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Lack of Cross-Sensitization of the Locomotor Effects of Morphine in Amphetamine-Treated Rats

Louk J.M.J. Vanderschuren, Ph.D., Anton N.M. Schoffelmeer, Ph.D., Arie H. Mulder, Ph.D., and Taco J. De Vries, Ph.D.

Repeated exposure to morphine and amphetamine induces long-lasting sensitization of their psychomotor stimulant properties, whereas pretreatment with morphine causes cross-sensitization of the locomotor effects of amphetamine. Here, we investigated whether pre-exposure to amphetamine also results in cross-sensitization to morphine. Rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.) displayed neither short-term (3 days post-treatment) nor long-term (3 weeks post-treatment) cross-sensitization of the locomotor effects of morphine (2 or 5 mg/kg, s.c.). Two other amphetamine pretreatment protocols (1 × 5 mg/kg, i.p. and 14 × 2.5 mg/kg, i.p.) also failed to induce cross-sensitization to morphine. In contrast, all amphetamine pretreatment regimens induced sensitization of the locomotor effects of amphetamine (1 mg/kg, i.p.) and pretreatment with morphine (14 × 10 mg/kg, s.c.) induced both short- and long-term sensitization of the locomotor effects of both morphine and amphetamine. These data suggest that the expression of sensitization of the locomotor effects of morphine and amphetamine, at least partially, involves distinct neuroadaptive phenomena.
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KEY WORDS: *Behavioral sensitization; Locomotor activity; Morphine; Amphetamine*

Upon repeated treatment with drugs of abuse of different pharmacological classes, such as psychostimulants, opioids, and ethanol, a long-lasting increment of the psychomotor stimulant and positive reinforcing effects of these drugs can be observed, which is termed behavioral sensitization. Cross-sensitization refers to the phenomenon that pretreatment with a given drug results in sensitization to the behavioral effects of another drug (Stewart and Badiani 1993). For instance, pre-exposure to opioids has been shown to induce long-lasting sensitization to opioids, as well as psychostimulants such as amphetamine and cocaine in rats (DuMars et al. 1988; Vanderschuren et al. 1997, 1999). Similarly, pretreatment with amphetamine and cocaine results in long-lasting cross-sensitization between these drugs (Kalivas and Weber 1988; Pierce and Kalivas 1995; Vanderschuren et al. 1999).

Sensitization to opioids in psychostimulant-pretreated rats has also been reported, but except for one study (Cador et al. 1995), this was short-term (up to 7 days post-treatment) cross-sensitization (DuMars et al. 1988; Vezina and Stewart 1990; Bjijou et al. 1996). This is an important difference since one of the core features of behavioral sensitization is its long-term character (Rob-

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inson and Berridge 1993; Pierce and Kalivas 1997). In addition, marked neurobiological differences have been reported between short-term (days post-treatment) and long-term (weeks to months post-treatment) behavioral sensitization (e.g., Wolf et al. 1993; Tjon et al. 1994, 1997; White et al. 1995).

There is a large body of evidence indicating that the expression of long-term behavioral sensitization depends on enhanced mesolimbic dopaminergic neurotransmission. Thus, repeated treatment with morphine, cocaine, amphetamine, nicotine, or ethanol has been shown result in a long-lasting increase in the sensitivity of nucleus accumbens dopaminergic nerve terminals to these drugs, as well as to depolarizing stimuli, both in vivo and in vitro (Kolta et al. 1985; Robinson et al. 1988; Kalivas and Duffy 1993; Spanagel et al. 1993; Heidbreder et al. 1996; Nestby et al. 1997; Balfour et al. 1998; for reviews see Kalivas and Stewart 1991; Pierce and Kalivas 1997). The locomotor activating properties of psychostimulant drugs are generally thought to be due to an increase in nucleus accumbens dopaminergic neurotransmission (Kelly et al. 1975; Pijnenburg et al. 1975; Delfs et al. 1990; for review see Amalric and Koob 1993). On the other hand, the psychomotor effects of opioid drugs have been shown to involve both dopamine-dependent and dopamine-independent components (Pert and Sivit 1979; Kelley et al. 1980; Stinus et al. 1980; Joyce et al. 1981; Kalivas et al. 1983; Vaccarino et al. 1986). This leaves open as to whether expression of opioid sensitization involves hyperresponsiveness of dopaminergic and/or non-dopaminergic mechanisms, and whether sensitization to opioids and psychostimulant requires different neuroadaptive phenomena.

The sensitizing properties of drugs of abuse have been suggested to be relevant for compulsive drug-seeking behavior and drug craving (Robinson and Berridge 1993). With respect to this hypothesis, we have recently found that the ability of a drug to induce a sensitized locomotor response predicted its ability to reinstate drug-seeking behavior in rats (De Vries et al. 1998, 1999; Vanderschuren et al. 1999). In those studies, heroin was found to be ineffective in inducing reinstatement of cocaine-seeking behavior, and animals with a history of cocaine self-administration did not display a sensitized locomotor response to heroin (De Vries et al. 1998).

Taken together, there is only limited evidence for long-term sensitization to opioids in psychostimulant-pretreated rats. In fact, studies from our laboratory have shown that psychostimulant self-administration does not cause long-term cross-sensitization to opioids (De Vries et al. 1998). Therefore, in the present study, we addressed this issue in detail. To that aim, rats were pretreated with morphine, or with one of three different amphetamine pretreatment regimens. After a short (three days) or a long (three weeks) post-treatment interval, their locomotor responses to amphetamine or one of two doses of morphine were assessed.

MATERIALS AND METHODS

Animals and Drug Pretreatments

All experiments were approved by the Animal Care Committee of the Free University of Amsterdam. Male Wistar rats (Harlan, Zeist, The Netherlands), weighing 180–200 g at the beginning of drug treatment, were housed two per cage in Macrolon cages under controlled conditions (lights on from 7.00 to 19.00 hr) for one week before use. Food and water were available ad libitum. Animals were briefly handled on the two days preceding drug treatment and on the two days preceding drug challenges. Rats received pretreatment injections with morphine or amphetamine in their home cages according to regimens that consistently induce long-term behavioral sensitization in our laboratory: five daily i.p. injections with 2.5 mg/kg amphetamine and 14 daily s.c. injections with 10 mg/kg morphine (Vanderschuren et al. 1997, 1999). In view of the lack of cross-sensitization to morphine initially observed in amphetamine-pretreated animals, two other amphetamine pretreatment regimens were also used. One consisted of 14 daily i.p. injections with 2.5 mg/kg amphetamine, to administer a similar number of amphetamine and morphine injections, and the other of one i.p. injection with 5 mg/kg amphetamine, to include a shorter amphetamine pretreatment protocol as well. Control groups received saline injections. Morphine-HCl and (+)-amphetamine-sulphate were purchased from O.P.G. (Utrecht, The Netherlands) and dissolved in sterile saline.

Procedure

Horizontal motor activity was measured in perspex cages (40 × 40 × 35 cm) using a video tracking system (EthoVision; Noldus Information Technology B.V., Wageningen, The Netherlands), which determined the position of the animal 5 times per second. All experiments were conducted between 9.30 AM and 4.30 PM, in the light phase of the day/night cycle. White noise was used to minimize the influence of surrounding sounds. Although behavioral sensitization is a long-term phenomenon (Robinson and Berridge 1993; Pierce and Kalivas 1997), mainly short-term cross-sensitization to morphine in psychostimulant-pretreated rats has been reported (DuMars et al. 1988; Vezina and Stewart 1990; Bjijou et al. 1996). Therefore, both three days and three weeks post-treatment, locomotor challenges were performed, to test for both short-term and long-term sensitization. These locomotor challenges were performed as follows. Animals were allowed to

habituate to the test cages for 2 hrs, during which activity was monitored. Then, the rats received an injection with saline (1.0 ml/kg, s.c. or i.p., depending on the route of administration of the challenge drug) and activity was monitored for 1 hr. Subsequently, animals were injected with morphine (2 or 5 mg/kg, s.c.) or amphetamine (1 mg/kg, i.p.), and activity was monitored for 1.5 hrs (amphetamine), 2 hrs (2 mg/kg morphine), or 3 hrs (5 mg/kg morphine). The challenge dose of amphetamine (1 mg/kg) represents a dose that elicits a submaximal stimulation of locomotor activity, to which long-term sensitization has been shown to occur in morphine- and amphetamine-pretreated rats (Vanderschuren et al. 1999). Morphine has been reported to have biphasic effects on locomotor activity: suppression followed by stimulation (Babbini and Davis 1972; Vasko and Domino 1978; Iwamoto 1984). To be able to interpret our morphine challenge experiments in terms of changes in the locomotor suppressant and stimulatory effects of morphine, we first tested the locomotor effects of two doses of morphine (2 and 5 mg/kg, s.c.) in drug-naive rats. In that experiment, a similar design as described above was used. The animals were habituated to the test cages for 2 hrs, after which all animals received an injection with saline. One hour after this saline injection, the animals received either saline (1 ml/kg, s.c.), or one of two doses of morphine (2 or 5 mg/kg, s.c.). Since these two morphine doses appeared to elicit different psychomotor effects, both doses were used in the subsequent challenge studies. All animals were tested only once.

Statistics

Horizontal locomotor activity, expressed as distance travelled (cm) was calculated either in 10 min blocks or for the entire test period. In the former case, locomotor activity was analyzed using two-factor repeated measures analysis of variance (ANOVA) for time block and (pre)treatment. Post-hoc comparisons were performed using Student-Newman-Keuls tests. In the latter case, locomotor activity was analyzed using one-way ANOVA.

RESULTS

Effects of morphine on locomotor activity in drug-naive rats

Morphine (2 and 5 mg/kg, s.c.) elicited significant alterations in locomotor activity, as compared to saline ($F(\text{treatment})(2,20) = 6.82$; $p < .01$; $F(\text{treatment} \times \text{time})(34,340) = 5.69$; $p < .001$). Except for during the first 10 min time block, saline-injected rats displayed low levels of locomotor activity. The 5 mg/kg dose of morphine almost completely inhibited locomotor activity in the first 10 min. For the next hr, levels of activity in the 5 mg/kg morphine group remained low. Since in the saline-treated rats levels of locomotion were very low as well, these do not indicate whether in this period morphine had a suppressant effect, or just lacked any effects on psychomotor activity. After this delay of about 60 min, locomotor activity gradually increased in the 5 mg/kg morphine group, hyperactivity being evident in the third hr of the test. The 2 mg/kg dose of morphine also suppressed locomotion in the first 10 min, albeit to a lesser extent than the 5 mg/kg dose did. Subsequently, stimulation of locomotor activity as compared to saline-treated rats was evident in the animals treated with 2 mg/kg morphine, without the delay observed in rats treated with 5 mg/kg morphine. The psychomotor stimulant effects of 2 mg/kg morphine lasted for 2 hrs (Figure 1).

Long-term effects of amphetamine and morphine pretreatment on the locomotor effects of amphetamine and morphine

Amphetamine (1 mg/kg, i.p.) induced a monophasic locomotor response. The psychomotor effect of amphetamine was significantly enhanced in rats pretreated with amphetamine (5 × 2.5 mg/kg, i.p.), three weeks after cessation of treatment ($F(\text{pretreatment})(1,25) = 5.35$; $p < .05$) (Figure 2A). In animals pretreated with

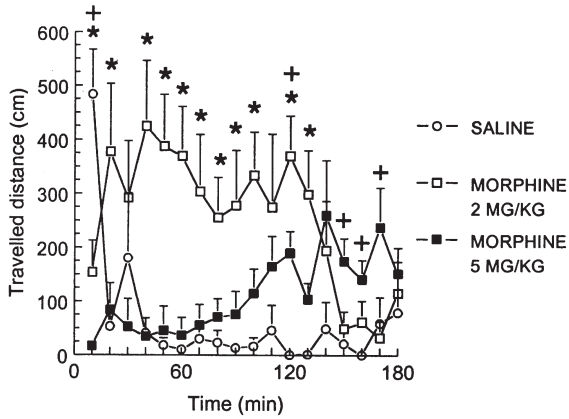

Figure 1. Locomotor responses to an injection with saline ($n = 8$), 2 mg/kg morphine ($n = 7$), or 5 mg/kg morphine ($n = 8$) in drug-naive rats. After 2 hrs habituation to the test cages, all animals were s.c. injected with saline. One hr thereafter, the animals received either saline (1 ml/kg, s.c.) or morphine (2 or 5 mg/kg, s.c.), and activity was monitored for 3 hrs. Data are expressed as mean travelled distance (in cm) ± S.E.M. per 10-min interval. * 2 mg/kg morphine differs from saline, $p < .05$ (Student-Newman-Keuls); + 5 mg/kg morphine differs from saline, $p < .05$ (Student-Newman-Keuls).

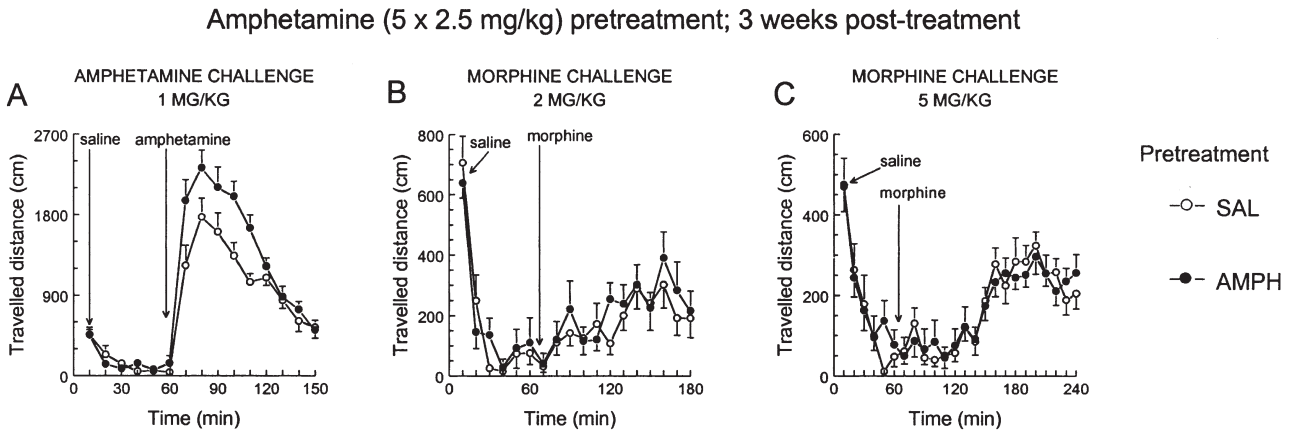

Figure 2. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg , or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with amphetamine (AMPH; 5 × 2.5 mg/kg, i.p.; **(A)** $n = 14$, **(B)** $n = 8$, and **(C)** $n = 16$) or saline (SAL; **(A)** $n = 13$, **(B)** $n = 8$, and **(C)** $n = 15$), 3 weeks post-treatment. After habituation to the test cages, the animals were injected with saline (1 ml/kg, i.p. or s.c.) and 1 hr later with amphetamine or morphine, after which activity was monitored for 1.5–3 hr. Data are expressed as mean travelled distance (in cm) ± S.E.M. per 10-min interval.

amphetamine, the locomotor effects of neither 2 mg/kg morphine (F(pretreatment)(1,14) = 0.68, NS; F(pretreatment × time)(11,154) = 0.51, NS) (Figure 2B), nor 5 mg/kg morphine (F(pretreatment)(1,29) = 0.01, NS; F(pretreatment × time)(17,493) = 0.55, NS (Figure 2C) were altered.

Pretreatment with morphine (14 × 10 mg/kg, s.c.) resulted in an augmentation of locomotion induced by amphetamine (F(pretreatment)(1,31) = 8.60; $p < .01$) (Figure 3A) three weeks post-treatment. The locomotor responses to morphine were profoundly sensitized in morphine-pretreated rats. In rats pretreated with morphine, 2 mg/kg morphine induced a hyperlocomotor response lasting the entire 2 hrs test period, which was markedly augmented as compared to saline-pretreated rats (F(pretreatment)(1,12) = 16.36; $p < .01$]; F(pretreatment × time)(11,132) = 0.66, NS) (Figure 3B). Morphine (5 mg/kg), in morphine-pretreated animals, induced an increase in locomotion, lasting 20 min, followed by a slight decline in activity. After 60 min, a second hyperlocomotor phase was observed, parallel to the gradual increase in activity seen in saline-pretreated animals. During the entire 3 hour-test period, levels of activity in morphine-pretreated rats were increased as compared to saline-pretreated rats (F(pretreatment)(1,15) = 26.64; $p < .001$; F(pretreatment × time)(17,255) = 1.12, NS) (Figure 3C; note the scale difference on the Y-axis as compared to Figure 2C).

Effect of amphetamine pretreatment regimen on the induction of sensitization of the locomotor effects of amphetamine and morphine

To investigate whether the failure to observe long-term sensitization to morphine in amphetamine pre-exposed rats was due to the nature of the amphetamine pretreatment regimen, the long-term effects of two different amphetamine pretreatment regimens were investigated. Pretreatment with a single injection of amphetamine (5 mg/kg, i.p.) or with 14 daily i.p. injections of 2.5 mg/kg amphetamine had essentially the same effects as the 5 × 2.5 mg/kg amphetamine pretreatment regimen. Animals that received a single pre-exposure to amphetamine (5 mg/kg, i.p.) displayed sensitization to the locomotor effects of amphetamine (1 mg/kg, i.p.) three weeks post-treatment (F(pretreatment)(1,27) = 9.34; $p < .01$) (Figure 4A), but not to the locomotor effects of 2 mg/kg morphine s.c. (F(pretreatment)(1,11) = 1.23, NS) (Figure 4B), or 5 mg/kg morphine s.c. (F(pretreatment)(1,13) = 0.56, NS) (Figure 4C). Similarly, three weeks after cessation of pretreatment with amphetamine (14 × 2.5 mg/kg, i.p.) the locomotion induced by amphetamine (1 mg/kg, i.p.) (F(pretreatment)(1,26) = 7.36; $p < .05$] (Figure 5A), but not morphine (2 mg/kg: (F(pretreatment)(1,11) = 1.86, NS) (Figure 5B); 5 mg/kg: (F(pretreatment)(1,29) = 0.00, NS) (Figure 5C), appeared to be sensitized. Since the temporal patterns of activity in these experiments did not differ from those presented in Figure 2, no time courses of locomotion are presented in Figures 4 and 5.

Short-term effects of amphetamine and morphine pretreatment on the locomotor effects of amphetamine and morphine

Pretreatment with amphetamine has been shown to result in short-term sensitization of the locomotor effects of morphine (Vezina and Stewart 1990; Bjijou et al. 1996). Therefore, we also investigated whether morphine- and amphetamine-pretreatment resulted in loco-

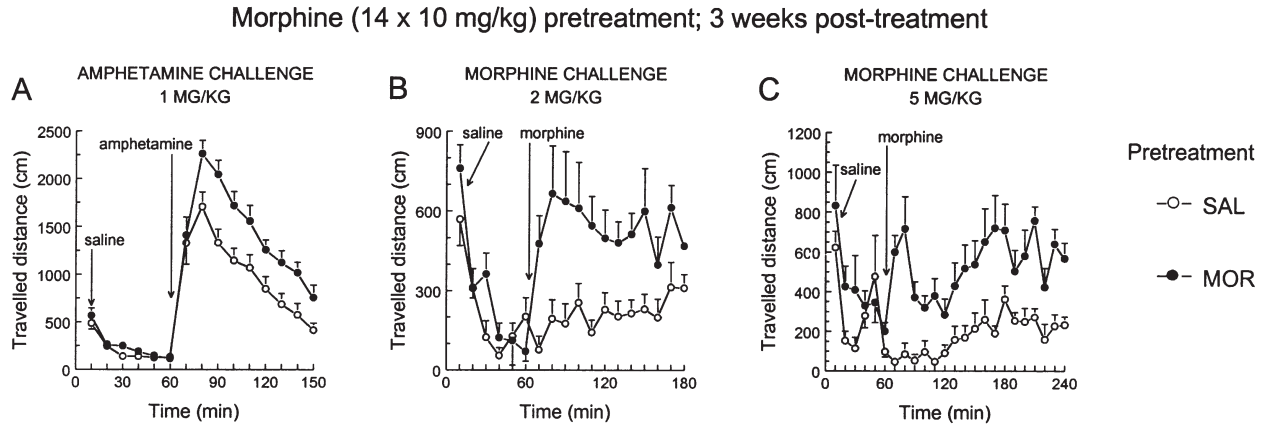

Figure 3. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg, or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with morphine (MOR; 14 × 10 mg/kg, s.c.; **(A)** $n = 17$, **(B)** $n = 7$, and **(C)** $n = 9$) or saline (SAL; **(A)** $n = 16$, **(B)** $n = 7$, and **(C)** $n = 8$), three weeks post-treatment. After habituation to the test cages, the animals were injected with saline (1 ml/kg, i.p. or s.c.) and 1 hr later with amphetamine or morphine, after which activity was monitored for 1.5–3 hrs. Data are expressed as mean travelled distance (in cm) ± S.E.M. per 10-min interval.

motor sensitization when challenge tests were performed three days, as opposed to three weeks post-treatment.

The short-term effects of amphetamine (5 × 2.5 mg/kg, i.p.) pretreatment are shown in Figure 6. The locomotor effects of amphetamine (1 mg/kg, i.p.) were augmented in amphetamine-pretreated rats (F(pretreatment)(1,25) = 0.94, NS; F(pretreatment × time) (11,275) = 2.86; $p < 0.01$), as apparent from the significant enhancement of amphetamine-induced locomotor activity in amphetamine-pretreated rats during the second 10 min time block (Figure 6A). The locomotor effects of 2 mg/kg morphine were not different between saline- and amphetamine-pretreated animals (F(pretreatment)(1,14) = 0.06, NS; F(pretreatment × time)(11,154) = 0.90, NS) (Figure 6B). When challenged with 5 mg/kg morphine (Figure 6C), animals pre-exposed to amphetamine did not show altered locomotor activity as compared to saline-pretreated rats (F(pretreatment)(1,14) = 1.85, NS; F(pretreatment × time)(17,238) = 0.36, NS]).

Three days after cessation of morphine pretreatment, the locomotor response to amphetamine (1 mg/kg, i.p.) was markedly enhanced in morphine-pretreated rats (F(pretreatment)(1,14) = 5.33; $p < .05$] (Figure 7A). The locomotor responses to morphine (2 and 5 mg/kg) were not only profoundly augmented in morphine-pretreated rats, the patterns of activity were also different. In morphine-pretreated animals, 2 mg/kg morphine evoked an immediate hyperactivity, which peaked during the 2nd 10 min block, then declined and reached the level of activity of saline-pretreated rats during the last

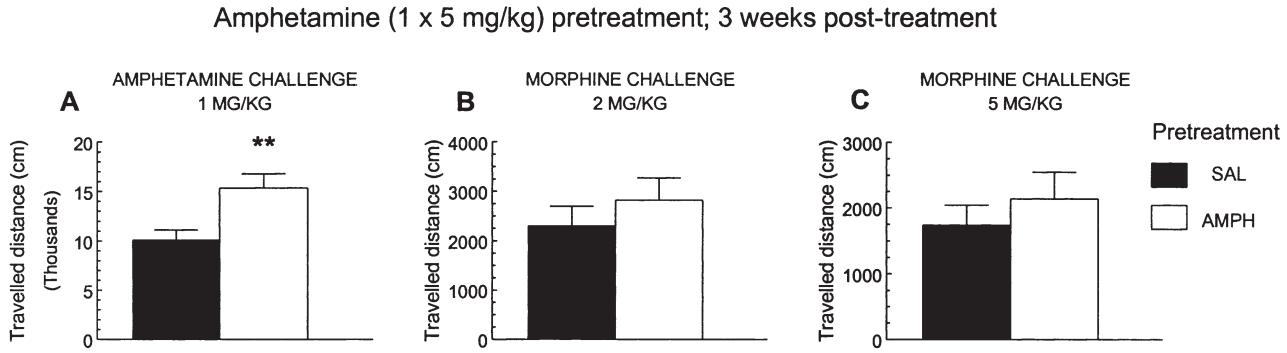

Figure 4. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg, or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with amphetamine (AMPH; 1 × 5 mg/kg, i.p.; **(A)** $n = 14$, **(B)** $n = 8$, and **(C)** $n = 7$) or saline (SAL; **(A)** $n = 15$, **(B)** $n = 5$, and **(C)** $n = 8$), three weeks post-treatment. Drug challenges were preceded by 2 hrs habituation to the test cages and 1 hr saline challenge. Activity was monitored for 1.5 (amphetamine), 2 (2 mg/kg morphine), or 3 hrs (5 mg/kg morphine). Data are expressed as mean travelled distance (in cm) ± S.E.M. during the entire test period. ** different from saline-pretreated rats, $p < .01$ (ANOVA).

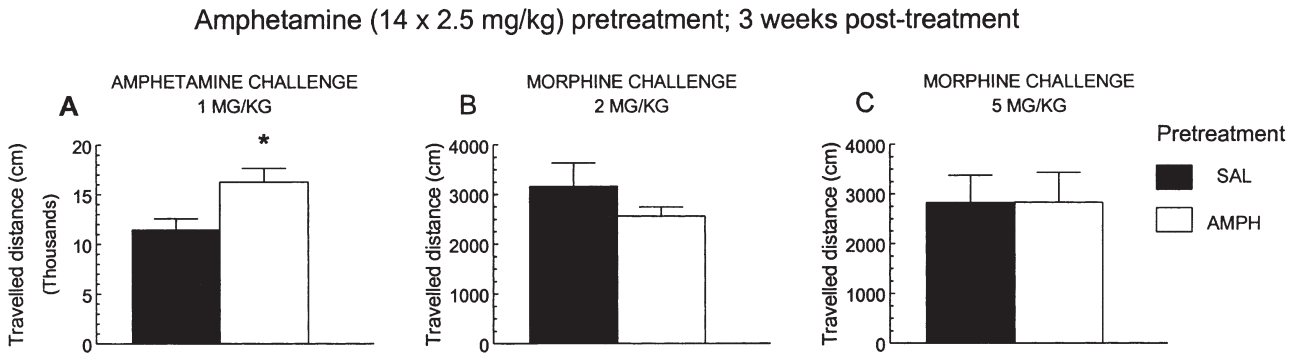

Figure 5. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg, or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with amphetamine (AMPH; 14 × 2.5 mg/kg, i.p.; **(A)** $n = 14$, **(B)** $n = 15$, and **(C)** $n = 7$) or saline (SAL; **(A)** $n = 14$, **(B)** $n = 16$, and **(C)** $n = 6$), three weeks post-treatment. Drug challenges were preceded by 2 hrs habituation to the test cages and 1 hr saline challenge. Activity was monitored for 1.5 (amphetamine), 2 (2 mg/kg morphine), or 3 hrs (5 mg/kg morphine). Data are expressed as mean travelled distance (in cm) ± S.E.M. during the entire test period. * different from saline-pretreated rats, $p < .05$ (ANOVA).

20 min of the test. In rats pre-exposed to saline (cf. Figure 1), 2 mg/kg morphine caused a brief hypoactivity, followed by an increase in locomotion (F(pretreatment)(1,14) = 23.89; $p < .001$; F(pretreatment × time) (11,154) = 4.72; $p < .001$) (Figure 7B; note the scale difference on the Y-axis as compared to Figure 6B). In rats pre-exposed to saline, 5 mg/kg morphine elicited delayed hyperactivity (cf. Figure 1). In morphine-pretreated rats, 5 mg/kg morphine immediately increased activity, with a peak during the 2nd 10 min block, after which the hyperactivity gradually declined (F(pretreatment)(1,14) = 10.39; $p < .01$; F(pretreatment × time) (17,238) = 3.91; $p < 0.001$) (Figure 7C; note the scale difference on the Y-axis as compared to Figure 6C).

As in our previous studies (Vanderschuren et al. 1997, 1999), pretreatment with neither amphetamine nor morphine resulted in altered locomotion during the habituation phases (not shown) and the challenges with saline, irrespective of whether testing was performed 3 days or 3 weeks post-treatment (see Figures 2, 3, 6, and 7).

DISCUSSION

Pre-exposure to amphetamine resulted in sensitization of the locomotor effects of amphetamine, but not in cross-sensitization to morphine. In contrast, and consistent

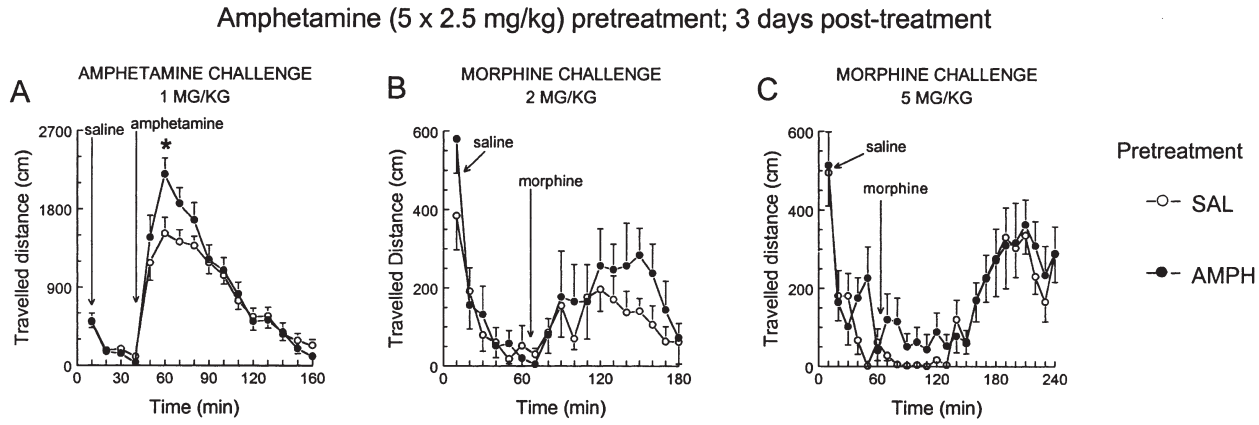

Figure 6. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg, or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with amphetamine (AMPH; 5 × 2.5 mg/kg, i.p.; **(A)** $n = 13$, **(B)** $n = 8$, and **(C)** $n = 8$) or saline (SAL; **(A)** $n = 14$, **(B)** $n = 8$, and **(C)** $n = 8$), three days post-treatment. After habituation to the test cages, the animals were injected with saline (1 ml/kg, i.p. or s.c.) and 1 hr later with amphetamine or morphine, after which activity was monitored for 2–3 hrs. Data are expressed as mean travelled distance (in cm) ± S.E.M. per 10-min interval. * different from saline-pretreated rats, $p < .05$ (Student-Newman-Keuls).

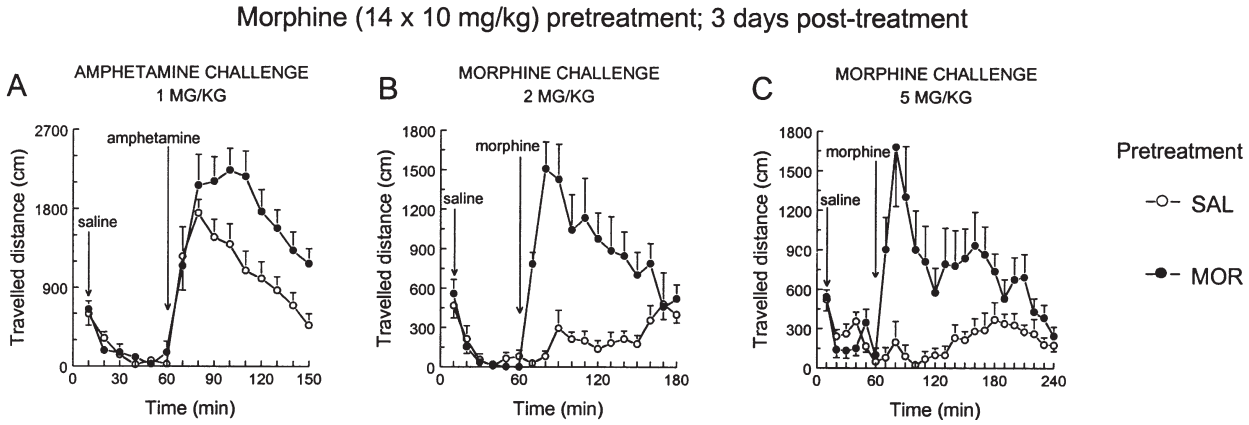

Figure 7. Locomotor responses to **(A)** 1 mg/kg amphetamine, i.p., **(B)** 2 mg/kg, or **(C)** 5 mg/kg morphine, s.c., in rats pretreated with morphine (MOR; 14 × 10 mg/kg, s.c.; **(A)** $n = 8$, **(B)** $n = 8$, and **(C)** $n = 8$) or saline (SAL; **(A)** $n = 8$, **(B)** $n = 8$, and **(C)** $n = 8$), three days post-treatment. After habituation to the test cages, the animals were injected with saline (1 ml/kg, i.p. or s.c.) and 1 hr later with amphetamine or morphine, after which activity was monitored for 1.5–3 hrs. Data are expressed as mean travelled distance (in cm) ± S.E.M. per 10-min interval.

with our previous work (Vanderschuren et al. 1997), pretreatment with morphine induced robust sensitization to both morphine and amphetamine. Morphine-pretreated rats seemed to display a stronger degree of sensitization to amphetamine than amphetamine-pretreated rats (cf. Figures 2A, 3A, 6A, and 7A). However, the lack of sensitization to morphine in amphetamine-pretreated rats is not likely to reflect a quantitative difference, viz. in the degree, rather than in the nature of locomotor sensitization induced by morphine and amphetamine. For instance, we have found that in the case of sensitization to cocaine and GBR-12909, amphetamine-pretreated animals seem to be sensitized to a somewhat larger degree than rats pre-exposed to morphine (Vanderschuren et al. 1999). In addition, sensitization to amphetamine in amphetamine-pretreated rats was much more marked three weeks, as compared to three days post-treatment (which is consistent with other reports showing that psychostimulant sensitization requires a considerable post-treatment interval (>1 week) to fully develop (Paulson et al. 1991; Pierce and Kalivas 1997)), but amphetamine-induced sensitization to the psychomotor effects of morphine was not observed at either post-treatment interval. Finally, the relatively small differences in the degree of sensitization to amphetamine, cocaine and GBR-12909 do not compare to the enormous differences observed with morphine challenges. We therefore argue that the present data indicate a qualitative difference in the neuroadaptive phenomena underlying the expression of sensitization to morphine and amphetamine. Support for this hypothesis comes from the observation that cholera toxin-induced stimulation of adenylate cyclase in the ventral tegmental area (VTA), the cell body area of the mesolimbic dopamine system, causes sensitization to amphetamine but not to morphine (Tolliver et al. 1996).

In rats, morphine has biphasic effects on locomotor activity- an initial suppression followed by hyperactivity. Upon repeated treatment with morphine, tolerance has been proposed to develop to its locomotor suppressant effects, and sensitization to its locomotor stimulant effects (Babbini and Davis 1972; Babbini et al. 1975; Vasko and Domino 1978). Morphine-pretreated rats appeared to be sensitized to both the 5 mg/kg dose (Vanderschuren et al. 1997) and the 2 mg/kg dose of morphine. In the present study, only a brief (10 min) suppression of locomotion by both doses of morphine could be demonstrated (Figure 1). However, a marked difference between the two doses was that after this hypoactivity phase, 2 mg/kg morphine immediately increased psychomotor activity, while in rats treated with 5 mg/kg morphine activity levels remained low for another 60 min. The finding that morphine pre-exposure induces sensitization to 2 mg/kg morphine, the locomotor suppressant effect of which is somewhat smaller and the hyperlocomotor effects more pronounced, suggests that sensitization to the locomotor effects of morphine involves increased psychomotor stimulant effects rather than just reduced suppressant effects. Since pretreatment with amphetamine fails to induce sensitization to 2 mg/kg morphine, it is therefore not likely that this lack of sensitization is solely due to a lack of tolerance to the locomotor suppressant effects of morphine. It is also worth noting that in morphine-pretreated animals, morphine can induce levels of psychomotor activity that cannot be evoked in morphine-naive rats (Vanderschuren et al. 1997; present study), indicating that in intermittent morphine-pretreated rats, the dose-response relationship for morphine locomotion is shifted upward, rather than leftward.

In apparent contrast to the present study, occurrence of sensitization to the locomotor effects of morphine in amphetamine-pretreated rats has previously been re-

ported (Vezina and Stewart 1990; Cador et al. 1995). For instance, an increased locomotor effect of morphine (1 mg/kg, i.p.) was observed in rats pre-exposed to amphetamine, 2 days post-treatment (Vezina and Stewart 1990). This resembles the experiment presented in Figure 6B. In that experiment however, we saw no significant effect of amphetamine pretreatment on the psychomotor effect of 2 mg/kg morphine, three days post-treatment. Amphetamine-induced sensitization to systemic morphine (2.5 mg/kg, s.c.) has also been reported at a longer (2 weeks) post-treatment interval (Cador et al. 1995). A possible confound in that study is that the morphine challenge was preceded by two challenges with amphetamine and one cocaine challenge. The most remarkable difference, however, between the studies cited above (Vezina and Stewart 1990; Cador et al. 1995) and the present study is that in the former studies, amphetamine was administered directly into the VTA during pretreatment, as opposed to i.p. injections in the present study. Although the available information suggests that intra-VTA amphetamine has the same sensitizing effects as i.p. amphetamine (Kalivas and Weber 1988; Perugini and Vezina 1994; Cador et al. 1995; Bjijou et al. 1996; Vezina 1996), this issue has never been addressed in detail. It could therefore be that different neuroadaptations are induced by intra-VTA and systemic amphetamine pretreatment, causing cross-sensitization to morphine in the former case only.

Cross-sensitization to intra-VTA administered morphine or DAMGO (a μ-opioid receptor agonist) has been shown in rats pretreated with systemic cocaine (DuMars et al. 1988), or with intra-VTA amphetamine (Bjijou et al. 1996), albeit at relatively short post-treatment intervals (3–7 days). A marked difference with systemic administration is that upon intra-VTA administration, the locomotor effects of opioids seem to be completely dopamine-dependent (Kelley et al. 1980; Stinus et al. 1980; Joyce et al. 1981; Kalivas et al. 1983), mediated by indirect stimulation of mesolimbic dopaminergic neurons (Johnson and North 1992; Klitenick et al. 1992). Given the findings that the mesolimbic dopaminergic system becomes hyperreactive upon repeated treatment with psychostimulant drugs (see introduction), it is therefore not surprising that in these studies cross-sensitization to intra-VTA morphine was observed. In this respect it is interesting to note that disruption of mesolimbic dopamine activity has been shown to result in supersensitivity to the locomotor effects of opioids, when administered into the nucleus accumbens (Kalivas and Bronson 1985; Stinus et al. 1985). A possible explanation for the lack of sensitization to morphine in amphetamine-pretreated rats is that amphetamine-induced hyperresponsiveness of the mesolimbic dopaminergic system results not only in an augmented locomotor response to intra-VTA administered opioids, but also in an inhibited locomotor response to intra-accumbens administered opioids; these two effects could neutralize each other. Since morphine-pretreatment also causes hyperreactivity of the mesolimbic dopaminergic system (Spanagel et al. 1993; Nestby et al. 1997), this would imply that upon morphine pre-exposure, neuroadaptations distinct from those occurring upon pretreatment with amphetamine will unmask, or perhaps even enhance the sensitization of the effects of morphine in the VTA.

It has been suggested that the neuroadaptations involved in behavioral sensitization play a pivotal role in the persistence of compulsive drug-seeking behavior and drug craving (Robinson and Berridge 1993). In this respect, we have recently shown that the occurrence of behavioral sensitization may predict the ability of a drug to induce reinstatement of drug-seeking behavior (De Vries et al. 1998, 1999; Vanderschuren et al. 1999). In agreement with the present observations, we observed that in rats with a history of cocaine self-administration, amphetamine induced both reinstatement of drug-seeking behavior and sensitized locomotor activity, whereas heroin caused neither reinstatement nor expression of locomotor sensitization. In contrast, in rats with a history of heroin self-administration, both heroin and amphetamine were effective in inducing reinstatement of drug-seeking behavior as well as sensitized locomotion (De Vries et al. 1998). Therefore, the present data support the hypothesis that expression of behavioral sensitization is a key phenomenon in the occurrence of drug-seeking behavior.

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