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Uncontrollable Stress Potentiates Morphine's Rewarding Properties

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WILL, M. J., L. R. WATKINS AND S. F. MAIER. *Uncontrollable stress potentiates morphine's rewarding properties.* PHARMACOL BIOCHEM BEHAV **60**(3) 655–664, 1998.—Strategies used to explore the role of stressors in drug addiction include measuring stressor's effects on drug's rewarding properties. The current investigation explored the effect of an acute stressor on morphine conditioned place preference. Twenty-four hours following either inescapable tail shock or home-cage control treatment, all subjects were conditioned with morphine (0, 1, 2, and 3 mg/kg SC) over 2 days, and later tested for conditioned place preference. Inescapably shocked subjects demonstrated a potentiated place preference compared to controls. The inescapable shock-induced potentiated place preference developed even when conditioning was delayed until 6 and 7 days following the stressor, while no longer occurring after a 14- and 15-day interval. The potentiation was not a result of reduced locomotion in the inescapably shocked subjects, as activity in inescapably shocked and home-cage control subjects was the same following "mock" saline conditioning. Furthermore, the anxiogenic methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) (0.3 mg/kg IP), which produces effects similar to those produced by inescapable shock, also potentiated morphine place preference. In addition, the potentiation in inescapably shocked subjects was dependent upon the stressor's uncontrollability, as identical escapable shock did not potentiate place preference above control subjects. Finally, the inescapable shock-induced potentiated place preference was drug specific, as amphetamine place preference was not affected. © 1998 Elsevier Science Inc.

Conditioned place preference Uncontrollable stress Reward Morphine Amphetamine DMCM Activity Learned helplessness Rat

THERE has been considerable interest in potential interactions between exposure to stressors and addiction to drugs. This has led to a variety of different research strategies including the study of the impact of stressor's on the rewarding properties of drugs. In specific regard to opiates, the majority of this work has assessed the effects of repeated daily intermittent stressors on opiate self-administration (55,57,59,60), locomotor activity in response to opiates (13,32,58), or opiate discrimination task performance (30,61). Other approaches have used chronic rather than repeated intermittent stressors and have examined opiate self-administration (2,6,24), locomotor activity (14,15,41), discrimination measures (71), or conditioned place preference (53,75).

It has frequently been shown that stressor exposure increases opiate self-administration and opiate-activated behaviors. However, these potentiating effects have developed gradually over days of the repeated or chronic stressor, and have been argued to be mediated by associative processes (56). This has been most systematically studied within the self-administration paradigm. Here, associative mediation of the potentiating effect of stress has been posited, because increased opiate self-administration depends on the occurrence of the stressor immediately preceding and signaling the self-administration session (56). Potentiated self-administration did not occur if the stressor was given in such a way that it did not reliably signal the drug session. This dependence on an association or signaling relation between the stressor and the opiate self-administration may be critical in producing repeated stressor-enhanced motor responding to morphine as well. Thus, the chronic or repeated stress situation may not be ideal for the analysis of mechanisms by which a stressor per se might modulate opiate reward. Acute stressors have not been studied for their potential impact on the rewarding properties of opiates in this regard.

An investigation of the effects of acute stressors on the rewarding properties of opiates requires choices of a stressor and a behavioral measure of reward. The impact of acute stressors on some of morphine's properties other than reward

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have been studied. For example, a number of acute stressors have been shown to potentiate the analgesic effects of morphine (7,23,27) and other opiates (8). Of the various stressors examined, a single session of 80–100 inescapable tailshocks (IS) delivered to restrained subjects has had by far the largest potentiating effect on morphine analgesia (67), and so was chosen for the present studies. Throughout this article the combination of IS and restraint will be referred to simply as IS.

In selecting a measure of reward, we considered the two measures most commonly used—self-administration and conditioned place preference. The self-administration paradigm is not well suited to a study of the effects of a single session of IS on opiate reward. The potentiating effects of IS on morphine analgesia persist for only 48–72 h following IS (67). An examination of the effects of IS on morphine reward, using acquisition of self-administration, would necessarily extend beyond this 48–72-h window. It would be possible to determine whether IS would shift established self-administration response rates, but exposure to IS also reduces motor activity for a 48–72-h period (16,28), thereby confounding any interpretation.

The place preference conditioning paradigm, however, does not have these difficulties. In this procedure, subjects are exposed to morphine in one environment and vehicle in another. They are later given a choice period in which they are free to choose between the two environments, and the relative time spent in the environments has been argued to reflect the relative rewarding (or aversive) properties of the drug (44,51). Because morphine place preference can be established with only a single pairing of morphine with an environment, the establishment of reward properties can be accomplished within 48–72 h following IS (5,44). In addition, motor activity is not necessary for conditioning to occur, because the subjects are placed in the environments during the conditioning phase. The testing for place preference can be conducted beyond the period during which IS reduces activity, and in any event, relative time spent in the two environments on the test day is not a direct function of the absolute amount of movement.

GENERAL METHOD

Subjects

Adult male Sprague–Dawley rats (Harlan–Sprague–Dawley, Inc., Indianapolis, IN) weighing 300–400 g, were housed in groups of two in Plexiglas cages in a climate-controlled colony room of 22°C. The subjects were maintained on a 12 L:12 D cycle, and all experiments were conducted during the light phase. They had free access to food and water prior to and throughout the experiment. All subjects were naive and allowed a minimum of 1 week adaptation followed by 2 days of handling before the beginning of all experiments. Experimental and control groups contained seven to nine subjects. All experimental procedures were in accord with protocols approved by the University of Colorado Institutional Animal Care and Use Committee.

Apparatus and Materials

The Plexiglas place preference apparatus measured 72 × 30 × 30 cm (length, width, height) and was composed of two distinct environments and a neutral zone. One environment was striped horizontally with alternating 3/4 inch black and white electrical tape on the walls, while the other environment was striped vertically in the same manner. The floor of the apparatus was black sanded Plexiglas with 1/2 inch wire grid on the floor of the horizontal side and 1/8 inch wire mesh on the vertical side. The neutral area measured 12 × 30 × 30 cm, was painted gray, and had no wire mesh or grid on the floor. During the conditioning phase, vertically and horizontally striped Plexiglas partition walls were inserted on the respective side of the neutral area to restrict the animal to their designated conditioning environment. The conditioning environments measured 30 × 30 × 30 cm. The entire apparatus was cleaned between the conditioning or testing of each subject.

The stressor environment was a dimly lit room with dimensions of approximately 3 × 2.5 × 2.5 m. Inescapable shock or restraint occurred in Plexiglas restraining tubes that were 17.5 cm in length and 7.0 cm in diameter. The rat's tail extended from the rear of the tube and could be taped at the base to a Plexiglas rod 4.0 cm in length. The front end of the tube was blocked by a Plexiglas plunger with several airholes drilled in it. Unscrambled shocks (1.0 mA) were delivered by a source modeled after the Grason-Stadler Model 700. Electrodes, coated with a small amount of electrode paste, were taped to the midsection of the tail.

The drugs used in the following experiments included morphine sulfate (Mallinkrodt; St. Louis, MO) and *d*-amphetamine (Sigma; St. Louis, MO), both dissolved in physiological saline. The benzodiazepine inverse agonist methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM) (RBI; Natick, MA) was dissolved in PBS-buffered saline (pH 3.0). Injection volume of all drugs and saline was 1.0 ml/kg body weight.

Statistical Analyses

Data were analyzed by repeated measures analysis of variance (ANOVA) followed by post hoc Newman–Keuls (alpha set at 0.05), which make all possible pair-wise comparisons, or orthogonal contrasts. The dependent variable in all experiments was expressed as the difference in time spent on the drug-paired side between the preexposure session and the test of conditioned preference session. This method of data presentation is often used in conditioned place preference paradigms (4,10,31).

EXPERIMENT 1: IS EFFECTS ON MORPHINE-CONDITIONED PLACE PREFERENCE

The purpose of the first experiment was to measure the effects of an acute stressor on morphine's rewarding properties, using a paradigm in which the stressor precedes the morphine administration by a substantial period of time. Rats were first given a 20-min preexposure to the test apparatus, and time spent in each compartment was measured. Twenty-four hours later they were given either a session of inescapable tailshocks identical to those used in previous experiments (23,27,67), or home-cage control treatment. The conditioning sessions were conducted over the following 2 days, with two pairings of both saline and drug to their respective environments. On the day following the last conditioning session, the rats were again exposed to the test apparatus in a drug-free state and time spent in each environment was measured.

METHOD

Procedure

On day 1, with the partitions removed, the animal was placed in the neutral area and allowed to explore the entire apparatus for 20 min. The session was video recorded, and the amount of time the rat spent in each environment was mea-

sured, using the placement of their front paws as the determining factor. This day served to assess the animals initial preferences and any possible box bias. A criteria was set to eliminate any rat that spent less than 4 min (20% of total time) in either side. On day 2, half of the rats received 100 inescapable tail shocks in Plexiglas restrainer tubes (5 s, 1 min ITI, 1 mA) in a different room, while the other half remained in their home cages. On day 3, the animals were weighed and given random counterbalanced assignment so that half were conditioned with morphine to the vertical-striped side and half to the horizontal-striped side. Morning conditioning occurred between the hours of 0900 and 1100, while afternoon conditioning occurred 4 h later. Half the animals received morphine conditioning in the morning and half in the afternoon. Animals were first injected and then 5 min later placed into the conditioning environment for 45 min. Separate groups were conditioned with either 0, 1, 2, or 3 mg/kg SC morphine, while all subjects received 1 mg/kg SC saline vehicle in the other context at the other time of day. On day 4, animals again were conditioned while counterbalancing the order of presentation. This counterbalancing was conducted to control for conditioned associations that could possibly occur between the subject's drug experience and the time of day. On day 5, testing of conditioned preference was conducted between the hours of 1100 and 1300. No injections were made. Subjects were simply placed in the preference apparatus for 20 min and time spent in each compartment measured.

RESULTS

The results are shown in Fig. 1. Morphine resulted in a dose-dependent place preference, and this conditioned preference was potentiated by prior IS. That is, the subjects spent more time in the drug-paired compartment after conditioning than prior to conditioning, and this increase was greater for the IS-treated subjects. A 4×2 ANOVA yielded reliable effects of dose, $F(3,52) = 14.06$, $p < 0.001$, and IS, $F(1,52) = 9.81$, $p < 0.01$, while the interaction between dose and IS was not reliable, $F(3,52) = 1.84$, $p = 0.15$. Post hoc orthogonal contrasts revealed significantly higher conditioned place preference in the IS-treated subjects at the 2 and 3 mg/kg doses of morphine, compared to controls.

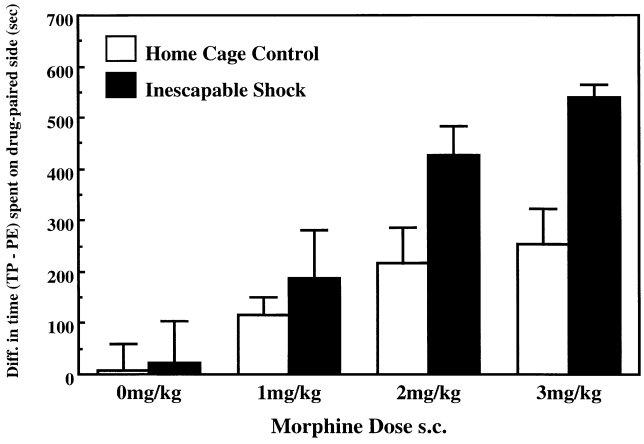

FIG. 1. Effects of IS on morphine (0, 1, 2, and 3 mg/kg SC)-induced conditioned place preference. Data are expressed as the mean (+SEM) of the difference in s spent on the drug-paired side between preexposure (PE) and test of conditioned preference (TP) sessions.

EXPERIMENT 2: TIME COURSE OF IS EFFECTS ON MORPHINE-CONDITIONED PLACE PREFERENCE

Experiment 1 demonstrated that IS potently increased the rewarding properties of morphine across a range of doses. Experiment 2 investigated the time course of this effect. Morphine conditioning was carried out either 1 and 2, 4 and 5, 6 and 7, or 14 and 15 days postshock. Investigation of the time course of morphine-conditioned place preference potentiation will allow comparison with other IS effects such as potentiation of morphine analgesia. In addition, if the effect is present at any time point after the 1 and 2 days postshock period used in Experiment 1, this would strengthen the argument that a signaling relationship between the stressor and the drug is not responsible for the potentiation of conditioned place preference.

METHOD

Procedure

Day 1 and 2 procedures were identical to the procedures used in Experiment 1. However, the next 2 days of conditioning occurred either 1 and 2, 4 and 5, 6 and 7, or 14 and 15 days postshock, with a 45-min pairing of 3 mg/kg SC morphine in one context, and 4 h later with a 45-min pairing of 1 ml/kg SC saline vehicle in the other context. On the second day, the order of presentation was reversed. Conditioning assignment was counterbalanced randomly. Testing of preference was conducted over a 20-min period approximately 24 h following the last conditioning pairing. Difference in time spent in compartments before and after conditioning with drugs was determined. Because initial compartment preference testing preceded IS, the various IS groups differed in the initial preference to final preference testing time interval, as well as in the IS to time of conditioning interval. Although there is no reason to believe that these intervals affect place preference conditioning, use of a single home-cage control was deemed to be inadequate. Two control groups were employed. One matched the shortest, and one matched the longest initial preference to final preference testing intervals. These controls did not differ.

RESULTS

The results of the time course experiment are shown in Fig. 2. The results show a clear IS-induced potentiation at the 1- and 2-day interval, with the IS-treated subjects reaching control levels 14 and 15 days after IS. A 2×2 ANOVA was first conducted comparing conditioned place preferences on days 1 and 2 with days 14 and 15. This was done because both IS and HCC groups were only used at these time points. The effect of time interval, $F(1, 27) = 7.0$, $p < 0.05$, IS, $F(1, 27) = 12.71$, $p < 0.01$, and the interaction between time interval and IS, $F(1, 27) = 6.26$, $p < 0.05$, were all significant. Orthogonal contrasts indicated that the IS group conditioned at the 1- and 2-day interval showed significantly greater conditioned place preference than did both control groups, which did not differ among themselves. Also, the IS group at the 14 and 15 day interval did not differ significantly from either control group. A one-way ANOVA was then conducted for the four time intervals with IS-treated subjects. A significant effect of time interval was observed, $F(3, 41) = 3.1$, $p < 0.05$.

EXPERIMENT 3: IS EFFECTS ON ACTIVITY LEVELS DURING TESTING PERIOD

Exposure to IS is known to reduce subsequent motor activity for some period of time (16,28). It is possible that this ef-

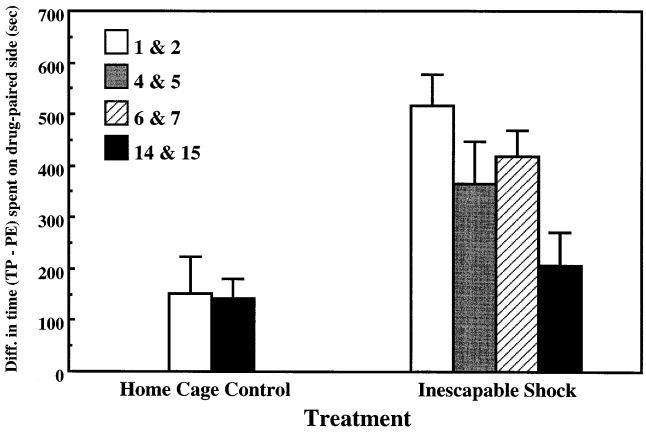

FIG. 2. Time course effect of conditioning interval (1 and 2; 4 and 5; 6 and 7; 14 and 15 days after IS) on the IS potentiation of morphine (3 mg/kg SC)-induced conditioned place preference. Data are expressed as the mean (+SEM) of the difference in s spent on the drug-paired side between preexposure (PE) and test of conditioned preference (TP) sessions.

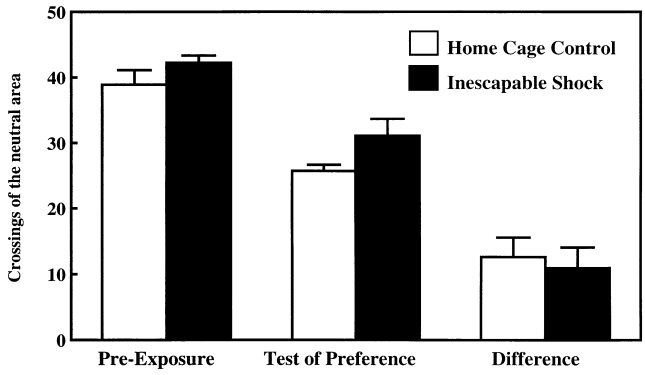

FIG. 3. Effect of IS and HCC treatment on crossings of the neutral area in subjects given "mock" saline (SC) conditioning. Data are expressed as the mean (+SEM) of the neutral area crossings during preexposure and test of conditioned preference sessions.

fect would manifest itself in reduced crossings of the preference test apparatus, possibly resulting in only an "apparent" enhancement of morphine place preference. Perhaps the IS subject remains in the morphine-paired environment because it simply has a reduced tendency to move between compartments. The finding that IS potentiates morphine place preference even 7 days after IS mitigates against this argument. This is because the activity reduction following IS persists for only 48–72 h (16,28). However, the testing conditions of the activity experiments were quite different from the conditions used for testing in the present experiments, and persistent changes in activity following a stressor have been reported (73). Thus, it is possible that IS rats might simply show an unconditioned reduction in crossings between choice boxes in the present paradigm. Experiment 3 was designed to directly assess this possibility.

METHOD

Procedure

Day 1 was identical to the procedure described in Experiment 1; however, the number of neutral area crossings were measured. On day 2, the stressor procedure was conducted in the same manner as in Experiment 1. On days 3 and 4, saline control mock "conditioning" was administered following the same methods as in the original morphine conditioning in Experiment 1, occurring 24 and 48 h after IS or HCC, with a 45-min pairing of 1 ml/kg SC saline in each context, separated by 4 h. Testing of locomotion was conducted over a 20-min period approximately 24 h following the last "conditioning" pairing. Differences in crossings before and after stress treatment were determined.

RESULTS

Activity changes in HCC- and IS-treated subjects are shown in Fig. 3. A significant reduction of crossings between PE and TP trials was observed for both groups, $F(1, 14) = 28.76$, $p < 0.001$. However, IS had no significant effect on the magnitude of this reduction, $F(1, 14)$ $p > 1.0$. Newman–Keuls post hoc analysis further confirmed that no significant activity differences existed as a function of stressor treatment.

EXPERIMENT 4: DMCM + RESTRAINT EFFECTS ON MORPHINE-CONDITIONED PLACE PREFERENCE

It is possible that IS does not increase the rewarding properties of morphine per se, but rather produces difficult to detect physical injury and persistent pain. IS does stimulate intense motor activity while the subject is confined to the restraint chamber, and injury or inflammation is certainly a potential outcome. Morphine might then produce increased place preference conditioning in IS subjects because it reduces this persistent pain while the subject is in the morphine-paired environment, in addition to merely exerting its normal rewarding effects. This potential confounding factor can be assessed by employing a "stressor" that mimics the behavioral and neurochemical effects of IS, but that does not elicit bursts of physical activity. The administration of inverse benzodiazepine agonists such as FG-7142 and DMCM produce classic indices of stress such as hypothalamo–pituitary–adrenal and sympathetic activity (46,64). In addition, these agents produce behavioral sequelae similar to those produced by IS, such as potentiated fear conditioning (38), poor escape learning (17, 63), and exaggerated morphine analgesia (27,67). It should be noted that benzodiazepine receptor inverse agonists do not elicit increased activity. Instead, they produce freezing (19). Experiment 4 sought to determine whether a single injection of DMCM would potentiate morphine-conditioned place preference conducted 1 and 2 days later, just as it produces other typical behavioral sequelae of IS at this time point.

METHOD

Procedure

Day 1 was identical to the procedure described in Experiment 1. On day 2, the inverse benzodiazepine receptor agonist DMCM (0.3 mg/kg IP) or vehicle was administered to separate groups immediately prior to placing subjects in restrainer tubes for a period equal to the shock session. Restraint was used following the administration of DMCM and vehicle to mimic the IS environment as closely as possible, as well as to control for the restraint environment by itself. Conditioning occurred 24 and 48 h after DMCM/restraint with 3 mg/kg-ml

SC morphine in one context and, 4 h later, 1 ml/kg SC saline vehicle in the other context. Conditioning assignment was counterbalanced randomly. Testing of preference shift was conducted over a 20-min period approximately 24 h following the last conditioning pairing. Difference in time spent in compartments before and after conditioning was determined.

RESULTS

Place preferences for DMCM and vehicle-treated subjects are shown in Fig. 4. The subjects treated with the inverse benzodiazepine agonist DMCM + restraint showed a significant potentiation of conditioned place preference relative to the subjects who received vehicle + restraint, $F(1, 13) = 5.51$, $p < 0.05$.

EXPERIMENT 5: STRESSOR CONTROLLABILITY EFFECTS ON MORPHINE-CONDITIONED PLACE PREFERENCE

Many of the sequelae of IS depend on the uncontrollability of the stressor and do not occur if the tail shock is escapable or controllable (17,38,63). This is true of the potentiation of morphine analgesia produced by IS (67). However, the potentiation of morphine-conditioned place preference follows a time course that is different from the effects of IS that have been shown to depend on stressor controllability (Experiment 2). Experiment 5, therefore, explored whether stressor controllability would influence subsequent morphine-conditioned place preference. Separate groups received either escapable tailshock, identical yoked inescapable tailshock, or restraint, followed by morphine conditioning and place-preference testing. A restraint group was included to allow assessment of whether restraint per se might have been responsible for the IS–HCC difference in prior experiments.

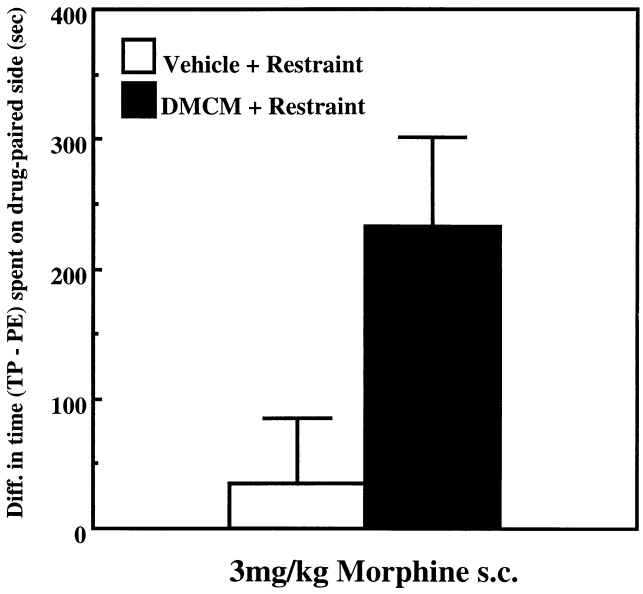

FIG. 4. Effect of DMCM + restraint and vehicle + restraint on morphine (3 mg/kg SC)-induced conditioned place preference. Data are expressed as the mean (+SEM) of the difference in s spent on the drug-paired side between preexposure (PE) and test of conditioned preference (TP) sessions.

METHOD

Apparatus

Escapable and yoked inescapable shocks were administered in small Plexiglas wheel-turn boxes. The entire box was made of clear Plexiglas. A small wheel extended 1.7 cm into the front of the chamber through a hole 8.0 cm from the floor of the box. The wheel required a force of about 0.50 N to turn. The tail of the rat was extended through a slot in the rear wall of the chamber and was taped to a Plexiglas rod parallel to the floor of the chamber. Shock was applied through electrodes attached to the rat's tail and augmented with electrode paste. The shock sources were modeled after Grason-Stadler Model 700.

Procedure

Day 1 was identical to the procedure described in Experiment 1. On day 2, rats either received 100 escapable tail shocks, 100 identical yoked inescapable tail shocks, or restraint in wheel-turn boxes for an equivalent period of time. In the escape condition, rats received 100 trials of an unsignaled 1.0 mA shock on a variable interval 60-s schedule (range: 30–90 s). The initial 0.8 s of the shock was not under the subject's control. The shock following this period could be terminated by the appropriate wheel-turn response. The initial response requirement was a 90° turn of the wheel, the basic unit of response that was measured, and the subsequent requirements were dependent upon the prior response latencies. Three responses under 5.0 s increased the requirement by one unit for the next trial. If that trial had a response latency under 5.0 s, the requirement was increased two units, and every subsequent trial response under 5.0 s resulted in a doubling of the previous unit requirement. The maximum response requirement was 16 units, or four full rotations of the wheel.

Any interruption of the increment sequence by response latencies over 5.0 s caused the sequence to restart with a requirement of three consecutive rapid-response trials. Response latencies of 10–29 s decremented the response requirement for the next trial by one unit; failure to complete a response, or a response latency of 30 s, the maximum shock duration allowed, reset the response requirement to one response unit. Response latency was measured from shock onset to the completion of the response requirement. ITI was measured from response completion to shock onset. The above procedure was used because it produces shock durations similar to those in Experiment 1 (i.e., 5 s shocks).

In the inescapable condition, each rat was paired with an escape rat. Each shock began for an inescapable subject at the same time as for the escape partner and was terminated whenever the escape subject performed the criterion escape response. Therefore, within each escape/yoked pair, both rats received the identical number, pattern, intensity, and duration of shocks. The responses made by the yoked animal had no effect on the shock's termination or onset. The restrained condition simply involved securing the rat in an identical Plexiglas box; however, no shock was delivered.

Conditioning occurred 24 and 48 h following the treatments described above, with a 45-min pairing of 3 mg/kg SC morphine in one context, and 4 h later with a 45-min pairing of 1 ml/kg SC saline vehicle in the other context. On the second day, the order of presentation was reversed. Conditioning assignment was counterbalanced randomly. Testing of preference shift was conducted over a 20-min period approximately 24 h following the last conditioning pairing. Difference in time

spent in compartments before and after conditioning was determined.

RESULTS

The effects of stressor controllability on morphine-conditioned place preference are depicted in Fig. 5. Yoked IS potentiated morphine conditioned place preference relative to restraint, while ES did not. ANOVA indicated a reliable effect of type of stressor, $F(2,24) = 8.19$, $p < 0.01$. Further Newman–Keuls post hoc comparisons also indicated that morphine place preference was greater in the IS than in the ES or restraint group, which did not differ among themselves. Furthermore, the magnitude of the conditioned place preference in the restrained subjects was very similar to that observed in HCC subjects in Experiments 1 and 2.

EXPERIMENT 6: IS EFFECTS ON AMPHETAMINE CONDITIONED PLACE PREFERENCE

The experiments described above indicate that IS potentiates morphine conditioned place preference conditioning. Whether IS would also augment conditioned place preferences produced by other rewarding drugs is unknown. Dopamine (DA) agonists such as amphetamine also produce conditioned place preference, but interestingly, IS might be expected to have a different effect on amphetamine-conditioned place preference. DA release in the nucleus accumbens has often been implicated as a mediator of the rewarding properties of drugs (20,74), and both morphine and amphetamine increase DA release in the nucleus accumbens (11). It is, thus, of interest that 5-HT_3 antagonists decrease the ability of morphine to produce DA release in the nucleus accumbens, but do not alter DA release following amphetamine (9). These findings are noted because IS produces alterations in 5-HT activity (18), and dorsal raphe nucleus serotonin has been shown to be a critical determinant of the IS-induced potentiation of morphine analgesia (66). It is, therefore, possible that the potentiation of morphine-conditioned place preference by IS also involves 5-HT, and so an augmentation of amphetamine conditioned place preference might not be expected.

METHOD

Procedure

Day 1 was identical to the procedure described in Experiment 1. On day 2, rats were given the same stressor treatment as in Experiment 1 or remained in their home cages. The first of four conditioning sessions began 5 h following the end of the stressor session. The subsequent conditioning sessions were held at the same time of the day separated by approximately 24 h, instead of 4 h as in prior experiments, due to amphetamine having a longer half-life than morphine. Assignment was counterbalanced, and on conditioning sessions 1 and 3 separate groups were administered either 0, 1, 2, or 3 mg/kg *d*-amphetamine SC 5 min before being placed into their conditioned environment for 30 min. On conditioning days 2 and 4, rats were administered 1 ml/kg SC saline vehicle in the opposite context from that in which they had received amphetamine. Twenty-four hours following the last conditioning session, rats were again placed into the testing apparatus to measure the conditioned preference.

RESULTS

Figure 6 shows amphetamine place preference for the various groups. Amphetamine-conditioned place preference showed an orderly increase with amphetamine dose in HCC subjects. This did not occur in IS subjects, and there was no clear amphetamine-conditioned place preference in these subjects at any of the doses. IS did not potentiate amphetamine-conditioned place preference and may have even interfered with amphetamine-conditioned place preference at the 3 mg/kg dose. A 4 × 2 ANOVA yielded no effect of treatment, $F(1, 60) = 0.033$, $p > 0.05$, dose, $F(3, 60) = 1.75$, $p > 0.05$, or interaction between dose and treatment, $F(1, 60) = 1.41$, $p > 0.05$. A subsequent one-way ANOVA revealed significant effects

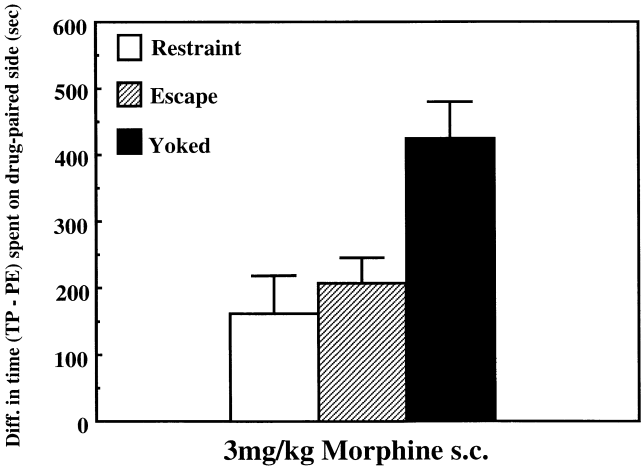

FIG. 5. Effect of ES, IS, and restraint on morphine (3 mg/kg SC)-induced conditioned place preference. Data are expressed as the mean (+SEM) of the difference in seconds spent on the drug-paired side between preexposure (PE) and test of conditioned preference (TP) sessions.

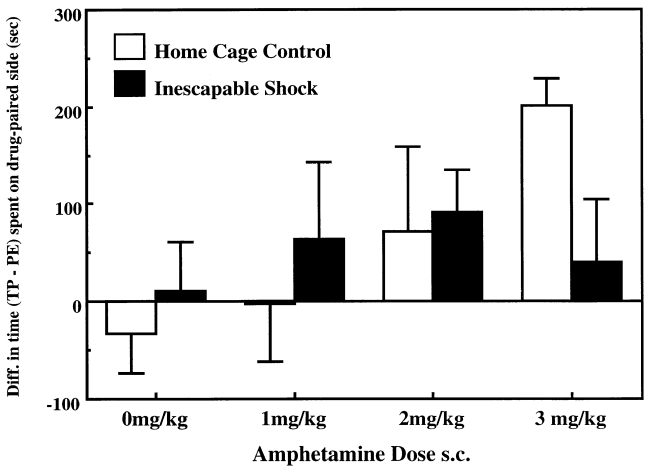

FIG. 6. Effect of IS on *d*-amphetamine (0, 1, 2, and 3 mg/kg SC)-induced conditioned place preference. Data are expressed as the mean (+SEM) of the difference in seconds spent on the drug-paired side between preexposure (PE) and test of conditioned preference (TP) sessions.

of dose, $F(3, 32) = 3.11$, $p < 0.05$, in HCC-treated subjects. Orthogonal contrast post hoc comparisons revealed a significantly larger conditioned place preference in HCC subjects conditioned with 3 mg/kg than those conditioned with 0 mg/kg morphine.

GENERAL DISCUSSION

The results presented here clearly indicate that IS potentiates morphine's rewarding properties, as measured by conditioned place preference. A significant potentiation of conditioned place preference in the IS-treated animals relative to control levels was observed at two of the three doses tested, while showing a tendency in this direction at the third and lowest dose. The IS-potentiated place preference developed even when conditioning was delayed until 6 and 7 days following the stressor. Potentiation no longer occurred when conditioning was delayed 14 and 15 days after the stressor. In addition, the potentiation of conditioned place preference was dependent upon the stressor's uncontrollability. Although the duration and intensity of the shocks were identical in both the IS- and the ES-treated animals, the two treatment groups demonstrated significantly different preference measures. IS-potentiated conditioned place preference relative to both restrained and HCC levels, while ES produced no change from control levels. This finding, along with the finding that the anxiogenic drug DMCM potentiates conditioned place preference, parallels the characteristics of more extensively explored learned-helplessness behaviors. Finally, the potentiation of conditioned place preference induced by IS may be particular to morphine's pharmacological properties. IS failed to significantly alter amphetamine place preference from control levels, while possibly disrupting a place preference at the highest amphetamine dose tested. The fact that a potentiation was not observed with the stimulant amphetamine might suggest that the state induced by IS selectively interacts with opiates.

The role of restraint deserves comment. IS was delivered to restrained subjects, and so the restraint might have exerted an independent effect on morphine-conditioned place preference. Several points can be made. First, ES was also delivered to restrained subjects, yet ES did not have a detectable effect on conditioned place preference. Second, across experiment comparisons between home-cage controls and the restrained controls in Figs. 4 and 5, there is no suggestion that restraint had an effect in the present paradigm. Finally, we have directly compared home-cage controls and restrained subjects using conditioning with 3 mg/kg morphine. The conditioned place preference mean scores for home cage and restrained subjects were [166 ± 69] and [180 ± 71], respectively.

The finding that IS potentiates morphine-conditioned place preference confirms other recent evidence suggesting a positive relationship between exposure to stressors and morphine's rewarding properties (55,57,59–61). However, previous investigations of a stressor's influence on the rewarding properties of morphine have been confounded by the involvement of associations forming between the stressor and the drug administration sessions. Indeed, it was demonstrated that the stressor became a signal for the upcoming drug administration session, and increased self-administration was dependent upon this signaling relationship (56). However, it is very unlikely that the current findings are a result of associations or a signaling relationship formed between the stressor and the drug. First, the stressor was administered only once, not repeatedly over several or many days. Furthermore, the stressor occurred on a separate day and in a completely different environment from that in which the subject was administered the drug. Finally, the potentiated place preference was observed even when conditioning was conducted as much as 6 and 7 days after the IS stressor.

Several alternatives to the possibility that the potentiated conditioned place preference reflects an augmentation of morphine's rewarding properties were investigated. It has been reported that IS identical to that used here will reduce activity in response to foot shock for a period up to 72 h (16,28). It was, therefore, conceivable that IS subjects might cross the preference test apparatus at a reduced rate, possibly resulting in only an "apparent" enhancement of morphine place preference. For instance, a subject maintaining lower activity might have remained in the morphine-paired environment because it simply had a reduced tendency to move between compartments. However, when activity changes were monitored between the preexposure trial and the test of preference trial in both IS and HCC saline-conditioned animals, crossing rates were unaffected by IS.

Another alternative involved the possibility that the potentiated conditioned place preference observed in the IS rats was due to morphine's analgesic properties. Because IS elicits intense struggling (16), it is possible that IS could lead to inflammation or injury that could persist into the period of testing. This issue was approached based on the findings that other behavioral effects that are produced by IS, but not ES, can be mimicked by the administration of the benzodiazepine inverse agonist DMCM (17,38,66). DMCM was administered prior to placing the rats in restrainer tubes for a period equal to that of an IS session. A potentiation of conditioned place preference was observed in DMCM-treated animals above the conditioned place preference produced in rats that were injected with vehicle, even though DMCM does not produce struggling. In addition, it should be noted that administration of the inflammatory agents Freund's adjuvant, formalin, and carrageenan into the paw, have no effect on, or actually attentuate, conditioned place preference produced by morphine (65,68).

Another alternative, not described previously, is that the IS treatment might have enhanced learning abilities above controls, allowing the IS-treated rats to form stronger associations between the drug and the environment. Although IS is most often thought to interfere with learning (12,28,35), it has been demonstrated that IS can facilitate the acquisition of conditioned responses when trained under a classical conditioning paradigm 24 h later (54,62). The potentiated morphine place preference observed after IS could, therefore, be a result of this effect. The animals treated with IS may have developed a stronger association between the drug and the environment through facilitated conditioning, rather than because the morphine was experienced as more rewarding. If this were in fact occurring, a potentiated conditioned place preference might be expected to occur following IS regardless of the rewarding drug chosen. However, when amphetamine was used as the reinforcer, IS failed to alter condtioned place preference levels.

In sum, the present experiments suggest that exposure to a single session of IS increases the rewarding properties of morphine for a period of 7–14 days. Because this effect is not likely to depend on an associative relation between IS and morphine, and because there were no obvious cues in common between the IS and the morphine place preference conditioning environments, the most likely possibility is that IS induces a change in a state of the organism that persists for 7–14 days and interacts with morphine to make the drug more reward-

ing. The experiments reported above do not indicate the nature of this state or the underlying persistent physiological alterations produced by IS. However, comparable findings in the literature to those currently reported have been further investigated, and various physiological substrates have been suggested.

The current finding that IS specifically increased morphine, but not amphetamine CPP, is rather interesting based on this literature. For example, it has been demonstrated that exposure to stress increases measures of self-administration (49,60) and locomotor activity (13) for both morphine and amphetamine. However, all of these findings have examined either effects of repeated stressors or have shown the effects to be glucocorticoid mediated. The IS-induced potentiation reported here is unlikely to be mediated by glucocorticoids. This is because with the parameters used here, exposure to ES produces the same increases in glucocorticoids as are produced by IS (34). However, the behavioral effect was only observed following IS, and not ES. This would suggest that the mechanism that here interacts selectively with morphine, but not amphetamine, is a neural system that is selectively activated by IS, but not equal and identical ES.

Indeed, biological correlates have been discovered that underlie the various behaviors that follow IS but not ES, and the IS-induced potentiation of morphine-conditioned place preference bears a resemblance to these behaviors. For example, the shuttlebox escape learning deficit and the potentiation of morphine analgesia produced by IS depend on the uncontrollability of the IS and do not follow equivalent ES (3,37), are produced in equivalent fashion by DMCM (38,66), and have a decaying, although somewhat shorter, time course (29,66). These behavioral changes have been argued to result from a state of "anxiety" induced by IS that persists for some number of days (37,63), and it can be noted that IS leads to anxious behavior as measured by the social interaction test (21) for 3–7 days (63). By anxiety in this context is meant exaggerated fear (36), and it is interesting that conditioned fear cues potentiate morphine analgesia (52) and withdrawal (1). Thus, it may be that IS produces a persistent but decaying anxiety/fear state that interacts with morphine to enhance its rewarding properties.

Alterations in 5-HT processes within the dorsal raphe nucleus (DRN) appear to be key elements in the IS-induced anxiety and other behavioral sequelae of IS such as poor escape performance and potentiated morphine analgesia. Lesion of the DRN blocks the subsequent occurrence of the anxious behavior, poor escape, and potentiated morphine analgesia (33). In addition, microinjection into the DRN of pharmacological agents that inhibit 5-HT activity also block the behavioral consequences of IS, when administered before IS or the subsequent behavioral testing (39,40,66). Furthermore, agents that increase 5-HT DRN activity, such as DMCM, induce these behavioral effects by themselves when injected into the DRN (38,66). These and other data have led to the view that IS sensitizes 5-HT neurons within the DRN so that subsequent test stimuli (e.g., foot shock in the escape task) produce an exaggerated activation of these neurons, with the release of abnormally high quantities of 5-HT in projection regions being the putative cause for the effects observed.

Importantly, systemically administered morphine produces potent activation of 5-HT neurons by inhibiting GABA neurons that tonically inhibit 5-HT neurons (69), thereby augmenting release of 5-HT in projection regions of the DRN (70). Thus, if IS does sensitize these neurons, then increased 5-HT release following morphine would be expected in regions that receive 5-HT innervation from the DRN. The mesolimbic system, consisting of the ventral tegmental area (VTA) and its projection areas of the nucleus accumbens (NAcc), amygdala, and the prefrontal cortex, receives direct neuronal input from the DRN (48,72). Moreover, it has been shown that this input onto the dopaminergic terminals, as well as their cell bodies originating in the VTA, is excitatory in nature, presumably via 5-HT_3 receptors (42,45).

5-HT_3 receptors have been suggested to have a permissive role in the reinforcing properties of morphine and nicotine, but not amphetamine, as demonstrated by place preference conditioning (10,26). In addition, 5-HT_3 antagonists have selectively reduced DA levels in the NAcc following morphine and nicotine, but not amphetamine. (9). Furthermore, although systemic morphine increases DRN 5-HT activity, systemic *d*-amphetamine does not. Indeed, a number of reports suggest that amphetamine inhibits DRN 5-HT neurons (25,47, 50). Thus, the hypothesis that IS potentiates morphine-conditioned place preference by sensitizing DRN 5-HT neurons so that they respond in an exaggerated manner to stimuli that activate these neurons, is capable of explaining the lack of potentiation of amphetamine-conditioned place preference.

A final issue needs to be noted. It is possible that the potentiated morphine-conditioned place preference is unrelated to a hyperresponsive 5-HT system, or even an increase in the rewarding properties of morphine per se. Rather, the augmented conditioned place preference might be a result of morphine reducing the putative anxiety of the IS subject. It is known that morphine can be weakly anxiolytic (22,43), and perhaps it reduced the persistent anxiety that follows IS while the IS subject was in the morphine-paired environment. Amphetamine is not anxiolytic, and so anxiety reduction would not have occurred in the amphetamine-conditioned place preference experiment. Under this scenario, IS would not have increased the rewarding properties of morphine per se, but rather would have added a second source of possible reward for these subjects—anxiety reduction. The present experiments do not allow a test of this possibility. However, under this possibility the potentiated conditioned place preference still reflects an increase in the total morphine-induced reward experienced by the IS subject, and potential implications for a role of uncontrollable aversive events in addiction are still suggested.

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