

## Intraadministration Associations: Conditional Hyperalgesia Elicited by Morphine Onset Cues

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There is evidence that exteroceptive cues associated with drug administration elicit conditional compensatory responding (e.g., hyperalgesia in organisms with a history of morphine administration). Recently it has become apparent that, within each administration, interoceptive early-drug onset cues (DOCs) may become associated with the later, larger drug effect (intraadministration associations). The present experiments evaluated DOC-elicited conditional hyperalgesia in rats intravenously infused with morphine. The results indicated that DOC-elicited hyperalgesia contributes to tolerance to the analgesic effect of morphine, and such DOC-elicited hyperalgesia is an associative phenomenon, rather than a sensitized response to the opiate. The findings suggest that associative analyses of tolerance should acknowledge the conditional responding elicited by DOCs, and extinction-based addiction treatments should incorporate extinction of DOC-elicited conditional responding.

Results of many experiments indicate that drug tolerance is modulated by drug-associated cues present at the time of tolerance testing. The contribution of such cues has been incorporated in a Pavlovian conditioning analysis of tolerance. Using the usual conditioning terminology, cues accompanying the primary drug effect function as conditional stimuli (CSs). The direct effect of the drug constitutes the unconditional stimulus (US). Prior to any learning, this pharmacological stimulation unconditionally elicits responses (unconditional responses; URs) that compensate for the drug-induced disturbances. After some pairings of the predrug CS and pharmacological US, drug-compensatory responses are elicited as conditional responses (CRs). Such CRs that mimic the compensatory response unconditionally elicited by a drug have been termed *conditional compensatory responses* (CCRs; see Dworkin, 1993; Kim, Siegel, & Patenall, 1999). These CCRs attenuate the effect of the drug and contribute to tolerance.

The extensive literature indicating that CCRs contribute to tolerance has recently been reviewed (Siegel, Baptista, Kim, McDonald, & Weise-Kelly, 2000; Siegel & Ramos, in press). Briefly, consistent with the conditioning analysis, a variety of manipulations that attenuate the expression of conditional responding also attenuate the acquisition of tolerance. Thus, in common with other CRs, the expression of drug tolerance is disrupted by presenting a novel external stimulus (“external inhibition”) or by altering the

putative CS (changing the context of drug administration in an unpredictable manner). The acquisition of tolerance is retarded by partial reinforcement, CS preexposure, and inhibitory learning. Like other CRs, drug tolerance displays extinction, spontaneous recovery, stimulus generalization, and a flattening of the generalization gradient as a result of extending the interval between acquisition and assessment. Tolerance also displays sensory preconditioning and a variety of compound conditioning effects such as overshadowing and blocking. Posttrial events that affect memory consolidation similarly affect the rate of tolerance acquisition; thus, electroconvulsive shock or frontal cortical stimulation decrease the rate of acquisition of morphine tolerance, and glucose facilitates the rate of acquisition of morphine tolerance (Siegel et al., 2000; Siegel & Ramos, in press).

The most thoroughly established findings supporting the conditioning analysis of tolerance are demonstrations of environmental specificity of tolerance. After tolerance is established by repeatedly administering the drug in a particular environment, tolerance typically is more pronounced in that drug-paired environment than in an alternative environment. The fact that tolerance displays environmental specificity is expected on the basis of the conditioning analysis of tolerance: Drug-associated cues elicit CCRs that attenuate the drug effect, thus tolerance is greater when assessed in the presence of drug-associated cues than when assessed elsewhere.

Environmental specificity has been demonstrated with respect to tolerance to many effects of a variety of drugs (see Kim et al., 1999; Siegel et al., 2000): opiates, naloxone, ethanol, nicotine, pentobarbital, phencyclidine, immunoenhancing drugs, cholecystokinin, carisoprodol, haloperidol, and several benzodiazepines. It is seen in many species, from snails to humans. The most dramatic demonstrations of environmental specificity concern tolerance to the lethal effects of drugs. Altering the context of drug administration increases the lethality of several drugs, including heroin, morphine, and ethanol (see Siegel, 2001; Siegel & Kim, 2000).

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This research was supported by United States National Institute on Drug Abuse Grant DA 11865 and Natural Sciences and Engineering Research Council of Canada Grant 00298 to Shepard Siegel. The assistance of Doreen Mitchell is gratefully acknowledged.

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Experimental evaluations of the conditioning analysis of tolerance typically have manipulated exteroceptive signals for the drug. In addition to such exteroceptive stimuli, there are also interoceptive stimuli that are paired with a drug effect and thus may elicit CCRs that mediate tolerance. For example, interoceptive cues incidental to self-administration of a drug (self-administration cues; SACs) modulate tolerance. That is, tolerance to a variety of effects of various drugs is more pronounced in the organism that self-administers the drug than in the organism that passively receives the drug. The role of SACs in tolerance has been demonstrated in several species, including humans (see Weise-Kelly & Siegel, 2001).

Another interoceptive cue that has received considerable attention is the drug onset cue. That is, within each administration of a drug, early-drug onset cues (DOCs) are detected by rats. Recently, we demonstrated that these interoceptive pharmacological cues become associated with the later, larger drug effect, and such *intraadministration associations* contribute to tolerance to the analgesic effect of morphine (Kim & Siegel, 2001; Kim et al., 1999). Because intraadministration pairings inevitably result from many drug-administration procedures, researchers (e.g., Cepeda-Benito & Short, 1997; Dworkin, 1993; Kim et al., 1999; Siegel et al., 2000; Siegel & Ramos, in press; Walter & Riccio, 1983) have suggested that (a) DOCs importantly contribute to drug tolerance and (b) the effectiveness of extinction-based treatments for addiction might be enhanced if such cue exposure therapy incorporated extinction of DOC-elicited CCRs as well as CCRs elicited by exteroceptive predrug cues.

In experiments demonstrating the role of DOCs in tolerance, experimental-group rats were repeatedly intravenously infused with 5.0 mg/kg morphine during the initial tolerance-development phase of the experiment. Infusions were gradual—each infusion was about 30 min in duration (hereinafter termed a long morphine infusion, LMor). When rats had displayed tolerance to the analgesic effect of the drug, they received a probe morphine (pMor) test trial. The pMor consisted of about the first 10% of the morphine infusion used during tolerance development, that is, 0.5 mg/kg morphine infused over a period of 3 min (hereinafter termed a 10% pMor), and was designed to reproduce the early effect of the tolerance-development infusions. In these experimental-group rats, pMor elicited a CCR of hyperalgesia—extraordinary sensitivity to nociceptive stimulation.

### Experiment 1

Although a 10% pMor was used in prior studies of intraadministration associations (Kim & Siegel, 2001; Kim et al., 1999), it is possible that the CCR elicited by this DOC would be even larger with a more salient probe. Obviously, there are many reasonable combinations of infusion rate and probe duration that may be evaluated. As a further complexity, because of the nature of an intraadministration association, the putative CS (the DOC) is not, like most CSs, “neutral;” rather, it is a less intense version of the US. Thus, following tolerance acquisition with 5.0 mg/kg, a 1.0 mg/kg pMor may, by some measures, be a more effective signal than the 0.5 mg/kg morphine probe we used. However, the expression of a morphine-compensatory CR elicited by the 1.0 mg/kg probe would be complicated because the greater conditional hyperalgesia (compared with 0.5 mg/kg) would be expressed in

combination with a greater unconditional analgesia. The purpose of Experiment 1 was to investigate the effectiveness of several different pMor infusions.

### Design

During the initial tolerance-development phase of the experiment, rats received six LMor infusions—one every other day. They were then divided into four groups, and responsiveness to nociceptive stimulation was determined for rats in each group following one of four different pMor infusions. One of the probe infusions consisted of physiological saline (0% pMor). The remaining probe infusions consisted of the same morphine solution that was used during tolerance development but differed with respect to the infusion duration. The 10% pMor was the same as the pMor used in previous research (Kim & Siegel, 2001; Kim et al., 1999; 0.5 mg/kg infused in about 3 min). A smaller probe (5% pMor, i.e., 0.25 mg/kg infused in about 1.5 min) and a larger probe (20% pMor, i.e., 1.0 mg/kg infused in about 6 min) also were evaluated.

### Method

#### *Subjects and Surgical Preparation*

The subjects were 28 experimentally naive, male, Sprague-Dawley derived rats (purchased from Charles River, Saint Constant, Montreal, Quebec, Canada), ranging from 350–450g at the start of the experiment. The rats were individually housed with ad lib access to food and water throughout the experiment. They were handled daily for a week prior to surgery.

Between 10 and 15 days prior to the start of the experiment, intravenous catheters were implanted in the right jugular vein of each rat under general anesthetic (ketamine and xylazine cocktail), using a modified version of the technique of Brown and Breckenridge (1975). The tip of the catheter was implanted approximately 1 cm from the heart. The catheters were assembled from 22-gauge hypodermic needles and 9.5 cm of silastic tubing (Dow Corning; 0.51-mm inner diameter and 0.94-mm outer diameter). The cannula was brought out to the skull and secured to the skull using dental acrylic. On the surgery day, 0.5 ml of Novo-Trimel (Novopharm, Toronto, Canada) was administered orally to each subject, followed by further administration of this antibiotic by addition of 4.5-ml to 500-ml water bottles in the home cage. Each catheter was flushed with a mixture of heparin and ampicillin (16.25 units/ml sodium heparin and 1.25 mg ampicillin) once daily during the recovery period (7–10 days).

#### *Apparatus, Drugs, Infusion Rates, and Analgesia Assessment*

Drug administrations were conducted in chambers (30.4-cm long × 20.5-cm wide × 19.0-cm tall; Lehigh Valley Electronics, Beltsville, MD), constructed of clear Plexiglas with a grid floor and placed in a sound-attenuating cubicle. The rat's cannula was connected to a variable rate syringe infusion pump (Sage Model 341A) through flexible tubing (Tygon, Size 13, No. 6409), attached to a 0.025-micrometer micropore filter (Sartorius, AG, Göttingen, Germany).

During tolerance-development sessions, 5 mg/ml morphine sulfate solution was delivered. Intravenous administrations were in a volume of 1 ml/kg and infused at a rate of 0.0166 ml/min. The infusion time varied between rats (24–34 min), depending on body weight, and was adjusted to deliver a dose of 5.0 mg/kg. The probe doses were delivered at the same rate of infusion as in the tolerance-acquisition and retraining sessions. The duration of probe infusion was also based on body weight adjusted to

deliver 0.25, 0.5, and 1.0 mg/kg of morphine for 5%, 10%, and 20% probes, respectively. The 0% pMor infusion duration was constant (3 min) independent of the subject's weight.

Analgesia was measured with the tail-flick procedure (Fennessy & Lee, 1975). The tail of a lightly restrained rat was immersed 5 cm into a water bath (located in the same room as the infusion chambers). The latency for the rat to lift its tail out of the water (tail-flick latency, TFL) was noted. The water bath was maintained at 50 °C during the tolerance-development phase. During the test sessions the temperature was 48 °C to increase the likelihood of observing a hyperalgesic response. Failure to respond within 30 s resulted in termination of tail immersion to prevent tissue damage.

### Procedure

#### Tolerance Development

All rats received six sessions of morphine administration, one session every other day. For each tolerance-development session, rats were transported, in their home cages, from the colony room to the room containing the infusion chambers. Prior to each session, each rat was weighed, and its cannula was flushed with 0.05 ml heparin solution (16.25 units/ml). The rat's cannula was then connected to the infusion pump, and the rat was placed in the infusion chamber. The rats were allowed free movement within the chamber while they were connected to the apparatus. Fifteen minutes after placement in the chamber, the morphine infusion started. Following the infusion, the subjects were detached from the apparatus, and 0.05 ml of a dextrose solution (3.3% dextrose and 0.3% sodium chloride) was injected into the cannula to help maintain patency between sessions. The animals were then returned to the infusion chamber. Thirty minutes postmorphine infusion, tail-flick latency was assessed and the subjects were returned to home cages.

#### Probe Tests

The rats were randomly assigned to one of the four test groups ( $n = 7$ ), each group receiving a different test probe: 0% pMor (i.e., saline), 5% pMor, 10% pMor, or 20% pMor. Rats received four pMor tests, with two LMor infusions interpolated between tests. Each rat was administered the same pMor on each of the test sessions. During testing, as during tolerance development, rats participated in the experiment on alternate days. There was a 5-day interval between pMor tests. Rats received LMor infusions on the second and fourth days between each pMor test and were left undisturbed on the first, third, and fifth days between tests. Tail-flick latencies were recorded at 5, 15, 30, and 45 min following each pMor infusion.

### Results and Discussion

#### Tolerance Development

The mean ( $\pm 1$  SEM) TFLs following each tolerance-development LMor infusion for rats assigned to each pMor test group are shown in Figure 1. As can be seen in Figure 1, tolerance to the analgesic effect of morphine was apparent (i.e., TFLs decreased across tolerance-development sessions). As would be expected, the rats in the four groups, not yet subjected to differential treatment, displayed similar response latencies. These observations were confirmed by a mixed-design analysis of variance

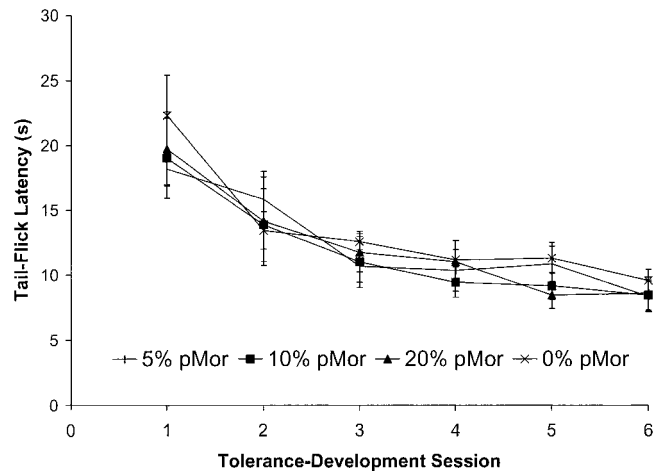


Figure 1. Mean tail-flick latencies ( $\pm 1$  SEM) following each morphine infusion for rats assigned to each probe morphine (pMor) test group during the tolerance-development phase of Experiment 1.

(ANOVA) of the data summarized in Figure 1. The effect of sessions was statistically significant,  $F(5, 120) = 30$ ,  $p < .001$ . Neither the effect of group nor the Group  $\times$  Sessions interaction was statistically significant ( $F_s < 1$ ).

#### Probe Tests

Rats assigned to each test group were treated identically on each of the four test sessions. Figure 2 displays the mean ( $\pm 1$  SEM) TFLs for each group at each post-pMor infusion test interval, collapsed across the four test sessions. As is apparent in Figure 2, the shortest response latencies were observed in the 10% pMor group.

A mixed-design ANOVA of the data summarized in Figure 2 revealed a significant dose effect,  $F(3, 24) = 9.62$ ,  $p < .001$ . Subsequent pairwise comparisons (Tukey's honestly significant difference test [HSD]) indicated that rats in the 10% pMor group displayed shorter TFLs than did rats in each of the other groups (all  $ps < .03$ ). None of the pairwise comparisons involving groups 0% pMor, 5% pMor, and 20% pMor were statistically significant.

The results of Experiment 1 indicated that rats with a history of LMor infusions display hyperalgesia in response to a 10% pMor, confirming the results of previous experiments (Kim & Siegel, 2001; Kim et al., 1999). We have interpreted this hyperalgesia as a CCR elicited by DOCS. In this earlier research, the choice of the first 10% of the LMor infusion as an effective DOC was arbitrary. The results of Experiment 1 indicate that it was a fortuitous choice.

Because the experiment was designed to assess the effectiveness of various proportions of the LMor infusion in eliciting conditional hyperalgesia, infusion duration and infused dose were necessarily confounded in the various pMor groups. Moreover, only a limited range of pMor values were evaluated thus although the 10% pMor was a more effective DOC than was the smaller (5% pMor) or larger (20% pMor) pMor values, it is possible that another combination of morphine dose and/or infusion rate would be an even more effective DOC. Nevertheless, because the results of Experiment 1 indicated that a 10% pMor elicits greater hyperalgesia than

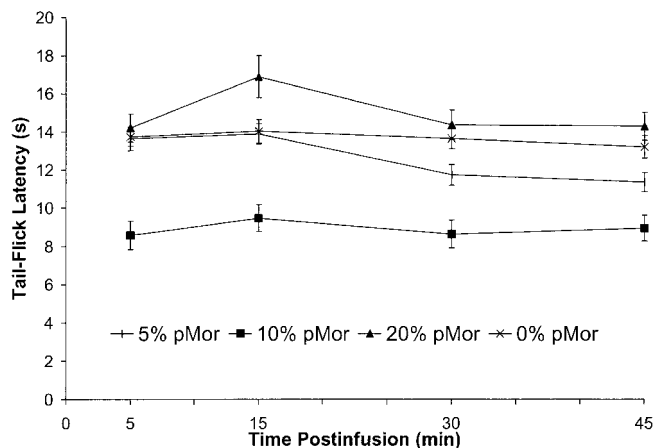


Figure 2. Mean tail-flick latencies ( $\pm 1$  SEM) for each group at each postprobe morphine (pMor) infusion test interval during the probe test of Experiment 1.

the smaller or larger proportion of the LMor infusion evaluated in the experiment, this 10% pMor DOC was used in Experiment 2.

### Experiment 2

The conditioning analysis of tolerance has generated a considerable amount of research. Many findings support the model, but some do not. For example, although there are numerous reports that the drug-experienced organism does not display tolerance in an environment not previously paired with drug administration (a nondrug environment), there are some reports to the contrary. That is, the animal with a history of drug administration may display about the same level of tolerance in the drug-paired environment as in an alternative environment (e.g., Griffiths & Goudie, 1986; Pinel & Puttaswamaiah, 1985; Sherman, 1979; Wolgin & Benson, 1991).

There are several reasons why tolerance, although associative, may be seen in a nondrug environment. For example, some drug-administration signals are common to the drug-paired and nondrug environments (e.g., handling, insertion of the hypodermic needle; see Dafters & Bach, 1985). Of special relevance to the present experiments are suggestions (e.g., Dworkin, 1993; Goudie, 1990; Kim et al., 1999; Siegel et al., 2000; Walter & Riccio, 1983) that tolerance may be seen in the nondrug environment, because the DOCs that enter into intraadministration associations are especially salient, and these DOCs overshadow (Kamin, 1969; Pavlov, 1927, pp. 142–143 and pp. 269–270) simultaneously present environmental cues. Unlike typical exteroceptive CSs (which likely generalize to stimuli encountered outside the conditioning situation), DOCs are both novel and presented in a perfectly positively contingent manner with the subsequent drug effect. Also, there is evidence that CSs that are physically similar to the USs with which they are paired are especially salient (see review by Mackintosh, 1983, pp. 213–214), and the CS and US that are paired to form an intraadministration association are very similar indeed. Pre-drug cues can be characterized as compound stimuli with both environmental and interoceptive elements (exteroceptive cues and DOCs,

respectively). Thus, tolerance seen in a nondrug environment may be mediated by CCRs elicited by highly salient DOCs that overshadow less salient environmental cues.

According to an intraadministration association interpretation of trans-environmental tolerance, tolerance should be displayed in the nondrug environment when the drug has been administered in a way that promotes the association between the early drug effect and the later, larger drug effect. In agreement with this prediction, Grisel, Wiertlak, Watkins, and Maier (1994) demonstrated that rats with a history of subcutaneous morphine administrations, but not rats with a history of intravenous drug administrations, display analgesic tolerance when they are assessed in a nondrug environment. Grisel et al. reasoned that the relatively more gradual onset of the subcutaneous opiate effect (compared with the intravenous opiate effect) resulted in an association between DOCs and the later, larger drug effect, and these pharmacological cues overshadowed simultaneously present environmental cues. However, as discussed by Grisel et al., the kinetics of morphine action after subcutaneous administration, rather than after intravenous administration, differ in a number of ways that might complicate interpretation of their findings.

Kim et al. (1999, Experiment 1) avoided the complications of comparing across different routes of administration by comparing two types of intravenous administration of the same dose of morphine: the slow rate of infusion used during the tolerance-development phase of Experiment 1 (LMor) or a rapid intravenous infusion occurring at over 100 times the speed of the LMor infusion (the more rapid infusion being termed short morphine, SMor). Kim et al. found that tolerance to LMor, but not tolerance to SMor, was apparent in the nondrug environment. On the basis of an associative interpretation, DOCs served as a highly salient CS in the LMor condition (where they signaled a subsequent larger drug effect) but not in the SMor condition (because the maximum effect of the drug occurred so rapidly that it was not effectively signaled by a pharmacological cue). The purpose of Experiment 2 was to essentially replicate the design of the Kim et al. (Experiment 1) study but test animals with pMor in the presence of exteroceptive nondrug cues. We would expect that pMor should elicit a CCR of hyperalgesia in rats that had acquired tolerance to LMor infusions but not in rats that had acquired tolerance to SMor infusions. On the basis of results of Experiment 1, a 10% pMor was used in this experiment to elicit a CCR.

The design of Experiment 2, like that of Kim et al. (1999, Experiment 1), used the “discriminative control of tolerance” procedure (Siegel, 1983). During the tolerance-development phase of Experiment 2, two groups of rats received 24 intravenous infusions—6 morphine infusions and 18 saline infusions. Each morphine infusion was preceded by a distinctive exteroceptive cue (CS+). Similarly, each saline infusion was preceded by another distinctive exteroceptive cue (CS−). The two morphine groups differed with respect to the rate of infusion, long or short (LMor and SMor, respectively). Two additional groups of rats also received 6 infusions in the presence of CS+ and 18 infusions in the presence of CS−, but the infused substance was always physiological saline. These two saline groups also differed with respect to infusion duration (either long or short saline infusions, LSaI and SSaI groups, respectively).

## Method

### Subjects, Surgical Preparation, and Apparatus

The subjects were 30 experimentally naive rats of the same gender, strain, and age as those used in Experiment 1. All rats were implanted with chronic intravenous cannulae as described previously. The chamber and apparatus used for intravenous infusions were the same as those described previously. Each chamber was equipped with a houselight and speaker. The houselight was provided by a 15-W (nominal at 120 VAC) bulb (luminance was approximately 225 cd/m<sup>2</sup>). Flashing the houselight (3 flashes/s) constituted the CS+. A clicking sound (5 clicks/s) constituted the CS-. The clicks were generated by a Scientific Prototype (New York, NY) Model 4041J click generator, set at a nominal scale intensity volume of 2, which corresponded to a volume of approximately 6 dB(C) above the ambient background of 73 dB SPL (C). The CS+ and CS- were the same as those used by Kim et al. (1999). The tail-flick assessment used in this experiment was the same as that used in Experiment 1. Eight rats were assigned to each of the morphine groups (LMor and SMor), and 7 rats were assigned to each of the saline groups (LSal and SSal).

### Procedure

The experiment consisted of two phases: tolerance development and CCR test. The design of the experiment is summarized in Table 1.

**Tolerance development.** Rats received two trials on each of 12 days, with about 5 hr between trials. On even-numbered days, both trials con-

sisted of presentations of CS-. On odd-numbered days, the first trial consisted of presentation of CS-, and the second trial consisted of presentation of CS+. For all rats, presentation of CS- was followed by saline infusion. For rats assigned to LMor and SMor groups, CS+ presentations were followed by infusion of morphine. For rats assigned to LSal and SSal groups, CS+ (like CS-) was followed by a saline infusion.

The concentration of the morphine solution for LMor, SMor, and pMor infusions was 5 mg/ml. As in Experiment 1, the LMor infusion rate was 0.0166 ml/min. The SMor infusion rate was 1.7 ml/min. For both infusions, the dose of morphine administered was 5.0 mg/kg, and the exact duration of the infusions depended on the weight of the rat. The mean duration of the LMor and SMor infusions was approximately 26 min and 15 s, respectively. As in Experiment 1, the pMor infusion consisted of the first 10% of the LMor infusion (i.e., 0.5 mg/kg infused at a rate of 0.0166 ml/min for a duration of approximately 2.6 min).

On each tolerance-development session, a rat was placed in the chamber, its cannula was flushed with heparinized saline (as described in Experiment 1), and the cannula was connected to the syringe pump. The CS was then presented for 15 min. Coincidental with CS termination, the infusion started. Following completion of the infusion, the cannula connector was disconnected from the rat, the cannula was filled with a dextrose solution (as also described in Experiment 1), and the rat remained in the chamber for an additional 90 min before being returned to its home cage.

To minimize the possibility of tissue damage, morphine-induced analgesia was assessed following every second morphine infusion (i.e., following the first, third, and fifth infusion of the opiate, corresponding to Days 1, 5, and 9 of tolerance development). In addition, analgesia level following saline infusion was determined for rats in the two saline groups at the corresponding times. As was the case in the Kim et al. (1999) experiment, TFL (from 50 °C water) was assessed on three occasions following infusion: immediately after the infusion (0 min) and again at 45 and 90 min after the infusion. For the 0-min and 45-min determinations, the rat was briefly removed from the conditioning chamber for TFL assessment and then returned to the chamber. Following the 90-min determination of TFL, the rat was returned to its home cage.

**CCR test.** Following the tolerance-acquisition phase, all subjects were infused with pMor in the presence of the saline-associated exteroceptive cue (CS-). On the basis of the compound-CS analysis of predrug signal, the presentation of exteroceptive cue signaling saline administration (CS-) with the interoceptive pharmacological cue (the DOC) should induce a hyperalgesic response in LMor rats but not in SMor rats. Tail-flick latencies were assessed at 5, 15, 30, and 45 min post-pMor infusion. As was the case in Experiment 1 and in the Kim et al. (1999) study, the water temperature (which had been 50 °C for TFL determinations during tolerance development) was reduced to 48 °C during assessment of pMor-elicited hyperalgesia.

## Results

### Tolerance Development

Analgesia was assessed at 0, 45, and 90 min following infusion on the first, third, and fifth tolerance-development sessions. The mean TFLs ( $\pm 1$  SEM) for each group for each postinfusion assessment are shown in Figure 3.

The data summarized in Figure 3 were subjected to a mixed-design ANOVA. The statistical analyses confirmed the trends apparent in the figure: (a) The two morphine-injected groups (that differed infusion duration) displayed similar levels of responsivity to the thermal stimulation, as did the two saline-injected groups ( $F_s < 1$ ); (b) morphine had an analgesic effect, that is, tail-flick latencies were longer for morphine- than for saline-injected rats (combined across infusion durations),  $F(1, 28) = 331, p < .001$ ;

Table 1  
Design of Experiment 2

Phase of Experiment		
Tolerance development		CCR test <sup>a</sup> (Day 13)
Odd days <sup>b</sup>	Even days <sup>c</sup>	
Morphine groups		
CS- → LSal and CS+ → LMor	CS- → LSal and CS- → LSal	CS- → pMor
CS- → SSal and CS+ → SMor	CS- → SSal and CS- → SSal	CS- → pMor
Saline groups		
CS- → LSal and CS+ → LSal	CS- → LSal and CS- → LSal	CS- → pMor
CS- → SSal and CS+ → SSal	CS- → SSal and CS- → SSal	CS- → pMor

**Note.** CS = conditioned stimulus. CCR = conditional compensatory responses.

<sup>a</sup> Rats were infused with the first 10% of the long morphine (LMor) infusion (probe morphine [pMor]) following CS- presentation. <sup>b</sup> Subjects in morphine groups were presented with CS+ (a flashing light) prior to either long or short morphine infusion (LMor and SMor, respectively) and CS- (a clicking sound) prior to physiological saline administration delivered in the same rates as morphine. Rats in saline groups received infusions of physiological saline via either the long or short delivery rate (LSal and SSal, respectively) following both CS+ and CS- presentations. <sup>c</sup> Rats were administered physiological saline at either the long (LMor and LSal) or short (SMor and SSal) infusion rate preceded by CS-.

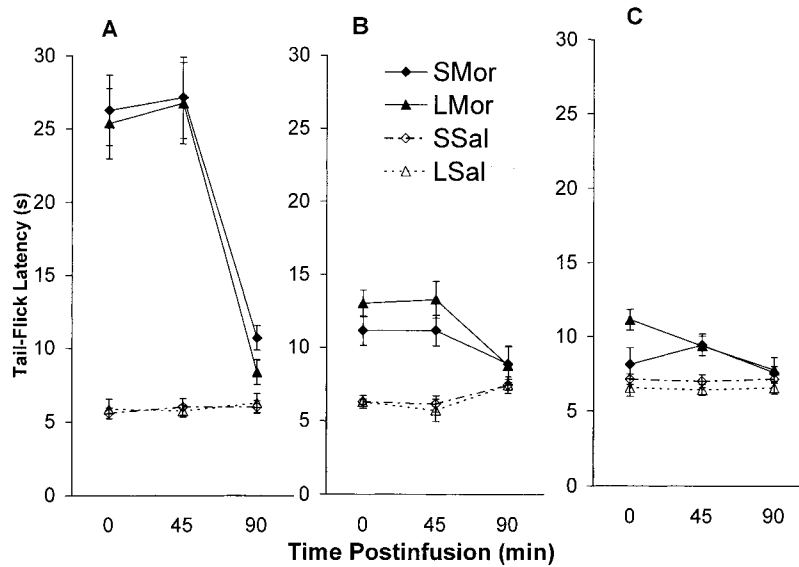


Figure 3. Mean tail-flick latencies ( $\pm 1$  SEM) during the tolerance-development phase of Experiment 2. Rats were administered 5.0 mg/kg morphine as either a long or a short intravenous infusion (LMor and SMor, respectively) or were administered physiological saline at the long or short infusion durations (LSal and SSal, respectively). Tail-flick latencies were determined at 0, 45, and 90 min following the first (A), third (B), or fifth (C) tolerance-development session.

(c) tolerance developed, that is, the Drug  $\times$  Session interaction was significant,  $F(2, 56) = 109, p < .001$ , and the analgesic effect of morphine (but not saline) decreased from the first to the fifth tolerance-acquisition assessment (unequal  $n$  HSD tests,  $p < .001$  for morphine,  $p > .3$  for saline).

CCR Test

On the CCR test all rats were presented with CS- for 15 min prior to the 10% pMor infusion. The mean TFLs ( $\pm 1$  SEM) for

each group for each postinfusion assessment are shown in Figure 4. As can be seen in Figure 4, LMor rats displayed more rapid TFLs than did rats in the other groups. A mixed-design ANOVA of the data summarized in Figure 4 indicated a significant groups effect,  $F(3, 26) = 11, p < .001$ . Pairwise comparisons (unequal  $n$  HSD tests) indicated that the difference between LMor rats and rats assigned to each of the other groups was significant (all  $ps < .04$ ). None of the pairwise comparisons between SMor, SSal, and LSal groups were statistically significant (all  $ps > .40$ ).

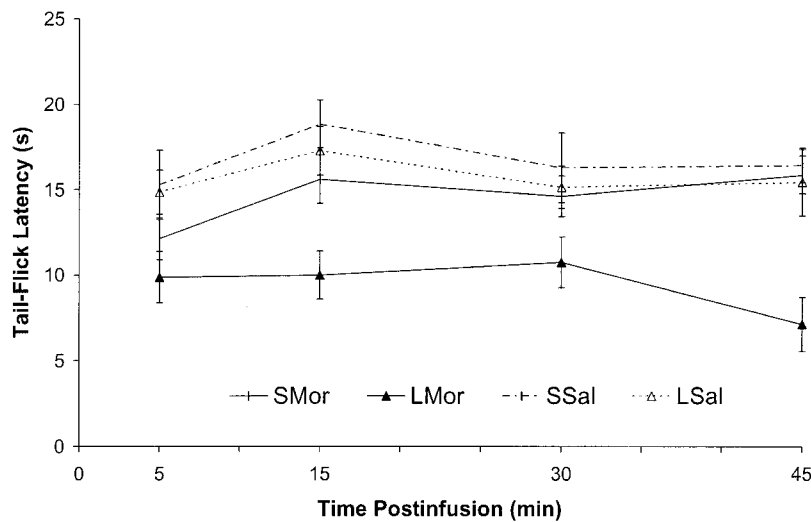


Figure 4. Mean tail-flick latencies ( $\pm 1$  SEM) for each group for each postinfusion assessment during the conditional compensatory responses test of Experiment 2. SMor = short intravenous morphine infusion; LMor = long intravenous morphine infusion; SSal = short physiological saline; LSal = long physiological saline.

### Discussion

Results of prior research demonstrated that LMor rats, but not SMor rats, displayed tolerance when the drug was administered following CS– (Kim et al., 1999). It was hypothesized that this tolerance was mediated by a CCR elicited by DOCs in LMor rats. The results of Experiment 2 demonstrated this hypothesized CCR. LMor rats, but not SMor rats, display hyperalgesia in response to 10% pMor (a pharmacological stimulus designed to duplicate DOCs).

The results of Experiment 2 further implicate intraadministration associations in tolerance. With such intraadministration associations, the CS and US are intrinsic parts of the same stimulus. This is in contrast with the typical Pavlovian conditioning situation in which the CS and US are two very different stimuli presented in different modalities (e.g., light and shock). Dworkin (1993) distinguished between these two types of conditioning situations. He applied the term *heteroreflexes* (“heterotopic conditioned reflexes”) to the traditional, two-stimulus conditioning preparation and distinguished heteroreflexes from *homoreflexes* (“homotopic conditioned reflexes”). In the case of homoreflexes, the CS and US are presented in the same modality and differ only in intensity. The type of learning studied in the present experiments, in which (within each administration) DOCs serve as cues for a later drug effect, is an example of a homoreflex.

As discussed by Dworkin (1993), “the heteroreflex makes for a clearer and more dramatic experimental demonstration. . . . Ultimately, however, homoreflexes may prove to be more basic and more ubiquitous” (p. 79). Although homoreflexes may be basic and ubiquitous, the experimental analysis of this type of conditioning presents special methodological challenges. The homoreflex CS is not, like the typical heteroreflex CS, “neutral”—rather, the homoreflex CS is a less intense version of the US. We know little about the optimal way of evaluating conditional responding with this sort of CS. For example, in the case of homoreflexes (and in contrast with heteroreflexes) the CS is an inherent part of the US. As discussed by Dworkin, the traditional control procedures used by learning researchers, which have been developed in heteroreflex studies, are not readily applicable to the study of homoreflexes (e.g., unpaired CS–US control groups). As suggested by Kim et al. (1999), “future research on homoreflexes in general, and intra-administration associations in particular, will have to develop control procedures suitable to this type of learning” (p. 502). Experiment 3 was conducted to evaluate CCRs elicited by DOCs using such a control procedure.

### Experiment 3

We have suggested that pMor duplicates DOCs that had previously signaled a larger effect of morphine and that pMor-elicited hyperalgesia is a CCR elicited by these DOCs. However, it is possible that this hyperalgesia represents a sensitized response, rather than a CR. For example, a small morphine dose (such as pMor) might elicit hyperactivity as a nonassociative, sensitized response in morphine-experienced rats (e.g., Powell & Holtzman, 2001), and the short response latencies seen in response to pMor on the tail-flick test may be secondary to this hyperactivity. The results of Experiment 2 suggest that the effects of pMor are indeed associative, as this small dose of the drug elicited hyperalgesia in

LMor rats, but not in SMor rats. Prior to the test, rats in both groups received the same dose of the drug (5.0 mg/kg) equally often (six times) and at the same intervals (once every other day). However, it is conceivable that the protracted opiate effect resulting from the LMor administration procedure favored the development of sensitized responding more than did the rapid effect resulting from the SMor administration procedure. The purpose of Experiment 3 was to evaluate the ability of DOCs to elicit CCRs in a preparation not subject to an alternative nonassociative interpretation, such as sensitization.

On the basis of an intraadministration analysis, the putative CS (DOCs) and US (later, larger drug effect) are inevitably paired with each other. Experiment 3 used a procedure in which the pharmacological CSs and USs need not be presented in the order that is inevitably present in intraadministration association studies. To evaluate the role of sensitized responding in pharmacological cueing, Experiment 3 used an intradrag conditioning procedure, rather than the intraadministration procedure used in Experiments 1 and 2. The two types of pharmacological conditioning procedures were distinguished by Siegel et al. (2000). Intraadministration associations are hypothesized to inevitably form following certain types of drug administration and are assessed by presenting the small, early drug effect on a test trial. Intradrag associations are explicitly trained by administering a small dose of a drug prior to a larger dose of that same drug. There is evidence that, following such paired presentations of two doses of the same drug, the first, smaller dose serves as a cue for the later, larger dose (Cepeda-Benito & Short, 1997; Greeley, Lê, Poulos, & Cappell, 1984). For example, Greeley et al. (1984) used an intradrag conditioning procedure to demonstrate that a small dose of ethanol could serve as a CS for a larger dose of ethanol. Rats in one group (paired) were intraperitoneally injected with a low dose of ethanol (0.8 g/kg) 60 min prior to a high dose of ethanol (2.5 g/kg). Another group of rats (unpaired) received the low and high doses on an unpaired basis. When tested for the tolerance to the hypothermic effect of the high dose following the low dose, paired rats, but not unpaired rats, displayed tolerance. Moreover, if the high dose of ethanol was not preceded by the low dose, paired rats failed to display their usual tolerance. This tolerance, dependent on an ethanol–ethanol pairing, was apparently mediated by a thermic CCR; paired rats, but not unpaired rats, evidenced hyperthermia in response to the low dose of ethanol.

In Experiment 3 we used a procedure, with intravenous morphine, similar to that used by Greeley et al. (1984) with intraperitoneal ethanol. During each day of the tolerance-development phase of Experiment 3, rats assigned to a forward-paired group received a brief intravenous infusion of a small dose of morphine (1.0 mg/kg) 10 min prior to a brief intravenous infusion of a large dose of the opiate (10.0 mg/kg). Rats assigned to a backward-paired group received the large dose 10 min prior to the small dose. Following tolerance development, the effect of 1.0 mg/kg intravenous morphine on TFL was assessed. If the small dose serves as a CS, it would be expected that forward-paired rats should display hyperalgesia on the test session. However, if this hyperalgesia results from sensitization to the drug, it would be expected that the equivalently drug-exposed backward-paired rats should also display hyperalgesia on the test session.

## Method

### Drugs and Design

Depending on group assignment and phase of the experiment, rats received various intravenous infusions: a small dose of morphine (m; 1.0 mg/kg), a large dose of morphine (M; 10.0 mg/kg), or physiological saline. The saline infusions were volumetrically equated with either the m infusion or the M infusion (s and S, respectively). The parametric characteristics of the infusions used in Experiment 3 are summarized in Table 2. The exact duration of all infusions depended on the weight of the rat but ranged from approximately 17 to 21 s.

The design of Experiment 3 is summarized in Table 3. Rats were assigned to one of six independent groups. All rats received eight daily sessions during the tolerance-development phase of the experiment. During each tolerance-development session, rats received two intravenous infusions, with a 10-min interval between the infusions. Groups differed with respect to the content of each of the tolerance-development infusions—either m, M, or S, and the order in which the substances were infused. A CCR test session was conducted on the day following the last tolerance-development session. For this test session, rats received a single infusion, and TFL was assessed. Groups differed with respect to the substance infused on the CCR test session, m or s. The tolerance-development and testing conditions are indicated by group abbreviations, thus rats in group mM–m were forward-paired experimental rats. These mM–m rats received m followed by M during each tolerance-development session and were tested with m on the CCR test session. Rats assigned to the Mm–m group were backward-paired experimental rats. These Mm–m rats were treated like mM–m rats, except the order of the small and large doses was reversed during tolerance development. Control rats assigned to the mM–s and Mm–s groups were treated like rats assigned to the mM–m and Mm–m groups, respectively, during tolerance development, but they were infused with saline, rather than the opiate, on the CCR test session. Control rats assigned to the remaining two groups, mS–m and Sm–m, were tested with m on the test session but had no pretest experience with the large morphine dose. Rather, these rats received the small morphine dose either 10 min before (mS–m) or 10 min after (Sm–m) a saline infusion on each tolerance-development session.

### Subjects, Surgical Preparation, and Apparatus

Eighty-three experimentally naive rats of the same gender and strain as those used in Experiments 1 and 2 (weighing 275–375 g at the start of the experiment) were implanted with chronic intravenous cannulae. In Experiment 3 the cannula design was modified from that used in Experiments 1 and 2 such that the cannula exited from the rat's back (rather than the top of its head). The catheters used in Experiment 3 were assembled from a commercially available guide cannula assembly (Plastic One, Roanoke, VA; Model C313G). Twenty centimeters of silastic tubing (used for the cannula in Experiments 1 and 2) were attached to the guide cannula. Under

Table 2  
Infusions Used in Experiment 3

Infused substance	Morphine concentration (mg/ml)	Infusion rate (ml/min)	Volume of infusion (ml/kg)	Morphine dose (mg/kg)
M	5	2.70	2.0	10
m	5	0.24	0.2	1
S	0	2.70	2.0	0
s	0	0.24	0.2	0

Note. M = large dose of morphine; m = small dose of morphine; S = large dose of saline; s = small dose of saline.

general anesthetic the tip of the catheter was implanted into the right jugular vein, approximately 1 cm from the heart, and the cannula was brought out to the rat's back and secured between shoulder blades. Rats were randomly assigned to one of the six groups indicated in Table 3. The apparatus used for intravenous infusions and the assessment of TFL were the same as those described previously.

### Procedure

**Tolerance development.** Prior to each tolerance-development session, the rat's cannula was flushed with heparinized saline, and at the end of the session, the cannula was filled with dextrose solution (as described in Experiment 1).

On each of the eight tolerance-development sessions, rats received two infusions, with the content of the infusions for rats assigned to each of the six groups indicated in Table 3. At the start of each tolerance-development session, the rat was placed in the infusion chamber, with its cannula attached to the infusion pump. Fifteen minutes later, rats were intravenously infused with either 1.0 mg/kg morphine (mM–m, mM–s, and mS–m groups), 10.0 mg/kg morphine (Mm–m and Mm–s groups), or saline (Sm–m group). Ten minutes after the termination of the first infusion, each rat was infused with either 10.0 mg/kg morphine (mM–m and mM–s groups), 1.0 mg/kg morphine (Mm–m, Mm–s, and Sm–m groups), or saline (mS–m group).

Following the second infusion, the rat was detached from the apparatus and was kept in the infusion chamber for 30 min. Thirty minutes after the second infusion, TFL (from 50 °C water) was assessed, and the rat was returned to its home cage.

**CCR test.** The CCR test was conducted on the day following the last tolerance-development session. Rats were infused either with 1.0 mg/kg morphine (mM–m, Mm–m, mS–m, and Sm–m groups) or saline (mM–s and Mm–s groups). Tail-flick latency (from 48 °C water) was measured at 15, 30, 45, and 75 min postinfusion.

## Results

### Tolerance Development

The mean TFLs ( $\pm 1$  SEM) seen in all groups for each of the eight tolerance-development sessions are shown in Figure 5. Rats infused with a total of 11.0 mg/kg during each tolerance-development session (the four groups receiving m and M during each session, in whatever order) displayed substantial analgesia, compared with rats infused with only 1.0 mg/kg morphine (the two groups receiving m and S during each session, in whatever order). Moreover, rats infused with 11.0 mg/kg during each session displayed analgesic tolerance over the eight sessions.

A mixed-design ANOVA of the data summarized in Figure 5 indicated a significant groups effect,  $F(5, 77) = 44, p < .001$ . Tukey's unequal  $n$  HSD tests indicated that rats in all four groups infused both m and M on each session (mM–m, Mm–m, mM–s, and Mm–s groups) displayed longer TFLs than did rats in either group infused with m and S on each session (mS–m and Sm–m groups; all  $ps < .001$ ).

The ANOVA also revealed a significant Groups  $\times$  Sessions interaction,  $F(35, 539) = 14, p < .001$ . As may be seen in Figure 5, this interaction resulted because rats in the four groups infused with 11.0 mg/kg morphine on each tolerance-development session, but not rats infused with 1.0 mg/kg morphine on each session, displayed decreased TFLs across sessions. A mixed-design ANOVA of only the four groups infused both m and M on each session revealed a significant sessions effect,  $F(7, 371) =$

Table 3  
Design of Experiment 3

Group	Tolerance development <sup>a</sup>	CCR test	n
mM-m	morphine (1 mg/kg) → Morphine (10 mg/kg)	morphine (1 mg/kg)	14
Mm-m	Morphine (10 mg/kg) → morphine (1 mg/kg)	morphine (1 mg/kg)	14
mM-s	morphine (1 mg/kg) → Morphine (10 mg/kg)	Saline	14
Mm-s	Morphine (10 mg/kg) → morphine (1 mg/kg)	Saline	15
mS-m	morphine (1 mg/kg) → Saline	morphine (1 mg/kg)	13
Sm-m	Saline → morphine (1 mg/kg)	morphine (1 mg/kg)	13

Note. m = small dose of morphine; M = large dose of morphine; s = small dose of saline; S = large dose of saline; CCR = conditional compensatory responses.

<sup>a</sup> There was a 10-min interval between the two infusions on each tolerance-development session.

136,  $p < .001$ . A similar analysis for the two groups infused with m and S on each tolerance-development session revealed no significant sessions effects,  $F(7, 168) = 1.4$ ,  $p = .20$ .

### CCR Test

Two control groups were tested with m, differing only with respect to the order in which S and m were presented during tolerance development (mS-m and Sm-m groups). There was no appreciable difference in test session TFLs between these two groups, and for simplicity in data presentation, they are collapsed into a combined m-control group. Similarly, the two control groups tested with s that differed only with respect to the order in which M and m were presented during tolerance development (mM-s and Mm-s groups) were combined into an s-control group. The mean TFLs ( $\pm 1$  SEM) for the forward-paired experimental group (mM-m), the backward-paired experimental group (Mm-m), and the two combined control groups for each postinfusion assessment are shown in Figure 6. As can be seen in Figure 6, despite the fact that rats assigned to the mM-m and Mm-m groups

were exposed to both the high and low morphine doses prior to the test, rats assigned to the mM-m group displayed shorter TFLs than did rats assigned to the Mm-m group. A mixed-design ANOVA of the data summarized in Figure 6 indicated a significant groups effect,  $F(3, 79) = 8.0$ ,  $p < .001$ . Subsequent unequal  $n$  HSD tests indicated that, following m infusion, rats assigned to the mM-m group responded significantly more quickly on the CCR test than did rats assigned to the Mm-m group ( $p < .001$ ). Moreover, mM-m rats responded significantly more rapidly than did rats assigned to control groups (all  $ps \leq .05$ ). The control groups did not differ significantly from each other. Examination of Figure 6 indicates that the backward-paired (Mm-m) rats responded more slowly than did rats assigned to control groups, suggesting that the backward pairings resulted in an inhibitory association between the DOC and the later, larger drug effect (Siegel & Domjan, 1971, 1974); however, the differences between Mm-m rats and rats assigned to control groups did not attain conventional levels of statistical significance.

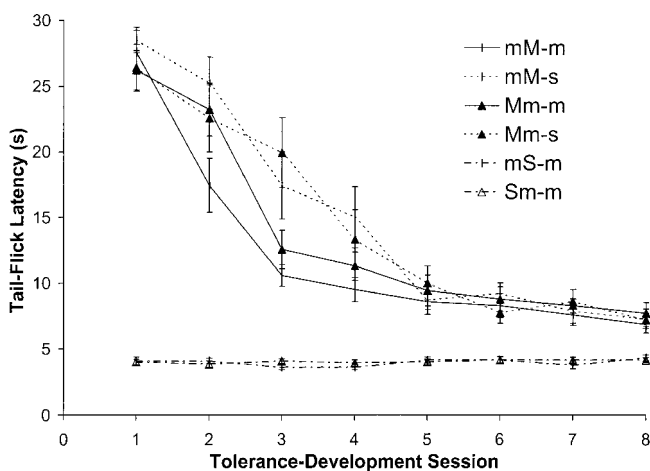


Figure 5. Mean tail-flick latencies ( $\pm 1$  SEM) for each group during the tolerance-development phase of Experiment 3. The first two letters of the group designation indicate the sequence of two infusions that occurred on each tolerance development session (m = 1 mg/kg morphine, M = 10 mg/kg morphine, S = 2 ml/kg physiological saline), and the third letter indicates the substance infused on the conditional compensatory response test session (m = 1 mg/kg morphine, s = 0.2 ml/kg physiological saline).

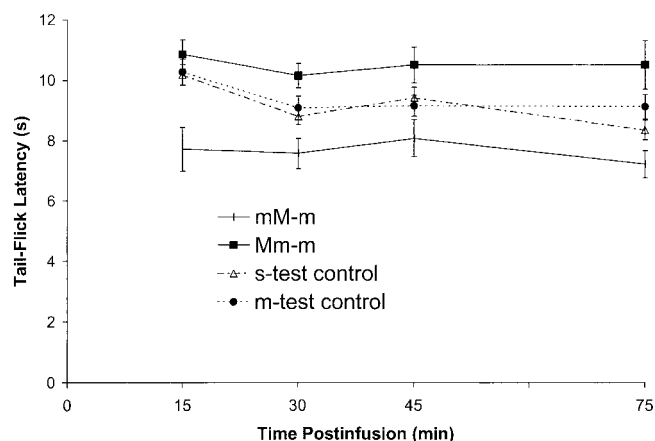


Figure 6. Mean tail-flick latencies ( $\pm 1$  SEM) for each postinfusion assessment for the conditional compensatory responses test of Experiment 3 for the forward-paired experimental group (mM-m; trained with 1 mg/kg morphine followed by 10 mg/kg morphine and tested with 1 mg/kg morphine), the backward-paired experimental group (Mm-m; trained with 10 mg/kg morphine followed by 1 mg/kg morphine and tested with 1 mg/kg morphine), and the two combined control groups (s-test control are groups tested with saline following either mM or Mm training; m-test control are groups tested with 1 mg/kg morphine following mS or Sm training).

### Discussion

The purpose of Experiment 3 was to evaluate whether *m* elicits hyperalgesia, conditional on that small dose having been a signal for *M*. The results indicated such conditional compensatory responding. Rats in a forward-paired experimental group received tolerance-development sessions in which 1.0 mg/kg morphine was intravenously administered 10 min prior to 10.0 mg/kg morphine. Rats in a backward-paired experimental group received the two doses of the opiate in the reverse order. When TFLs subsequently were assessed following 1.0 mg/kg morphine, forward-paired rats (*mM*-*m* group) responded more quickly than did backward-paired rats (*Mm*-*m* group), despite similar pretest exposure to morphine. In fact, *mM*-*m* group rats responded to *m* on the CCR test with shorter latencies than did (a) rats tested with *m* but with no prior exposure to *M* (*mS*-*m* and *Sm*-*m* groups) and (b) rats with prior exposure to both *m* and *M* but tested with *s* (*mM*-*s* and *Mm*-*s* groups). The results indicate that *m* elicited a CCR of hyperalgesia in *mM*-*m* rats.

The association that develops between a small dose of a drug administered prior to a larger dose of that same drug has been termed an *intradrug association* (Siegel et al., 2000). The results of the present experiment, demonstrating an intradrag association as revealed by conditional compensatory responding in rats trained with intravenously infused morphine, are similar to results previously reported by Greeley et al. (1984) in rats trained with intraperitoneally injected ethanol. Greeley et al. reported that a CCR of hyperthermia was apparent in response to the smaller dose of ethanol that previously had signaled a larger dose of ethanol (and its hypothermic effect). Neither the results reported here nor the Greeley et al. results are readily explained by a nonassociative interpretation, such as drug sensitization. For example, in the present experiment both *mM*-*m* and *Mm*-*m* rats have the same pretest exposure to morphine—they should be equally sensitized to the effects of the opiate. The fact that *mM*-*m* rats but not *Mm*-*m* rats displayed hyperalgesia in response to *m* indicates that the order in which *m* and *M* are presented prior to the test determines whether *m* elicits hyperalgesia (as would be expected if an intradrag association formed during tolerance development). The results of Experiment 3 support the suggestion that the hyperalgesia seen in the response to the drug-onset cue in rats with a history of gradual morphine infusions (Experiments 1 and 2 of this article; Kim & Siegel, 2001; Kim et al., 1999) results from an intradrag administration association, rather than drug sensitization.

### General Discussion

According to a conditioning analysis, chronic drug tolerance results because cues present at the time of drug administration function as CSs and elicit CCRs that attenuate the effect of the drug. Several researchers have hypothesized that, within each drug administration, DOCs become associated with the later, larger drug effect and that these DOCs, in common with exteroceptive cues, are CSs that elicit CCRs (e.g., Goddard, 1999; King, Bouton, & Musty, 1987; Mackintosh, 1987; Tiffany, Petrie, Baker, & Dahl, 1983). Recently, Kim et al. (1999) provided evidence for such an intradrag administration association. They demonstrated that following the development of analgesic tolerance acquired by repeated gradual intravenous infusions of morphine, rats respond with a CCR of

hyperalgesia following a *pMor* infusion—an infusion consisting of only the initial 10% of the gradual morphine infusion used.

Kim et al. (1999) acknowledged that there was some arbitrariness in selecting as a DOC the first 10% of the longer morphine infusion used during tolerance development. That is, a *pMor* infusion of a different duration and/or dose may better capture the CS properties of the initial drug effect. However, in the case of an intradrag administration association (as in the case of other homotopic CCRs; see Dworkin, 1993) the fact that the CS is simply a weaker version of the US complicates analysis of effective CS characteristics. For example, increasing the intensity of the *pMor* cue (to increase its salience and thus its ability to conditionally elicit hyperalgesia) also increases its unconditional analgesic effect. Experiment 1 was designed to evaluate the effectiveness of various *pMor* infusions in eliciting conditional hyperalgesia. The concentration of morphine used in the *pMor* assessment was the same as that used during tolerance development, and various lengths (and thus doses) of *pMor* were evaluated: 5%, 10%, and 20% of the gradual infusion used during pretest tolerance-development sessions. The results of Experiment 1 indicated that the 10% *pMor* was more effective than the shorter or longer versions of the pharmacological CS, thus this 10% *pMor* was used in subsequent experiments.

Kim et al. (1999) reasoned that DOCs are more salient than simultaneously presented environmental cues; thus, if the drug is administered in a way that promotes the development of DOCs as signals for the later and larger drug effect, there may be little evidence of environmentally specific tolerance. Kim et al. developed two morphine administration procedures, both involving intravenous infusion of 5.0 mg/kg of the opioid, that differed only in terms of infusion duration. The *LMor* infusion, but not the *SMor* infusion, should promote the development of intradrag administration associations. As would be expected if DOCs overshadow environmental cues, Kim et al. found that morphine tolerance was seen when the drug was administered following a nondrug cue (CS-) when rats were trained and tested with *LMor* infusions but not when rats were trained and tested with *SMor* infusions. Experiment 2 was designed to assess whether the tolerance seen following *LMor* infusions (but not following *SMor* infusions) in the presence of CS- results because *LMor*-trained rats (but not *SMor*-trained rats) learn an intradrag administration association. In Experiment 2, following tolerance development, both *LMor*- and *SMor*-trained rats were infused with *pMor* following CS- presentation. Only *LMor* rats displayed conditional compensatory responding. Thus, the results of Experiment 2 confirm Kim et al.'s suggestion that tolerance seen in a nondrug environment is mediated by CCRs elicited by DOCs.

In Experiments 1 and 2, we (like Kim et al., 1999) suggested that the *pMor*-elicited hyperalgesia seen in rats with a history of *LMor* administration is a CCR elicited by DOCs. However, it is possible that this *pMor*-elicited hypersensitivity to nociceptive stimulation is a manifestation of a nonassociative sensitized response, rather than an intradrag administration association. Experiment 3 was designed to determine whether *pMor*-elicited hyperalgesia in morphine-experienced rats represents an unconditionally elicited sensitized response or a CCR. During the tolerance-development phase of Experiment 3, rats received an intravenous infusion of *m* (1.0 mg/kg) either before (forward) or after (backward) infusion of a *M* (10.0 mg/kg). Following tolerance devel-

opment, rats in the forward group displayed hyperalgesia in response to m. Inasmuch as both forward and backward rats had the same exposure to morphine during tolerance development, the hyperalgesia seen in forward rats likely represents an associative effect rather than a nonassociative effect.

The results of Experiments 1–3 complement those presented in prior reports of intraadministration associations (Kim & Siegel, 2001; Kim et al., 1999) and intradrag associations (Cepeda-Benito & Short, 1997; Greeley et al., 1984). It is clear that a small dose of a drug can serve as a cue for a larger dose of that drug, and such associations form (even if there are no experimenter-presented pairings of pharmacological CS and US) if the drug-administration procedure results in a protracted period of drug effect—DOCs are ineluctable signals of the subsequent larger drug effect.

Examination of the tolerance development data and CCR test data of these experiments might suggest that the magnitude of conditional compensatory responding is modest, in comparison with the magnitude of tolerance that is hypothesized to be mediated by these CCRs. However, in these studies of intraadministration associations, the CCR is assessed on pMor test trials following LMor administrations (Experiments 1 and 2) or following a 1.0 mg/kg dose of morphine in rats that had previously received 1.0 mg/kg–10.0 mg/kg pairings of the drug (Experiment 3). These procedures correspond to the use of CS-alone test trials to assess conditioning that occurs following CS–US pairings. As noted by several investigators (e.g., Mackintosh, 1983, p. 210; Rescorla, 1980), the extent of conditioning evaluated on CS-alone test trials likely is underestimated because of generalization decrement resulting from the difference between training (CS followed by US) and testing (CS alone) conditions. Furthermore, a distinctive feature of the intraadministration CS is that it unconditionally elicits a response that attenuates the expression of the CCR.

Recognition that intraadministration associations contribute to drug effects not only has important implications for theories of tolerance but also for conditioning-based treatments of drug addiction. There is evidence that the CCRs that mediate tolerance also mediate withdrawal distress and craving (see Childress, McLellan, & O'Brien, 1986; Siegel, 1999); thus, some drug-addiction treatment protocols incorporate procedures to extinguish the association between predrug cues and the drug: "These treatments reflect a logical extension of classical conditioning theory. If addicts' responses to drug-related stimuli reflect CRs, then extinction of these CRs may be achieved through repeated unreinforced exposure to the CS" (Carter & Tiffany, 1999, p. 329). There are mixed reports of the efficacy of such cue-exposure treatments (see Drummond, Tiffany, Glautier, & Remington, 1995; Siegel & Ramos, in press), but generally the results have been disappointing: "The value of these [cue-exposure] procedures in producing clinically meaningful reductions in substance use has been met with only modest success to date" (Carroll, 1999, p. 261). There are many reasons why cue-exposure treatments, as currently implemented, may have met with only limited success (Siegel & Ramos, in press; Ramos, Siegel, & Bueno, in press). Of special relevance to the results of the present experiment is Siegel and Ramos's (in press) observation that these treatments typically do not incorporate extinction of DOCs. As noted by Cepeda-Benito and Short (1997), "the inclusion of small drug doses during cue-exposure treatments may better reproduce the CSs responsible for craving" (p. 239). Indeed, some investigators have described suc-

cessful cue-exposure treatment procedures for problem drinking that incorporate priming doses of alcohol (e.g., Sitharthan, Sitharthan, Hough, & Kavanagh, 1997).

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Received December 5, 2001

Revision received March 27, 2002

Accepted March 28, 2002 ■

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