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Sensitization to Daily Morphine Injections in Rats With Unilateral Lesions of the Substantia Nigra

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VOLPICELLI, L. A., K. W. EASTERLING, H. L. KIMMEL AND S. G. HOLTZMAN. *Sensitization to daily morphine injections in rats with unilateral lesions of the substantia nigra.* PHARMACOL BIOCHEM BEHAV **64**(3) 487–493, 1999.—Morphine indirectly enhances dopaminergic activity in the nigrostriatal system, and repeated administration of morphine progressively increases the locomotor activity of rats. We used the rotational behavior model to determine if daily morphine produces an increase in turning and produces cross-sensitization to *d*-amphetamine and cocaine. Rats with unilateral nigrostriatal lesions received daily injections of saline or morphine (10 mg/kg). Repeated morphine administration produced a progressive increase in turning over 13 days. Next, a morphine dose–response curve (1.0–30 mg/kg) was determined. Both the saline and morphine-treated groups showed dose-dependent increases in turning, but, the peak effect in the morphine group was higher than that in the saline group, indicating sensitization to morphine. The morphine-treated group did not show cross-sensitization to either *d*-amphetamine (0.1–3 mg/kg) or cocaine (1.0–30 mg/kg); in fact, it showed less cocaine-induced turning than the saline group. Seventy-one days after saline or morphine injections began, the morphine group was still significantly more sensitive to turning induced by 10 mg/kg morphine than the saline group was (200 vs. 750). Therefore, repeated daily injections of morphine produce a progressive sensitization to turning induced by morphine in the absence of cross-sensitization to turning induced by psychomotor stimulants. © 1999 Elsevier Science Inc.

Morphine *d*-Amphetamine Cocaine Nigrostriatal lesions Turning behavior
6-Hydroxydopamine Rotational behavior Stimulants Opioids

THE rotational behavior of the rat provides a convenient dependent measure of the effects of drugs that act upon the nigrostriatal dopamine system (31). In this model, rats are given a unilateral lesion of the nigrostriatal tract with 6-hydroxydopamine (6-OHDA), destroying presynaptic dopamine terminals that project to the ipsilateral striatum. When drugs that interact with brain dopamine systems are administered, these rats rotate away from the side with the greater increase in postsynaptic dopamine receptor activity. For example, when a direct postsynaptic dopamine receptor agonist, such as apomorphine, is administered systemically, the upregulated receptors of the striatal neurons on the lesioned side causes a greater increase in neuronal activity relative to the nonlesioned side. Consequently, the animals turn contralateral to the lesion (36). In contrast, drugs that increase dopamine levels by a presynaptic action, such as amphetamine and cocaine, will cause these lesioned rats to rotate ipsilaterally because the drug-induced increases in synaptic dopamine levels occur only on the nonlesioned side (35).

Drugs that act upon opioid receptors also influence dopaminergic activity. For example, μ- and δ-opioid receptor agonists increase extracellular levels of dopamine in the striatum and nucleus accumbens (5,6,34). In addition, opioids indirectly increase the activity of dopamine neurons in the substantia nigra (12) and ventral tegmental area (25). Opioid receptors are localized on neurons in the mesolimbic and nigrostriatal dopamine tracts of rats (7,23,24,32,42). Activation of μ- and δ-opioid receptors decrease the activity of in-

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hibitory GABA-ergic neurons that project from the substantia nigra to the striatum (15,22), thus removing the inhibitory GABAergic influence upon dopamine neurons.

The behavioral effects of opioids can also reflect the actions of these drugs on brain dopamine systems. Acute administration of morphine or other μ-opioid agonists produces rotational behavior in nigrally lesioned rats in some studies (2,11,20,28), but not in others (30,40). Although acute morphine administration may not produce large amounts of turning, there is some evidence from studies of locomotor activity that repeated administration may increase the behavioral effects of opioids (i.e., sensitization). Daily repeated administration of morphine to rats results in a progressive increase in locomotor activity over time and a decrease in the latency to begin activity (1,13,14,18). A study of rat rotational behavior examined the effects of morphine dispensed continuously through an SC osmotic pump (19). Acute administration of morphine (0.3–10 mg/kg, SC) in these chronically morphine-treated animals produced only a slight increase in ipsilateral turning, despite evidence that the animals were tolerant to the behavioral depressant effect of a 10 mg/kg challenge dose.

It appears that the specific drug treatment regimen can affect the production of sensitization. Rats given a single daily injection of morphine (10 mg/kg) for 2 weeks showed more sensitization to the locomotor stimulant effects of morphine than did rats given the same total amount of morphine in thrice-daily escalating doses for 5 days (37). Similarly, cocaine administered to rats by intermittent daily injections produced sensitization to its behavioral-stimulant effects, whereas cocaine administered by continuous infusion did not, and even produced tolerance to those effects (18).

In the present study, we sought to determine if the turning behavior of nigrally lesioned rats increases progressively over the course of single daily morphine injections. By using the rotational model, we can investigate if the nigrostriatal dopamine system contributes to sensitization, and if the effects are mediated pre- or postsynaptically. Preexposure to morphine may also produce cross-sensitization to the effects of psychomotor stimulants (3,4,37,39). Therefore, we also compared the effects of amphetamine and cocaine on the rotational behavior of morphine-treated and saline-treated rats.

METHOD

Subjects

Sixteen male Sprague–Dawley-derived rats (Sasco, Inc., Omaha, NE), weighing 240–260 g at the time of surgery, were used. Food (Purina Rodent Chow, Purina Mills, St. Louis, MO) and water were provided ad lib. All rats were housed (three per cage) in polycarbonate hanging cages in a temperature-controlled room maintained on a 12 L:12 D cycle (lights on at 0700 h).

Surgery

Prior to screening and testing, rats were given a unilateral lesion of the right nigrostriatal pathway with 6-OHDA. Equithesin™ (pentobarbital and chloral hydrate, 3.3 mg/kg, IP), was given as an anesthetic before the rats were placed in the stereotaxic frame. To create the lesion, 8 μg/4 μl of 6-OHDA was injected at a rate of 1.0 μl/min for 4 min, injected using a 25-μl Hamilton syringe. Stereotaxic coordinates relative to bregma were AP = −4.5, ML = −2.3, DV = −7.1 (29). Rats were allowed to recover from surgery for at least 7 days before behavioral screening.

Rotational Behavior

Rotational behavior was measured in eight round stainless steel bowls (rotometers), 40.6 cm diameter and 25.4 cm high, with a Plexiglas collar, 40.6 cm diameter and 25.4 cm high (Roto-Rat™, MED Associates, Inc., East Fairfield, VT). A Velcro belt wrapped around the midsection of the rat was attached to a spring tether that was connected to a rotation sensor. Sensors were interfaced to an IBM desktop computer running specialized software (Roto-Rat version 1.2©, MED Associates Inc.) that recorded full (360°) clockwise and counterclockwise turns during testing.

Screening

Rotational behavior is not reliably produced until approximately 90% of nigrostriatal neurons are destroyed, and this can be determined by the turning response of the animals to apomorphine (10). Therefore, the animals in this study were screened by injecting them with 0.3 mg/kg SC apomorphine twice weekly, with a 2–3-day intertest interval, for 2 weeks. Following each apomorphine injection, rotational activity was recorded for 1 h. Rats showing at least 200 contralateral turns/h (with no ipsilateral turns) in any screening period continued in the study.

Testing

Following screening, animals were randomized into two groups of eight rats each, the morphine or saline treatment groups. (However, two rats were excluded from the study, one from each group: one rat died after receiving the first injection of morphine, and the other became ill.) On the first day of testing (day 0), all animals received an injection of saline. On day 1, all animals received an injection of morphine (10 mg/kg). On days 2–14, the animals received either daily injections of saline (SAL treatment group $n = 7$) or 10 mg/kg morphine (MOR treatment group $n = 7$). All subjects were tested every day, and rotational activity was recorded continuously for 4.0 h following injections.

After 13 days of treatment with either saline or morphine (experimental days 2–14), several doses of morphine (1.0–10 or 1.0–30 mg/kg) were administered in a random order to all animals. Drug tests were conducted at 3-day intervals. On the 2 days between drug tests, animals received daily injections of morphine or saline, appropriate to the experimental condition, in the home cage and were not tested for rotational behavior. Out of concern for the safety of the animals, the SAL-treated group did not receive the 30 mg/kg dose of morphine. If a rat in the MOR treatment group received less than 10 mg/kg morphine on a test day, an additional amount of morphine was given immediately following the session so the rat received a total of 10 mg/kg morphine that day.

After a morphine dose–response curve was determined in each group, all animals received doses of *d*-amphetamine (0.1–3.0 mg/kg) or cocaine (1.0–30 mg/kg) administered in a random order. Amphetamine was administered SC, and cocaine was administered IP. As before, rotational behavior was tested every third day. On the intervening 2 days, 10 mg/kg morphine or saline was administered in the home cage, according to group assignment.

Two days after the last dose of amphetamine or cocaine was administered the subjects were challenged with 10 mg/kg morphine. This challenge occurred after 71 total daily injections. During the 2 days between the last injection of a stimu-

lant and the morphine challenge, subjects received 10 mg/kg morphine or saline.

Drugs

Morphine sulfate (Penick Corp., Newark, NJ), *d*-amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) and cocaine hydrochloride (National Institute on Drug Abuse) were dissolved in 0.9% saline. 6-OHDA hydrobromide (Sigma Chemical Co.) and apomorphine hydrochloride (Research Biochemicals, Inc., Natick, MA) were dissolved in 0.1% ascorbic acid in 0.9% saline. Morphine, amphetamine, and apomorphine were given SC, and cocaine was given IP; doses refer to the free base. All drugs except 6-OHDA were given in a volume of 1.0 ml/kg body weight.

Data Analysis

Data were analyzed by analysis of variance (ANOVA) appropriate for repeated measures. If necessary, post hoc comparisons were made using Fisher's LSD protected *t*-tests. The alpha level set for all comparisons was $p < 0.05$. When amphetamine or cocaine was administered, turning was recorded for 4 h but analyzed only for the initial 2-h period, the time during which most rotational activity occurs (20).

RESULTS

Control Data

There were no significant differences in ipsilateral, $t(1, 6) = -0.02$, p = NS, or contralateral, $t(1, 6) = 0.68$, p = NS, turning between the two treatment groups during the initial (day 0) saline challenge. Both groups (pooled) made approximately 6.5 (±4.2) full ipsilateral turns, and 2.7 (±1.9) contralateral turns in 4 h.

On day 1, when both groups received morphine for the first time, there were no significant differences in ipsilateral, $t(1, 6) = -0.7$, p = NS, or contralateral, $t(1, 6) = 0.4$, p = NS, turning between the two treatment groups. The saline and morphine groups made 14.9 (±10.6) or 43.3 (±38.4) full ipsilateral turns, respectively. Few contralateral turns were made by either the saline 3.3 (±2.4) or morphine 1.9 (±1.9) groups.

At this time, and when data for the first 14 days of the experiment were analyzed, there were no significant morphine-induced changes in contralateral turning, either between groups, $F(1, 12) = 2.20$, p = NS, or over the 14 days, $F(12, 144) = 1.21$, p = NS. Further, when the morphine dose–response curve was determined (days 17–29), there were no morphine-induced changes in contralateral turning in response to any dose of morphine, $F(1, 12) = 3.65$, p = NS. Therefore, the contralateral turning data were not analyzed in subsequent experiments.

Changes in Morphine-Induced Turning Across Days

In the morphine-treated group, there was a progressive increase in ipsilateral turning over the course of the daily injections of morphine (Fig. 1A). A two-factor ANOVA was performed on the ipsilateral turning data from days 2 through 14, the 10 mg/kg dose of morphine from the dose–response curve and from the morphine challenge on day 71. The results of the ANOVA indicated significant effects of treatment group, $F(1, 12) = 31.5$, $p < 0.05$, day of treatment, $F(14, 168) = 10.1$, $p < 0.05$, and a significant treatment × day interaction, $F(14, 168) = 9.46$, $p < 0.05$. Planned comparisons indicated that on days 5–14, the morphine group showed significantly ($p < 0.05$) more turning than the saline group. Also, on days 7–14 the rats in the morphine group turned significantly more than they did on day 2.

Figure 1B compares ipsilateral turning in the morphine-treated group over 15-min bins for days 2, 14, and 71. There was a significant increase in turning on day 14 compared to day 2, $F(1, 12) = 5.2$, $p < 0.05$, and across 15-min bins, $F(15, 180) = 2.3$, $p < 0.05$, but no significant interaction between bins and days, $F(15, 180) = 1.01$, p = NS. On day 14, by 30 min, ipsilateral turning was significantly higher ($p < 0.05$) than turning at the corresponding time point on day 2, and this generally continued throughout the session—except at 105, 120, 135, 165, and 180 min.

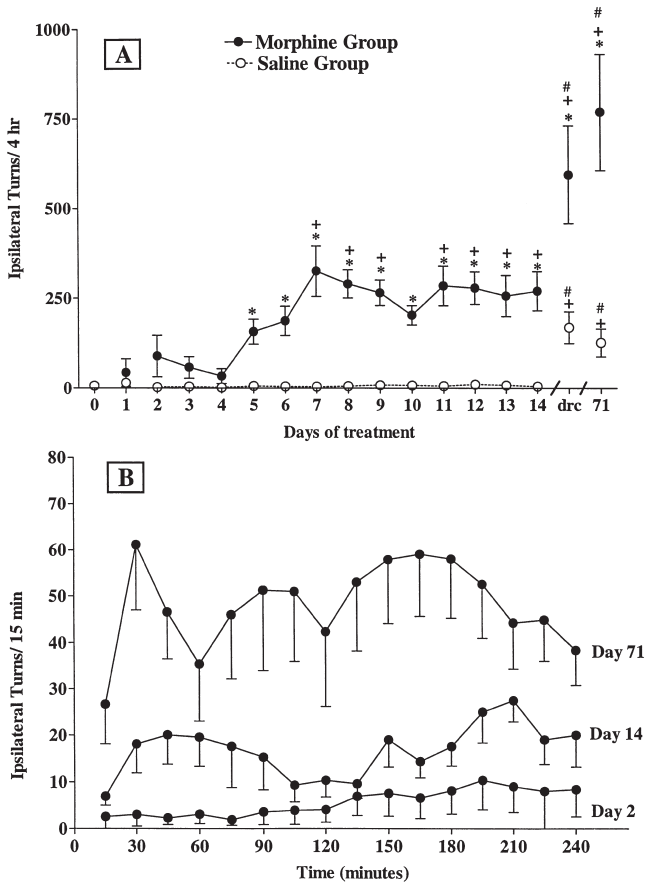

FIG. 1. (A) The number of full ipsilateral turns produced in 4 h by saline or 10 mg/kg morphine over 71 days of the study. On day 0, all subjects received saline, on day 1, all subjects received morphine, and on days 2–14, subjects received either saline or morphine, according to treatment condition. As part of the dose–response curve (days 17–29) and on day 71, subjects received another morphine (10 mg/kg) challenge. Each point represents the mean (±SEM) of observations in seven subjects. (B) Time course in the morphine group of ipsilateral turning produced by saline or 10 mg/kg morphine on days 2, 7, and 71 of daily drug administration. Each point represents the mean (and SEM) number of turns produced in seven subjects during each 15-min interval. On day 14, the morphine-treated group showed significantly greater turning than day 1, beginning at 45 minutes. On day 71, turning was significantly higher than it was on day 1 beginning at 15 min. *Indicates a difference between groups ($p < 0.05$); +indicates a difference from day 2 ($p < 0.05$); #indicates a difference from day 14 ($p < 0.05$).

In the morphine group, a morphine challenge (10 mg/kg) either during the determination of the morphine dose–response curve or at the end of the study (day 71) significantly elevated ipsilateral turning above levels seen on day 14 ($p < 0.05$; Fig. 1A). In addition, morphine-induced turning on day 71 was significantly higher than that produced by the same dose (10 mg/kg) during the determination of the dose–response curve. At equivalent time points within the session, turning by the morphine group was significantly greater than turning by the saline group. In the saline group, morphine (10 mg/kg) given during the dose–response determination (days 17–29), produced increases in turning that were significantly greater than those seen on day 14 (within group), but not significantly different from those seen on day 71.

Within the morphine group, when turning was compared between days 2 and 71 over 15-min bins, there were significant differences among days, $F(1, 12) = 15.5$, $p < 0.05$, and among bins, $F(15, 180) = 1.78$, $p < 0.05$; Fig. 1B), but no significant interaction between bins and days, $F(15, 180) = 1.33$, p = NS. Post hoc comparisons revealed that, by 15 min, turning within the session was higher on day 71 than on day 2, and this difference remained throughout the 4 h.

Morphine Dose–Response Curve

Morphine produced a dose-dependent increase in turning in both treatment groups (Fig. 2A). ANOVA of the ipsilateral turning data (for the saline, 1.0, 3.0, and 10 mg/kg morphine) indicated that the effects of morphine, in addition to being dose dependent, $F(3, 36) = 21.8$, $p < 0.05$, were also a function of treatment group [interaction, $F(3, 36) = 10.9$, $p = 0.05$]. However, there was no main effect of treatment group, $F(1, 12) = 1.5$, p = NS. Relative to saline, both the 3.0 and 10 mg/kg doses of morphine produced dose-dependent increases in ipsilateral turning in both treatment groups ($p < 0.05$). Administration of 30 mg/kg morphine to the morphine-treatment group did not produce a significant increase in turning relative to saline. Morphine (10 mg/kg) produced the greatest increase in turning for the morphine-treatment group, and this was significantly greater than the effects of this same dose on the saline treatment group ($p < 0.05$). The peak amount of turning produced by 3.0 mg/kg morphine in the saline group was significantly lower than the peak amount of turning produced by 10 mg/kg of morphine in the morphine group.

When the two groups were compared by 15-min intervals over the course of the 4.0-h session following the 10 mg/kg dose of morphine (Fig. 2B), there were significant main effects of both time within the session, $F(15, 180) = 2.58$, $p = 0.02$, and treatment group, $F(1, 12) = 8.92$, $p = 0.01$, and a significant interaction, $F(15, 180) = 1.94$, $p = 0.02$, between time and group. For example, by 45 min the morphine group showed significantly more turning than the saline group, and this difference lasted until the final 15 min of the test session when the saline group began to increase turning (Fig. 2B).

Cocaine and Amphetamine Dose–Response Curves

Cocaine dose dependently increased ipsilateral turning in both groups (Fig. 3A). ANOVA indicated significant dose-dependent effects of cocaine, $F(4, 48) = 31.3$, $p < 0.05$, and a significant interaction between group and dose, $F(4, 48) = 2.91$, $p < 0.05$, but no significant effect of treatment group, $F(1, 12) = 3.56$, p = NS. The highest dose of cocaine tested (30 mg/kg) produced significantly more turning in both treatment groups relative to saline. In addition, this dose produced more turning in the saline group compared to the morphine group ($p < 0.05$).

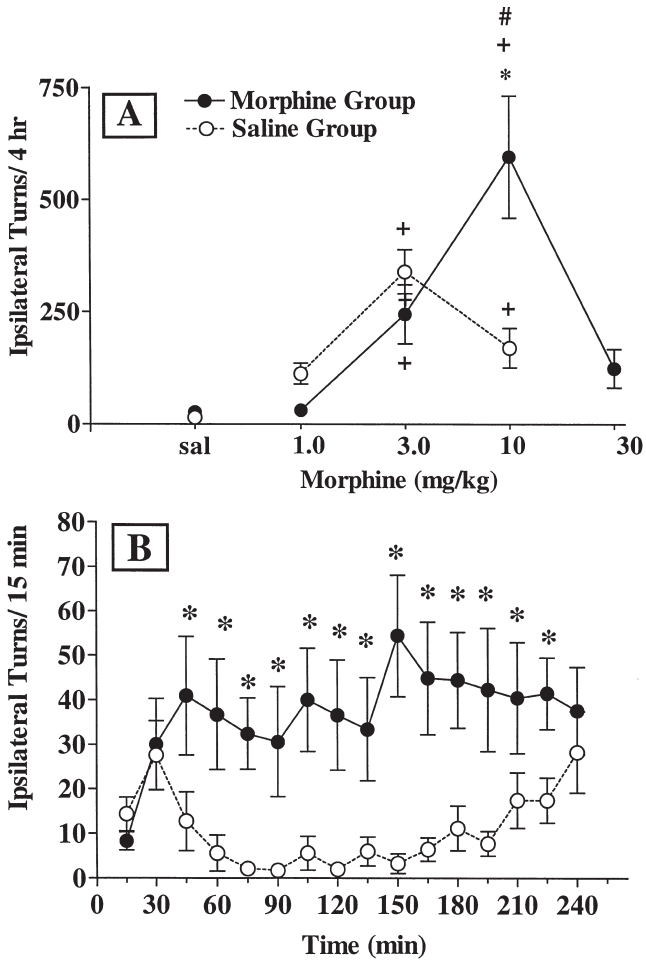

FIG. 2. (A) The number of ipsilateral turns in the saline or morphine groups by produced by saline or morphine (1.0–30 mg/kg). The 10 mg/kg doses are reproduced from Fig. 1. Morphine dose dependently increased turning in saline and morphine treated groups, but did so to a greater extent in the latter group. (B) The effects the 10 mg/kg dose of morphine at 15-min intervals within the 4-h session in both the saline and morphine groups. By 45 min, the morphine group showed a significantly greater amount of turning than the saline group until the last 15-min interval. *Indicates a difference between groups ($p < 0.05$); +indicates a difference from saline within groups ($p < 0.05$); #indicates a difference between the morphine group (10 mg/kg) and the saline group (3.0 mg/kg).

Amphetamine (0.1 to 3.0 mg/kg) produced significant dose-related increases in ipsilateral turning in both groups (Fig. 3B), $F(4, 48) = 15.2$, $p < 0.05$. However, there were no significant differences between groups, $F(1, 12) = 1.20$, p = NS, and no interaction between the factors, $F(4, 48) = 0.45$, p = NS. The 1.0 and 3.0 mg/kg doses produced significantly more turning in the saline group, whereas, relative to saline administration, only the 3.0-mg/kg dose produced more turning in the morphine group.

DISCUSSION

In rats with unilateral lesions of the substantia nigra, daily administration of morphine (10 mg/kg) over 71 days resulted

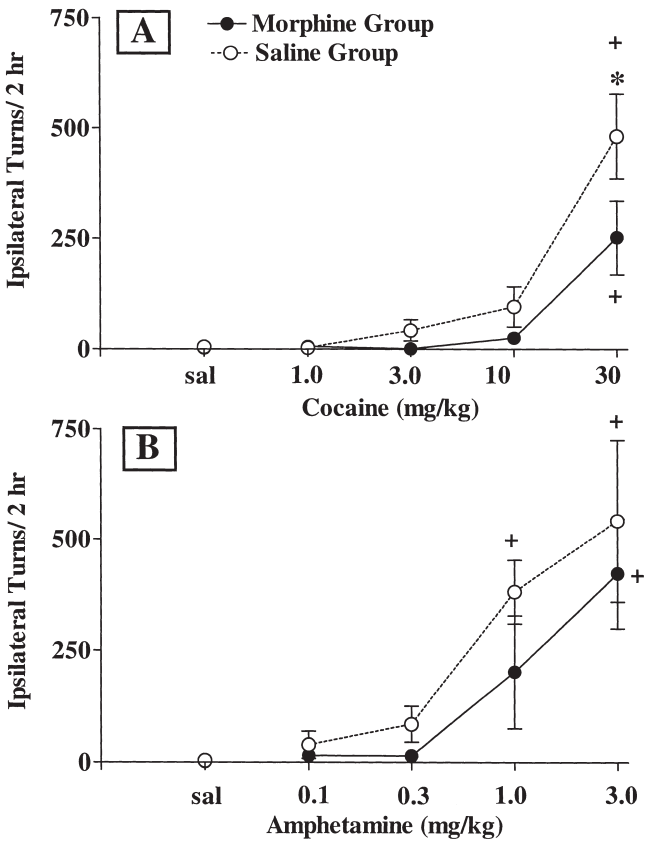

FIG. 3. The number of ipsilateral turns in both groups produced by saline, cocaine (1.0–30 mg/kg; A), or amphetamine (0.1–3.0 mg/kg; B). Curves were determined on days 17–29, after the morphine dose–response curves had been determined. Each point represents the mean (and SEM) of observations over a 2-h session. Cocaine and amphetamine produced dose-dependent increases in ipsilateral turning in both groups. *Indicates a difference between groups ($p < 0.05$); +indicates a difference from saline within groups ($p < 0.05$).

in progressive increases in ipsilateral turning. An initial period of sensitization occurred over the first 7–14 days of injection and daily testing, peaking at approximately 250 turns. When morphine dose–response curves were then (days 17–29) compared between the two groups, the peak amount of turning produced by 10 mg/kg morphine in the morphine group was 200% (equal or greater than 600 turns) greater than the peak turning in the saline group (produced by 3.0 mg/kg). By the end of the experiment (71 days), morphine produced up to 750 turns in the morphine group in a 4.0-h period.

In the present study, neither the saline group nor the morphine group turned significantly (<100 turns, day 1) when challenged with morphine (10 mg/kg) acutely. Following the initial morphine challenge (day 2), the saline group did not show any increases in turning during the 12-day period in which they received daily saline injections. Therefore, the morphine-induced increases in turning produced in this group on later test dates (i.e., dose–response curve), while they might have been induced by the initial (day 2) morphine challenge, were not reflected in any general increases in the behavioral baseline (saline only, days 3–14). The morphine dose–response curve determined in the saline group, days 17–29, did indicate that a significant degree of sensitization had occurred by this point. Clearly, this might be due to prior exposure to both the initial morphine challenge (day 2, 10 mg/kg) and any morphine exposure that occurred during the dose–response determination (1.0 or 3.0 mg/kg, randomized). Preexposure to apomorphine or the lesion may have affected the development of sensitization. For example, prexposure to apomorphine sensitizes rats to the effects of SKF 38393, a D_1 agonist, on contralateral turning (27). However, the saline and the morphine groups showed low levels of turning in response to the first dose of morphine, indicating that apomorphine did not sensitize the animals to the effects of morphine. In addition, the saline group consistently showed significantly lower levels of turning than the morphine group. Therefore, sensitization was produced by repeated exposure to morphine and not by prior exposure to apomorphine or other neuroadaptive changes that might occurred over time in lesioned rats.

The minimum morphine exposure a saline group rat may have received before being tested with 10 mg/kg in the dose–response curve would have been 10 mg/kg, received as one injection (on day 2); the maximum possible exposure would have been 14 mg/kg in three separate injections. In contrast, the rats in the morphine group had received twice this amount of morphine (30 mg/kg) by day 3 of the experiment, and yet did not show sensitization. Therefore, it may safely be concluded that sensitization is not simply a direct function of the amount of morphine received. Morphine-induced increases in turning may be dependent on treatment regimen. Continuous morphine administration produced a slight increase in turning (19), while intermittent daily injections of morphine (37) or cocaine (17) produce more sensitization than chronic administration of either drug.

It could be argued that the larger degree of morphine-induced sensitization seen in the morphine-treated group was due to the development of tolerance to the depressant effects of morphine. Indeed, the morphine dose–response curve (days 17–29) shifted to the right in the morphine group such that the10-mg/kg dose of morphine produced peak turning, whereas a lower dose (3.0 mg/kg) produced peak activity in the saline group. When morphine (10 mg/kg)-induced turning was compared between these two groups within the 4.0 h session, the saline group showed increased turning at the beginning and end of the session and decreased activity in the interim (60 to 180 min). In contrast, the activity of the morphine group increased over the first 60 min, and remained elevated throughout the rest of the 4.0-h session. Certainly, the lack of a depressant phase in the morphine group indicates that tolerance cannot be ruled out as a mechanism to account for the apparent behavioral sensitization seen in this group. However, the amount of turning seen in the morphine group in response to 10 mg/kg of morphine, was 200% greater than the peak turning produced by any dose of morphine given to the saline group during the determination of the dose–response curve. These results strongly suggest that some mechanism (i.e., sensitization) other than tolerance development is involved in producing the increased turning. The 10 mg/kg dose of morphine may also be a threshold dose for the development of sensitization while lower doses, such as 3.0 mg/kg would not produce a progressive increase in turning. For example, 10 but not 3.0 mg/kg of morphine potentiated an increase in extracellular striatal levels of dopamine produced by amphetamine (21). Therefore, the development of sensitization may be limited to higher doses of morphine.

The intervening exposure to psychomotor stimulants may have produced or enhanced the significant increase in turning

seen in the morphine group between the dose–response curve and the end of the study (day 71, Fig. 1). However, on the last day of the study (day 71) the saline/control group did not show an increase in morphine-induced turning compared to the dose–response determination (days 17–29). Therefore, exposure to amphetamine and cocaine after the morphine dose–response determination did not affect morphine-induced turning in the saline group. By extension, significant increases in turning in the morphine group, from the dose–response determination to day 71, must have been due to the continued exposure to morphine and not to the tests of the psychomotor stimulants.

The morphine-treated group showed a trend toward decreased turning relative to the saline-treated group when tested with cocaine and amphetamine. In some studies, prior exposure to morphine produced cross-sensitization to psychomotor stimulants (3,4,37,39). That did not occur in this study. However, there are several procedural differences between this study and the others. First, this study measured turning in animals with unilateral lesions of the substantia nigra and not locomotor activity in intact animals. In the other studies, morphine was administered every other day instead of every day (4,39). In addition, amphetamine was administered from 48 h (4) to 3 weeks (37) following the last morphine treatment. In all instances sensitization resulted. Therefore, there may be an effect of morphine withdrawal on cross-sensitization to psychomotor stimulants. Another procedural difference is that in the other studies morphine was administered for only 4 or 5 days prior to testing, while the animals in our study received morphine injections for approximately 32 days before receiving amphetamine or cocaine.

Environmental conditioning may also affect cross-sensitization. In a study on the effects of conditioning, one group of animals received morphine paired with the locomotor activity recording chamber and saline in the home cage ("conditioned group"), another group received morphine paired with the home cage and saline in the activity chamber ("unpaired group"), and a control group received saline in both the home cage and activity chamber (39). When amphetamine was administered to all groups, the conditioned group showed higher locomotor activity than the control group. However, the unpaired group showed less activity than the control group in response to amphetamine. The morphine-treated group in our study is similar to the unpaired group because our animals received morphine both in the home cages and in the rotometers, so morphine was not always paired with a particular environment.

The mechanisms of the development of sensitization to morphine appear to be distinct from those responsible for the development of sensitization to psychomotor stimulants. Morphine is thought to hyperpolarize GABA containing neurons in the substantia nigra and VTA to release the tonic inhibition of the dopaminergic neurons in these regions, thereby increasing their excitability (8,9,16,22,25,41). Repeated injections of morphine may enhance the disinhibition of the dopaminergic neurons, increasing the sensitivity of the dopaminergic neurons to stimulation and, therefore, increasing ipsilateral turning. Amphetamine and cocaine, however, act on the terminals of the dopaminergic neurons to either increase the release or inhibit the reuptake of DA (26). Injections of amphetamine into the cell body regions of the VTA have been shown produce sensitization (38). However, if sensitization to morphine in the nigrostriatal pathway develops via opioid receptor-mediated inhibition of GABA-containing interneurons, amphetamine and cocaine would not necessarily show cross-sensitization.

The progressive increase in rotational behavior indicates that sensitization is mediated, at least in part, by the nigrostriatal dopamine system. However, these effects may not be produced exclusively by increased activity of the substantia nigra projections. For example, morphine-induced turning does not correlate with extracellular dopamine levels in the intact striatum (21). In addition, it was demonstrated that ipsilateral μ opioid binding sites are decreased in rats with unilateral lesions (>90%) of nigrostriatal projections, and that these binding sites are not located on dopamine containing neurons (33). Because the lesioned side shows a decrease in μ receptor binding, morphine would be predicted to produce greater opioid receptor activation on the contralateral side, thus producing ipsilateral turning. Therefore, the present increase in ipsilateral turning may be produced by morphine's direct actions in the striatum. In addition to the effects of morphine on the nigrostriatal system, the ventral tegmental area and nucleus accumbens may also mediate the development of sensitization because morphine increases synaptic levels of dopamine in the nucleus accumbens (5,6,34). However, rotational behavior in nigrally lesioned rats do not allow analysis of the role of the nucleus accumbens in sensitization.

In conclusion, daily administration of morphine produces a progressive increase in ipsilateral turning in rats with unilateral lesions of the substantia nigra. The development of sensitization may be dependent on the daily, intermittent treatment regimen. Cross-sensitization to amphetamine and cocaine was not produced, indicating that sensitization to morphine can occur independently of sensitization to psychomotor stimulants, at least in the case of unilateral rotation. These data are consistent with the results of studies that show the repeated morphine administration produces progressive increases in locomotor activity (18) and extend the generality of the findings to rotational behavior.

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