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Single-Trial Conditioned Place Preference Using Intravenous Morphine

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BARDO, M. T. AND J. L. NEISEWANDER. *Single-trial conditioned place preference using intravenous morphine.* PHARMACOL BIOCHEM BEHAV **25**(5) 1101–1105, 1986.—Experiments were performed to investigate single-trial conditioned place preference (CPP) using intravenous morphine in rats. Single-trial CPP was obtained when morphine (8 mg/kg) was paired for either 15 or 30 min with a distinct white compartment. When morphine administration was delayed for either 15 or 25 min after the beginning of a 30-min exposure to the white compartment, single-trial CPP was not obtained. Intravenous naloxone (2 mg/kg) also blocked single-trial CPP when administered 15 min after the beginning of the 30-min exposure to the white compartment with morphine, but naloxone by itself did not alter place preference. The results from these experiments indicate that single-trial CPP using intravenous morphine may offer a useful animal model to assess the reinforcing efficacy of the initial drug experience.

Conditioned place preference Morphine Naloxone Classical conditioning Drug reinforcement

RECENT clinical evidence suggests that drug abuse in humans may be predicted, at least in part, by the degree of reinforcement derived from the initial drug experience [16]. In a population of male drug abusers, the initial reinforcement value was found to be related to subsequent long-term abuse of various drugs, including sedative-hypnotics, stimulants, hallucinogens and opiates. Despite this clinical evidence, however, little is known at present about the neuropharmacologic mechanisms involved in the reinforcing value of the initial drug experience. One animal model often used to assess the neuropharmacologic mechanisms of drug reinforcement is the self-administration procedure [43]. While this model has been valuable in assessing chronic drug administration, it does not measure directly the reinforcing value of the initial drug experience.

An alternative animal model of drug reinforcement is termed conditioned place preference (CPP). In CPP, animals are given a reinforcing drug in one distinct environment and are given saline in an alternative environment. Following conditioning, when the animal is in a drug-free state, a preference test is given in which the animal has free-choice access to both environments simultaneously. CPP is reflected in an increased duration spent in the presence of drug-associated stimuli relative to saline-injected control animals.

In the CPP model, the drug injection is presumed to act as an unconditioned stimulus (UCS) which elicits a reinforcing unconditioned response (UCR). By pairing the drug UCS with distinct environmental conditioned stimuli (CS), the CS comes to elicit a reinforcing conditioned response (CR). Unfortunately, one problem inherent in the CPP model is that the CR is not measured directly, but is inferred from an operant choice behavior, i.e., duration spent in the drug-associated environment. In fact, there is some controversy regarding whether the operant choice behavior reflects reinforcement *per se* or perhaps some other behavioral process (e.g., [39]). Indeed, it seems clear that the CPP model measures a component of drug action which differs from that measured by the self-administration model of drug reinforcement. For example, Spyraki and colleagues [35] found that cocaine-induced CPP was *not* blocked by depleting dopamine with 6-hydroxydopamine (6-OHDA) or by administering dopamine antagonists. This contrasts with the finding that cocaine self-administration is attenuated by either 6-OHDA [27] or dopamine antagonists [15]. Thus, until the neuropharmacologic mechanisms underlying CPP and self-administration are elucidated more fully, the degree to which they share a common reinforcement process may remain unclear.

Despite this discrepancy between CPP and self-administration, considerable evidence has accumulated to indicate that CPP measures some component of drug reinforcement. It is clear that environmental stimuli paired with a reinforcing psychoactive drug can elicit a CR that mimics the drug-induced UCR [13,38]. For example, various morphine-induced UCRs can be conditioned to environmental CSs such that the CR mimics the UCR, including changes in body temperature [12, 18, 21, 32], locomotor activity [24,42], catecholamine release into blood [20] and cortical evoked potentials [37,44]. Similarly, environmental stimuli paired with a reinforcing psychoactive drug may elicit a reinforcing CR. If this is the case, then these environmental stimuli would be capable of directing operant behavior. In support of this notion, research has demonstrated that monkeys and rats injected with morphine in association with an environmental stimulus will perform an operant response which delivers the stimulus alone [7, 9, 31, 40]. Furthermore, drugs which support self-administration behavior in animals also have been shown to support CPP, including amphetamine [10, 26, 34], cocaine [22,35], nicotine [14], heroin [6, 29, 30], and morphine [2–4, 17, 23, 28, 33, 36]. Moreover,

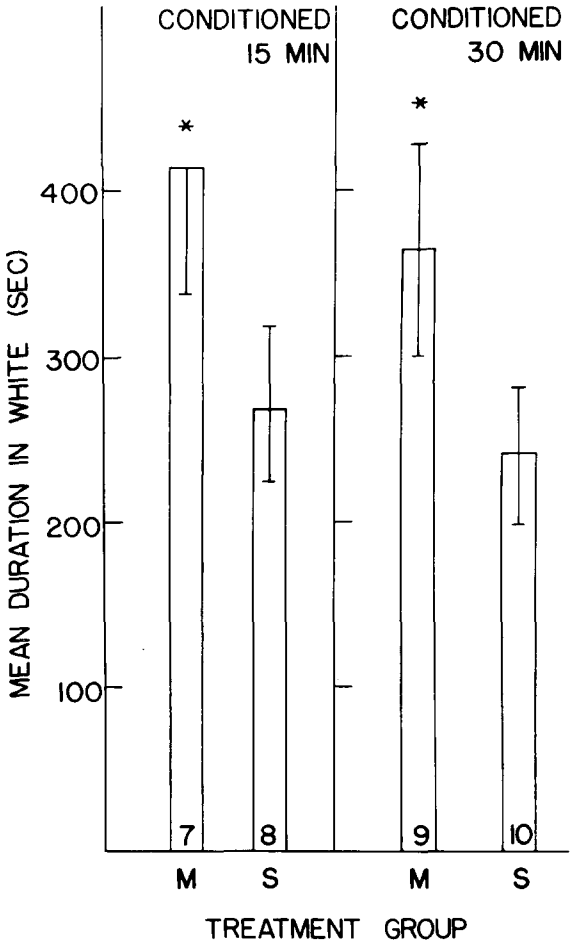

FIG. 1. Mean duration (±S.E.M.) spent in the white compartment for animals given either morphine (M) or saline (S) and confined in white conditioning compartment for either 15 or 30 min. Number of animals in each group are designated at the bottom of each bar. Asterisk (*) represents significant difference from saline control group, $p<0.05$.

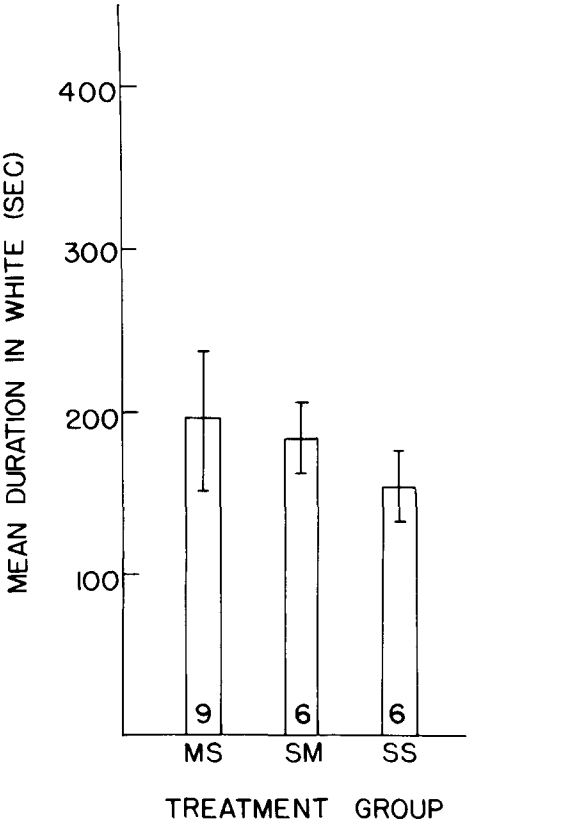

FIG. 2. Mean duration (±S.E.M.) spent in the white compartment for animals injected 15 and 25 min after 30-min placement into the white compartment. Injections were either morphine (M) or saline (S). Number of animals in each group are designated at the bottom of each bar.

drugs such as ethanol which do not readily support self-administration behavior in animals also do not support CPP [1, 8, 41]. Taken together, these results indicate that CPP measures some component of drug reinforcement.

To the extent that CPP measures a component of drug reinforcement, CPP may be used to assess the reinforcing efficacy of the initial drug administration. Although CPP is typically established by giving multiple drug conditioning trials prior to the preference test, at least one report has shown that CPP can be obtained by administering a single intravenous dose of morphine (4 mg/kg) to rats [22]. In the present report, we examined in detail the reinforcing effect of a single intravenous morphine injection in rats using the CPP model.

METHOD

Animals

The animals were male Sprague-Dawley albino rats (Harlan Industries, Indianapolis, IN), initial weight 225–400 g, housed individually with food and water ad lib. Two to three days prior to the start of drug conditioning, a PE50 polyethylene catheter was implanted into the left jugular vein under chloral hydrate anesthesia. The catheter was kept patent by daily injections of 0.5 ml saline.

Apparatus

Animals were conditioned in a chamber that had three different compartments separated by guillotine doors. The two end compartments measured 22×26×30 cm, while the middle compartment measured 22×14×30 cm. One end compartment had white walls, a wire-mesh floor, and pine wood chips under the floor. The other end compartment had black walls, a metal-grid floor, and cedar shavings under the floor. The middle compartment had gray walls and a solid wood floor which was also gray.

General Procedure

In all experiments, conditioning took place over a 2-day period. On one day, each animal was confined to the white compartment for 30 min, while on the other day, each animal

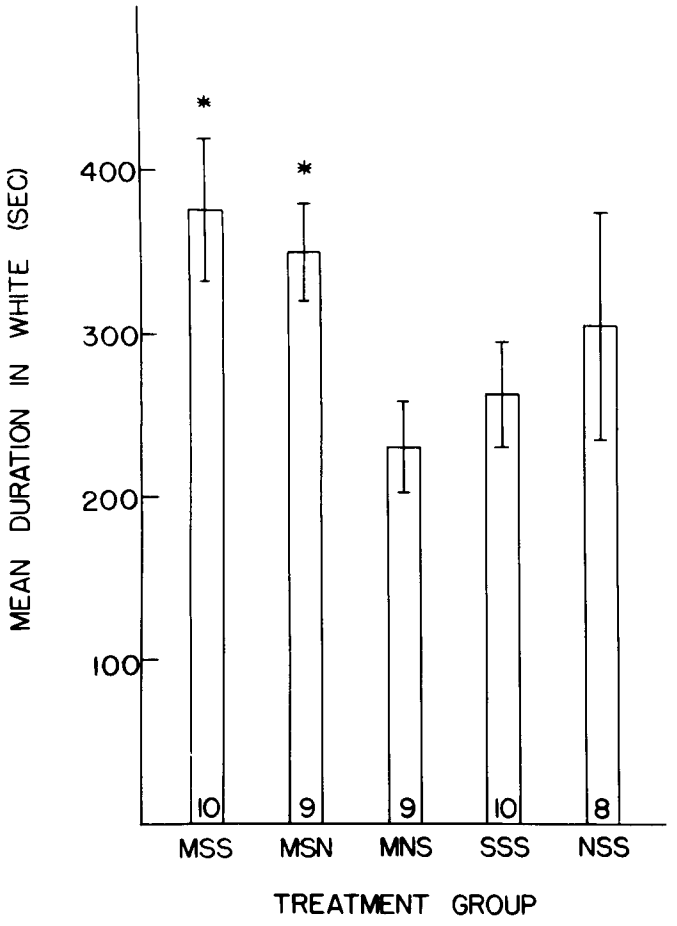

FIG. 3. Mean duration (±S.E.M.) spent in the white compartment for animals given three injections; one immediately upon being placed into white, one 15 min after placement into white and one immediately after the 30-min placement into white. Injections were either morphine (M), naloxone (N) or saline (S). Number of animals in each group are designated at the bottom of each bar. Asterisks (*) represent significant difference from Group SSS, $p<0.05$.

was confined to the black compartment for 30 min. In one experiment however, animals were confined for only 15 min to each compartment. Intravenous injections of morphine sulfate (4 or 8 mg/kg), naloxone hydrochloride (2 mg/kg) or saline were given when the animal was confined in the white compartment. Each drug infusion was followed by 0.5 ml saline in order to insure that the drug was flushed entirely from the catheter. Animals received no injection when confined to the black compartment. The order in which animals experienced the white and black compartments was counterbalanced across treatment groups.

On the day following this single-trial conditioning procedure, each animal was given a preference test by being placed into the middle gray compartment with the guillotine doors open. A blind observer recorded the duration spent in each compartment and the number of entries into each compartment over a 15-min period.

Statistical Analysis

The data were analyzed by factorial or completely randomized analyses of variance, and pairwise comparisons were made using Duncan's multiple range test [11].

RESULTS

Single-Trial CPP Following 15 or 30 Minute Exposure to the CS

Animals administered 4 mg/kg morphine and exposed to the white compartment for 30 min did not display significant 1-trial CPP (data not shown). In contrast, animals administered 8 mg/kg morphine immediately after being placed into the white compartment displayed significant CPP, regardless whether they were left in white for either 15 or 30 min post-injection (see Fig. 1). With this dose of morphine (8 mg/kg), the animals appeared cataleptic within 10 sec after injection. Single-trial CPP was evident in that the total duration spent in the white compartment was significantly longer in morphine-treated than in saline-treated animals, $F(1,26) = 9.56$, $p<0.01$. There was no significant interaction between the drug treatment and length of exposure to the white compartment, indicating that morphine was effective following either 15 or 30 min CS exposure.

Single-trial CPP was evident even though there was no significant difference in the number of entries into white between morphine- and saline-treated groups. The mean number of entries into white were 6.6 and 6.5 for morphine- and saline-treated groups respectively. Thus, CPP was due to an increased duration spent *per entry* into white, rather than an increase in the number of entries into white *per se*.

Delaying UCS After CS Onset Disrupts Single-Trial CPP

To assess the effect of delaying intravenous morphine during CS exposure, animals were injected twice in the white compartment, once at 15 and once at 25 min after the beginning of a 30-min exposure to white. For one group, the first injection was 8 mg/kg morphine and the second was saline (Group MS), another group received saline followed by morphine (Group SM) and a third group received saline twice (Group SS). A completely randomized analysis of variance revealed no significant differences in duration spent in the white compartment among treatment groups. Thus, single-trial CPP was not obtained in the morphine groups since they did not differ from saline controls (see Fig. 2). Further, there were no significant differences in the number of entries into white among groups (data not shown).

Terminating UCS Before CS Offset Disrupts Single-Trial CPP

To assess the effect of terminating intravenous morphine during CS exposure, animals were injected three times; once at the start of a 30-min placement into white; once 15 min after placement into white; and once immediately after removal from white. For one group, the first injection was 8 mg/kg morphine and the next two injections were saline (Group MSS). For a second group, the first injection was morphine, the second was saline, and the last was 2 mg/kg naloxone (Group MSN). For a third group, the first injection was morphine, the second injection was naloxone, and the last injection was saline (Group MNS). For a fourth group, all three injections were saline (Group SSS). In order to determine whether naloxone by itself produced any change in place preference, a fifth group was administered naloxone followed by two saline injections (Group NSS).

A randomized analysis of variance of the four main treatment groups (Groups MSS, MSN, MNS, and SSS) indicated a significant effect of treatment on the mean duration spent in the white compartment, $F(3,34)=4.09$, $p<0.01$. Pairwise comparisons revealed that Group MSS spent significantly more time in white than Group SSS (see Fig. 3). This finding replicates the single-trial CPP shown previously. Furthermore, Group MSN also displayed CPP, as they spent significantly more time in white than Group SSS. There were no significant differences between Groups MSS and MSN on the preference test, indicating that similar conditioning was obtained in these two groups.

In contrast to the single-trial CPP evident in animals that experienced morphine throughout the 30-min CS exposure, Group MNS did not differ significantly from Group SSS in duration spent in the white compartment. Further, naloxone by itself did not influence preference behavior significantly, as there was no significant difference between Groups NSS and SSS in duration spent in white. There were no significant differences among any treatments in the number of entries into the white compartment (data not shown).

DISCUSSION

The present experiments provide evidence that single-trial CPP may be used to assess the reinforcing efficacy of an acute intravenous dose of morphine. In contrast to multiple-trial CPP or self-administration procedures, the reinforcement value of the initial drug experience can be measured without any induction of tolerance or dependence. Also, a major advantage of single-trial CPP using intravenous morphine is that the temporal characteristics of drug action can be controlled more precisely than with either subcutaneous or intraperitoneal routes of administration. Compared to the subcutaneous and intraperitoneal routes, the onset of drug action is more rapid and stronger in magnitude following intravenous injection. This may explain why only one trial is required to obtain CPP when morphine is given intravenously, whereas three trials are required when morphine is given subcutaneously [23]. Further, the offset of drug action may be controlled precisely by administering naloxone intravenously at some interval following morphine administration. Using this intravenous procedure, the presentation of drug can be controlled precisely. By controlling the temporal characteristics of the CS-UCS pairing, single-trial CPP using intravenous morphine and naloxone may be comparable to other Pavlovian learning situations in which the CS (e.g., a tone) and UCS (e.g., shock) durations are defined precisely.

Since the original report by Pavlov [25], morphine administration has been examined as an UCS in a myriad of classical conditioning situations. In contrast to other general Pavlovian situations, however, we found that a simultaneous conditioning procedure produced stronger conditioning than a delayed conditioning procedure. With simultaneous conditioning, the CS and UCS presentations are initiated at the same time, whereas with delayed conditioning, the UCS is presented at some interval following CS presentation. In both instances, the CS and UCS are typically terminated at the same time. In the present study, CPP was not obtained when morphine administration was delayed for either 15 or 25 min after a 30-min placement into the white compartment. However, CPP was obtained when morphine administration was simultaneous with placement into the white compartment. In this situation, naloxone administration was used to terminate the morphine UCS as the animal was removed from the white compartment CS, thus defining a simultaneous conditioning procedure. The fact that simultaneous conditioning proved more effective than delayed conditioning suggests that single-trial CPP differs from traditional Pavlovian learning situations in which a delay procedure generally produces the strongest conditioning [19]. It should be noted however, that in traditional Pavlovian learning situations, multiple CS-UCS presentations are given and CS-UCS durations are usually measured in seconds rather than minutes.

Our results also demonstrate that simultaneous onset of the CS and UCS is not a sufficient condition to establish single-trial CPP. In the present study, some animals received morphine simultaneously with a 30-min exposure to the white compartment CS, but were given naloxone 15 min after the simultaneous CS-UCS onset. In this situation, CPP was not obtained. This failure to obtain CPP was not due to an aversive effect of naloxone, as naloxone by itself did not alter place preference. In fact, previous evidence suggests that three or more trials may be required to obtain conditioned place aversion with naloxone [23]. Further, this failure to obtain CPP was not because a 15-min CS-UCS overlap is an insufficient duration to establish CPP, as simultaneous administration of morphine with a 15-min CS presentation did produce CPP. Instead, these results indicate that the reinforcing effect of morphine can be conditioned during the first 15 min following intravenous injection, but that the CPP produced by this 15-min drug exposure may be extinguished rapidly when followed by exposure to the CS alone. Thus, exposure to the CS alone, either before or after the CS-UCS pairing, disrupts single-trial CPP with intravenous morphine.

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