without a unilateral 6-OHDA lesion (Uslaner et al. 1999).

The findings reported here indicate that both the acute and the sensitized psychomotor response to morphine are enhanced when the treatments are administered in a distinct environment. Similar results have been obtained previously with amphetamine and cocaine (Badiani et al. 1995a, 1995b, 1997; Crombag et al. 1996; Browman et al. 1998a, 1998b; Fraioli et al. 1999). Furthermore, other types of environmental manipulation have also been reported to alter drug sensitization (e.g., Kiyatkin 1992; Bardo et al. 1995). How environmental factors interact with the pharmacological effects of addictive drugs to produce the effects such as those described here is not well understood, but given the potential role of sensitization in the development of addiction (see Robinson and Berridge 1993), this remains an important topic of investigation.

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