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Morphine-Induced Place Conditioning is not Confounded by Drug-Induced Alterations in Locomotor Activity

T. S. SHIPPENBERG, M. W. EMMETT-OGLESBY* AND A. HERZ

Department of Neuropharmacology, Max-Planck Institute for Psychiatry
Am Klopferspitz 18a, D-8033 Planegg-Martinsried, Federal Republic of Germany
**Department of Pharmacology, Texas College of Osteopathic Medicine*
3516 Camp Bowie Blvd., Fort Worth, TX 76107

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SHIPPENBERG, T. S., M. W. EMMETT-OGLESBY AND A. HERZ. *Morphine-induced place conditioning is not confounded by drug-induced alterations in locomotor activity.* PHARMACOL BIOCHEM BEHAV 32(1) 129–132, 1989.—The influence of locomotor activity and environmental familiarity upon the reinforcing effects of morphine was examined in an unbiased place preference conditioning procedure. Groups of rats were trained to associate one distinctive environment with morphine and another with saline. One group was made tolerant to the locomotor activity effects of morphine by the SC administration of morphine (5.0 mg/kg/12 hr) for four days prior to conditioning. The other group received injections of saline. Administration of morphine, at doses which decreased locomotor activity, resulted in marked preferences for the drug-associated place in saline-treated rats. In contrast, chronic morphine treatment resulted in tolerance to the sedative effects of morphine and an abolition of the morphine-induced place preference. These results indicate that in the place conditioning procedure, measures of reinforcement are not confounded by drug-induced increases in activity.

Morphine Place conditioning Locomotor activity Reinforcement

A prominent behavioural effect of opioids and other psychoactive drugs is their ability to function as positive reinforcers, an action which may underlie their marked potential for abuse (5,7). The reinforcing properties of these agents have been demonstrated in several animal models including those of self-administration and intracranial self-stimulation (7, 8, 19, 20). In such procedures, the capacity of a drug to directly control behaviour is assessed, and data so derived provide a measure of a drug's primary reinforcing properties.

An alternative procedure for assessing the motivational properties of drugs is that of place preference conditioning. This classical conditioning paradigm is based on the observation that animals will approach and subsequently prefer stimuli associated with positive reinforcers and avoid those that induce aversive states (3,9). Thus, in this procedure, the associations developed between the effects of a drug and a stimulus are examined and evaluation of the subject's behaviour following presentation of the drug-associated stimulus provides a measure of secondary reinforcement processes. A potential advantage of this animal model is that subjects are tested in the drug-free state, thus circumventing the influence of drug-induced alterations in locomotor activity which could confound subsequent data interpretation. To date, this model has been used to evaluate the motivational effects of a variety of psychoactive drugs (10, 13, 15, 16).

Recent data, however, suggest (17,18) that place conditioning may result from processes that are independent of a drug's motivational properties and may, in fact, be confounded by drug-induced alterations in locomotor activity. Specifically, it has been hypothesized that due to neophobia, animals will acquired a preference for any environment in which they have previously experienced high levels of activity and is hence, more familiar. As a result, place preferences may not reflect the reinforcing properties of a drug, but rather its ability to increase locomotor activity. If, however, this hypothesis is valid, then all drugs which produce conditioned place preferences in this procedure should increase activity.

Accordingly, in the present study, we have examined the place conditioning produced by morphine in drug-naive rats and those made tolerant to its locomotor activity effects. The results of this investigation demonstrate that in the place preference conditioning procedure, the reinforcing properties of an opioid are independent of its ability to increase locomotor activity.

METHOD

Subjects

Male Sprague-Dawley rats (Charles River Wiga, FRG) weighing 160–180 g were housed individually in plastic cages in a climatically-controlled (temp: 22°C) colony room. They

were maintained on a 12:12 hr light/dark cycle with food and water available ad lib. Rats were divided into 2 groups. One group received subcutaneous (SC) injections of morphine (5.0 mg/kg/12 hr) for four days prior to conditioning; the other group received injections of saline (1.0 ml/kg/12 hr). The dose and schedule of morphine administration was selected on the basis of previous studies showing that such treatment produces tolerance to other behavioural effects of μ-opioid agonists [(4), Shippenberg *et al.*, in press]. All injections were given in the home cages and in a room different from that where conditioning was conducted unless otherwise specified.

Place Conditioning

Place conditioning commenced 12 hr after the last chronic injection. Conditioning was conducted as previously described using an unbiased procedure (10–12). The apparatus were shuttleboxes (30×60×30 cm: w × l × h) composed of Plexiglas and wood. Each box was divided into 2 equal sized compartments by means of a sliding partition. One compartment was white with a textured floor; the other was black with a smooth floor. For conditioning, rats were injected SC with saline or one of a number of doses of morphine. They were immediately confined to one compartment following morphine injections and to the other compartment following saline injections. Conditioning sessions (2 vehicle: 2 drug) were 50 min in duration. Two sessions were conducted daily, with each separated by 8 hr. Treatment compartment and the presentation order of drug and saline were counterbalanced across subjects for each dose.

Tests of conditioning were conducted 12 hr after the last conditioning session. For these sessions, the partition which separated the two compartments was raised 12 cm above the floor and a neutral platform, consisting of galvanized steel mesh (5 × 2 cm: w × h), was inserted along the seam separating the compartments. Preference for a particular place was assessed in the drug-free state by placing uninjected rats on the neutral platform and allowing them free access to both compartments. The time spent in each compartment during a 15-min session was then measured. An Olympus VX351 E video camera was used for behavioural observations and data recording. All sessions were conducted under conditions of dim illumination (14.5 lux) with masking white noise present. Under these conditions, drug-naive rats exhibit no preference for either of the place cues (12–14).

Locomotor Activity

Locomotor activity was monitored during conditioning sessions with morphine (5.0 mg/kg) and saline by recording the number of times an animal crossed a line that divided each environment in half. The number of crossing was recorded for 3-min intervals at 2, 8, 14, 20, 26, 36 and 46 min after injections.

Data Analysis

Place conditioning scores represent the time spent in the drug-associated place minus the time spent in the vehicle-associated place and are expressed as means±S.E. Dose-response curves were analyzed using a one-way random effects model factorial analysis of variance. The Student Newman-Keul's test (SNK) was used to determine whether doses of morphine produced conditioning significantly different from that produced by saline. A two-factor (treatment by dose) ANOVA and a two-factor repeated measures ANOVA were used to analyze the influence of chornic morphine treatment upon place conditioning and locomotor activity, respectively. The accepted level of significance for all tests was $p \leq 0.05$.

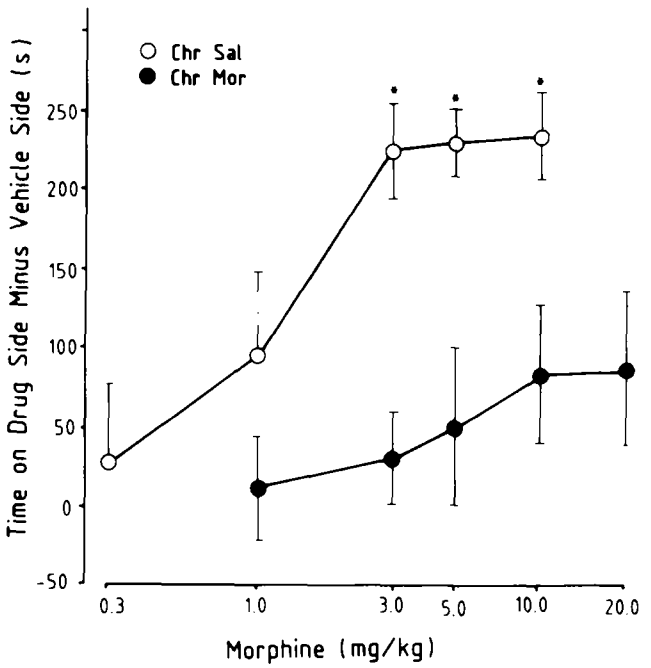

FIG. 1. Place conditioning produced by morphine in rats chronically treated with saline (Chr Sal) or morphine (Chr Mor). Rats received SC injections of saline (1.0 ml/kg/12 hr) or morphine (5.0 mg/kg/12 hr) for 4 days prior to conditioning. Ordinate: conditioning score defined as time spent in the drug-associated place minus time spent in the saline-associated place (sec). Abscissa: morphine dose (mg/kg). Each point represents the mean±S.E. of 8–10 rats. Asterisks denote significant place preference (ANOVA: see text, SNK: $^*p<0.05$).

Drugs

Morphine hydrochloride (Merck, FRG) was dissolved in saline and injected in a volume of 1.0 ml/kg. Doses are expressed as the base.

RESULTS

Place Conditioning

Rats conditioned with saline exhibited no significant preference for either compartment of the shuttlebox confirming that the conditioning procedure employed was of the unbiased type. The mean time spent in the white and black compartments were 354.1±18 sec and 365.2±19 sec (n=16), respectively.

Administration of morphine to control (saline-pretreated) rats resulted in a significant preference for the drug-associated place (Fig. 1). The magnitude of this effect was linearly related to dose, $F(1,39)=29.1$, $p \leq 0.001$. Significant conditioning was observed with doses of 3.0 mg/kg (conditioning score: 224.9±33 sec, n=10) and greater.

The chronic administration of morphine for four days prior to conditioning resulted in a complete abolition of the

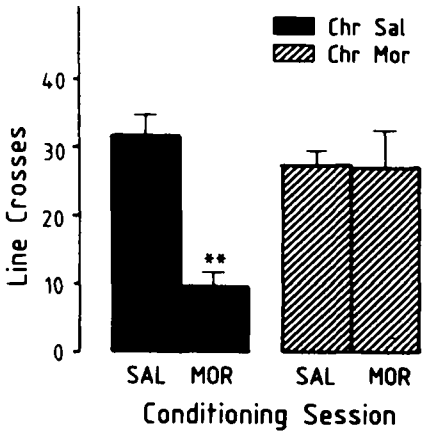

FIG. 2. Locomotor activity during conditioning sessions with saline and morphine (5.0 mg/kg). Rats were treated as described in Fig. 1 and activity defined as number of line crossings was measured during each conditioning session. Ordinate: line crosses. Abscissa: conditioning session. Each column represents the mean activity ±S.E. of 8 rats. Asterisk refers to significant treatment and interaction effects (ANOVA: see text).

morphine-induced place preference (Fig. 1). A two-factor ANOVA with treatment (chronic morphine vs. saline) and dose of conditioning drug as factors revealed significant effects of treatment, $F(1,83)=9.47$, $p\leq0.003$, and dose, $F(6,33)=4.17$, $p\leq0.001$, but no treatment by dose interaction ($p>0.05$). An ANOVA of the dose-response curve for morphine in chronic morphine-treated rats indicated that at no dose was the place conditioning produced by morphine significantly different from that produced by saline. Similarly animals chronically-treated with morphine in the same room as that where conditioning occurred were also tolerant to the effects of morphine (5.0 mg/kg) and there was no difference between the conditioning score of these animals (44±18 sec, n=8) and those which were pretreated in a room distinct from that where conditioning occurred (50±45 sec, n=8).

Locomotor Activity

As shown in Fig. 2, administration of morphine (5.0 mg/kg) to control rats resulted in a significant decrease in locomotor activity. Mean activity during morphine conditioning sessions were 9.6±2.3 line crossings as compared to 32.2±2.9 crossings during saline sessions. In contrast, no decrease in activity was observed in chronic morphine-treated rats. Mean activity during morphine conditioning sessions (27.4±2.1) did not differ from that produced by saline (26.9±5.6). A two-factor (treatment by conditioning session) repeated measures ANOVA of the data revealed a significant effect of treatment, $F(1,14)=42.5$, $p\leq0.001$, and a significant treatment by session interaction, $F(1,14)=7.9$, $p\leq0.01$.

DISCUSSION

Morphine at doses of 3.0, 5.0, and 10.0 mg/kg produced marked preferences for the drug-associated place in rats chronically pretreated with saline. Locomotor activity following administration of the 5.0 mg/kg dose of morphine was, however, significantly less than that following injections of saline. These results are consistent with previous place conditioning studies (10, 12, 16) and with data indicating locomotor depressant effects of morphine one hour after its administration in drug-naive rats (1,21). Furthermore, the finding that morphine produces place preferences at doses which decrease activity strongly suggests that, in the place preference conditioning procedure, the ability of a drug to function as a reinforcer is independent of increases in either locomotor activity or environmental familiarity.

Previous studies (13,21) have shown that chronic administration of morphine produces tolerance to its locomotor depressant effects and may, in fact, unmask the stimulatory actions of this drug. Therefore, it was hypothesized that if place conditioning confounds measures of reinforcement with drug-induced increases in activity (18), then rats chronically treated with morphine and then conditioned with morphine should exhibit a preference for the drug-associated place. Furthermore, this effect should be greater than that of rats exhibiting a locomotor depressant response to morphine. In the present study, rats which had received morphine for four days prior to conditioning developed tolerance to the locomotor depressant effects of this drug. Such treatment, however, resulted in a complete abolition of the morphine-induced place preferences. Thus, administration of morphine at doses equal to or 6-fold greater than those producing place preferences in saline-treated rats failed to produce significant conditioning in chronic morphine-treated rats. Such findings demonstrate that the place preferences induced by morphine do not result from the ability of this drug to increase activity. Furthermore, the abolition of place conditioning following chronic morphine treatment strongly suggests that, in this procedure, tolerance develops to the reinforcing effects of opioids. Whether or not such tolerance reflects a pharmacodynamic adaptation at the level of the opioid receptor or the influence of associative blocking and/or other learning processes (11), cannot be determined from the present data. This issue is, however, addressed in another report (14).

In summary, recent data (17,18) have suggested that the place preference conditioning procedure may confound measures of reinforcement with drug-induced increases in activity. The present findings that morphine produces place preferences in drug-naive animals at doses which decrease locomotor activity but fails to produce such effects in rats which are tolerant to its locomotor depressant effects provide no support for this hypothesis. Rather, these data and those (6) demonstrating place aversions in response to phencyclidine, an agent which produces marked behavioural stimulation, indicate that place conditioning in response to a drug does not result from drug-induced alterations in locomotor activity or the resulting degree of environmental familiarity.

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REFERENCES

1. Babbini, M.; Davis, W. M. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. Br. J. Pharmacol. 46:213–224; 1972.
2. Brady, L. S.; Holtzman, S. G. Locomotor activity in morphine-dependent and post-dependent rats. Pharmacol. Biochem. Behav. 14:361–370; 1980.
3. Cappell, H.; LeBlanc, A. E.; Endrenyi, L. Aversive conditioning by psychoactive drugs: effects of morphine, alcohol and chlordiazepoxide. Psychopharmacologia 29:239–246; 1973.
4. Emmett-Oglesby, M. W.; Shippenberg, T. S.; Herz, A. Tolerance and cross-tolerance to the discriminative stimulus properties of fentanyl and morphine. J. Pharmacol. Exp. Ther. 245:17–23; 1988.
5. Haertzen, C. A. Subjective effects of narcotic antagonists, cyclazocine and nalorphine on the addiction research center inventory (ARCI). Psychopharmacologia 18:366–377; 1970.
6. Iwamoto, E. Place aversion conditioned by phenacyclidine in rats: development of tolerance and pharmacological antagonism. Alcohol. Drug Res. 6:265–276; 1986.
7. Johanson, C. E.; Uhlenhuth, E. H. Drug self-administration in humans. In: Krasnegar, N., ed. Self-administration of abused substances: Methods for study. NIDA Research Monograph No. 20, DHEW Publication No. (ADM) 78-727. Washington, DC: U.S. Government Printing Office; 1978:68–85.
8. Kornetsky, C.; Esposito, R. U.; Mcdean, S.; Jacobson, J. O. Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. Arch. Gen. Psychiatry 36: 289–292; 1979.
9. Kumar, R. Morphine dependence in rats: secondary reinforcement from environmental stimuli. Psychopharmacologia 25:332–338; 1972.
10. Mucha, R. F.; Herz, A. Motivational properties of kappa and mu opioid receptor agonists studies with place and taste perference conditioning procedures. Psychopharmacology (Berlin) 86:274–280; 1985.
11. Randich, A.; LoLordo, V. M. Associative- and nonassociative theories of the UCS preexposure phenomenon: implications for Pavlovian conditioning. Psychol. Bull. 86:523–548; 1979.
12. Shippenberg, T. S.; Herz, A. Differential effects of mu and kappa opioid systems on motivational processes. Progress in opioid research. NIDA Research Monograph 75. Washington, DC: U.S. Dept. Health and Human Services; 1986:563–566.
13. Shippenberg, T. S.; Bals-Kubik, R.; Herz, A. Motivational properties of opioids: evidence that an activation of delta-receptors mediates reinforcement processes. Brain Res. 436:234–240; 1987.
14. Shippenberg, T. S.; Emmett-Oglesby, M.; Ayesta, J.; Herz, A. Tolerance and selective cross-tolerance to the motivational effects of opioids. Psychopharmacology (Berlin) 96:110–115; 1988.
15. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Res. 253:185–193; 1982.
16. Stolerman, I. P. Motivational properties of opioids: Evidence on the role of endorphins in the mediating reward or aversion. Pharmacol. Biochem. Behav. 23:877–881; 1985.
17. Swerdlow, N. R.; Koob, G. F. Restrained rats learn amphetamine-conditioned locomotion but not place preference. Psychopharmacology (Berlin) 84:163–166; 1984.
18. Swerdlow, N. R.; Gilbert, D.; Koob, G. F. Conditioned drug effects on spatial preference: critical evaluation. In: Boulton, A. A.; Baker, G. B.; Greenshaw, A., eds. Neuromethods, vol. 13, Psychoharmacology I. Clifton, NJ: Humana Press Inc.; in press.
19. Woods, J. H. Behavioral pharmacology of drug in self-administration. In: Lipton, M. A.; DiMascio, A.; Killam, K. F., eds. Psychopharmacology, a generation of progress. New York: Raven Press; 1978:595–607.
20. Woods, J. H.; Young, A. M.; Herling, S. Classification of narcotics on the basis of their reinforcing, discriminative, and antagonist effects in rhesus monkeys. Fed. Proc. 41:221–227; 1982.
21. Yasko, M. R.; Domino, E. F. Tolerance development to the biphasic effects of morphine on locomotor activity and brain acetylcholine in rats. J. Pharmacol. Exp. Ther. 207:848–858; 1978.