CONDITIONED STIMULUS CONTROL OF THE PHYSIOLOGICAL EFFECTS OF MORPHINE

by

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ABSTRACT

Classical conditioning has been shown to play a role in how an organism responds to drugs administered chronically. For example, after repeated exposures to drug in the presence of certain cues (e.g., a "distinctive" room), an animal may show tolerance or sensitization to the drug effects only in the presence of those cues. Placebo administration in the presence of drug-paired cues often results in a conditioned response which either mimics or is opposite the drug effect. The purpose of the first experiment was to determine whether drug responses could be conditioned when intravenous morphine administration was paired with an explicit conditioned stimulus (CS).

In the first experiment, adult male rats were implanted with a jugular vein cannula and a biotelemetry device (MiniMitter) for monitoring body temperature. The animals were housed 24 hrs/day in light-and sound-attenuating chambers in which all testing occurred. The CS was a compound light/noise which lasted 15 min. The unconditioned stimulus (US) was an infusion of morphine (5 mg/kg) which has been shown to produce hyperthermia. Rats were assigned to one of two groups. The Paired group (n=7) received morphine 30 sec after the onset of the CS (ISI = 30 sec), and the CS remained on for an additional 14.5 min. The Unpaired group (n=8) received explicitly unpaired presentations of the CS and US (ISI = 90 min). One trial per day was given. Every seventh day, the Paired group received a Delayed-morphine test in which morphine was administered at the end of the 15-min CS to test for a conditioned response. At the end of the training phase, both groups were given morphine with and without the CS.

A learned association between the CS and US in the Paired group was demonstrated by a significant increase in temperature to the CS in the absence of morphine. Also, morphine produced a greater hyperthermic response in the presence of the CS relative to its absence. Furthermore, the Paired group responded with greater hyperthermia relative to the Unpaired group during the CS. In the absence of the CS, there was no difference between the groups in their response to morphine.

The second experiment was designed to determine how CS-US overlap duration affects conditioned changes in both heart rate and body temperature, and to evaluate predictions derived from Wagner's SOP theory (cf., Donegan & Wagner, 1987). Briefly, Wagner's theory suggests that the function relating strength of conditioning to CS-US overlap duration should resemble an inverted U, with optimal conditioning occurring at some intermediate CS duration. This prediction is based on hypothesized changes in the time course of activity in "memory nodes" that are activated by presentation of the CS and US.

Adult male rats were implanted with a cannula, a MiniMitter and heart-rate electrodes. The animals were housed and maintained as in Experiment 1. The CS and US were also the same. Three Paired groups (P5, P15 and P60) and one explicitly unpaired control group (Group U)

were used. As in Experiment 1, the ISI was 30 sec; therefore, duration of CS-US overlap varied among the Paired groups. Group P5 received a 5-min CS. Group P15 received a 15-min CS, and Group P60 received a 60-min CS. Every eleventh day, all rats were given a placebo test in which saline infusion was paired with the CS to test for a conditioned response. At the end of the training phase, all groups were given morphine with and without the CS to test for cue-specific changes in the heart rate and temperature responses.

In agreement with previous results, morphine produced hyperthermia and a biphasic heart rate response: bradycardia followed by tachycardia (Schwarz, Peris & Cunningham, 1987) in all rats. In the Placebo tests, all of the Paired groups showed a hyperthermic and tachycardic response to the CS. However, the temperature response in Group P5 was not significantly different from that of Group U. Both Groups P5 and P15 evidenced cue-specific sensitization to morphine's hyperthermic response and cue-specific tolerance to morphine's bradycardic response. When comparing the response of the Paired groups with that of Group U, all Paired groups showed a significantly faster rate of temperature change relative to Group U during the CS. Only Group P15 showed more tolerance to morphine's bradycardic effect relative to Group U.

With respect to CS duration, the results generally supported predictions based on Wagner's SOP theory. Optimal CS duration was thought to be around 15 min due to overlap of CS and US nodal elements in the A1 state. The 5- and 60-min overlap durations were predicted to result in less excitatory conditioning. Group P15 showed evidence of excitatory learning in both the heart-rate and temperature response in all tests. Groups P5 and P60 showed some excitatory learning, but not in all of the tests, suggesting less net excitatory strength relative to Group P15.

Overall, the results of both experiments clearly show that learning is capable of affecting responses to morphine administered repeatedly. In contrast to previous studies, conditioned hyperthermia was elicited within 15 min by a discrete CS in a situation where the response was not confounded by handling or the stress of injection and where there was an appropriate control for nonassociative factors. Conditioned tachycardia was elicited within 30-sec after CS onset. The results of Experiment 2 illustrate the generality of the results with the addition of the heart rate measurement. These results also contribute to the few studies on CS-US overlap duration.

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Effects of many drugs change as a result of repeated exposure to the drug. These changes may have important implications not only for the therapeutic use of drugs but also for determining the strength of drug-seeking behavior (Goudie & Demellweek, 1986; Jaffe, 1985). Basic pharmacological research has focused on two effects of repeated exposure to drugs: tolerance and sensitization. Tolerance is defined as a reduced drug effect to a given dose following repeated administration of the same dose of drug (Dews, 1978). Tolerance can also be defined by showing that a drug dose, administered repeatedly, must be increased in order to produce effects of equal intensity or duration to those originally observed. Sensitization is another phenomenon that results from chronic drug exposure and is the complement of tolerance (Carlton, 1983). Sensitization is defined as an increased drug effect to a given dose. Sensitization can also be defined by showing that a drug dose must be decreased to reinstate the initial effect.

Pavlovian Conditioning with a Drug US

Learning also alters drug responses by the formation of associations between environmental stimuli (e.g., sights, sounds, smells, people) and the physiological effects of the drug. Pavlov (1927) described how drug injection is a classical conditioning trial. He thought the conditioned stimulus (CS) to be a set of cues including the experimenter, the syringe, and all of the other stimuli surrounding injection of morphine, the unconditioned stimulus (US). After repeated injections of morphine in dogs, these cues developed the ability to elicit restlessness, profuse salivation and nausea (effects similar to those produced by morphine) even when placebo was injected. The greater

the number of previous injections received, the fewer the cues needed to elicit the drug-like response.

Other investigators have also shown classical conditioning of drug effects. Learned changes in heart rate (Bykov, 1957; Rush, Pearson & Lang, 1970) and evoked potential (Stein, Lynch & Rushkin, 1977) have been studied using morphine as the US.

Stein et al. (1977) reported learned changes in cortical evoked potential after repeated pairings of an auditory click with morphine in rats. Generally, the conditioned response (CR) was an increase in the amplitude of the evoked potential to the CS. Stein used a control group which received saline paired with the CS. The major problem with this type of control group is that it does not have the same amount of experience with the US as the experimental group. Differences between these two groups could be attributed solely to the difference in experience with the morphine US (i.e., "nonassociative factors"). There are a variety of more suitable control procedures that equate groups for exposure to both the CS and US (cf. Rescorla, 1967): (1) An explicitly unpaired control group that receives unpaired presentations of the CS and US; (2) A backward conditioning control group that receives paired presentations of the CS and US, but the US always precedes the CS; (3) A discriminative conditioning procedure that uses two CSs--one (CS+) which is always paired with the US and the other (CS-) which is never paired with the US; (4) A truly-random control group that receives presentations of both the CS and US which occur randomly and independently of each other. In this procedure, some CS-US pairings may occur by chance. The relative advantages and disadvantages of each of these procedures have been discussed elsewhere (e.g., Rescorla, 1967).

In a related experiment, Stein (1976) measured heart rate while pairing an auditory click with morphine in rats. Stein gave two trials per day with an intertrial interval of 10-30 min. Each trial lasted 4 min during which a 1-min baseline was measured followed by a 2-min CS presentation. The interstimulus interval was 90 sec, and a 1-min post-drug measurement period followed morphine infusion, the dose of which increased from 0.5 to 60 mg/kg over the course of the experiment. The unconditioned response (UR) was bradycardia to which tolerance developed rapidly within days (i.e., from trial 1 to trial 2 each day) and gradually during the 4 weeks of conditioning. As in the evoked potential study, the control group received saline paired with the CS. Stein reported no learned changes in heart rate as measured during placebo trials. The lack of learning may have been due to the short interval between the two daily trials, i.e., allowing 10 to 30 min between trials was probably not long enough for the drug effects to wear off, thus masking the contingency between the CS and US (Stewart & Eikelboom, 1987), and causing Trial 2 to be both a forward and backward CS-US pairing.

Other reasons for Stein's (1976) failure to observe a heart-rate CR could be due to his conditioning procedure or the parameters he used. For example, he gave the rats 10 pre-exposures to the CS before conditioning which may have produced latent inhibition. Pre-exposure did not deleteriously affect conditioning of an evoked potential (Stein, 1977); however, there are examples in the literature of differences in latent inhibition in various response systems. Fitzgerald and Hoffman (1976) showed that 50 preconditioning exposures to the CS actually resulted in faster heart rate conditioning in rats relative to 0 or 10

pre-exposures. On the other hand, Chacto and Lubow (1967) showed that 20 and 40 pre-exposures to the CS resulted in attenuated conditioning of spontaneous tail movement. Successful latent inhibition has been achieved in heart rate conditioning in rabbits (Gallagher, Meagher & Bostock, 1987).

With respect to conditioning parameters, another reason for Stein's (1976) failure to elicit a heart-rate CR may have been that the ISI was too long (90 sec). Generally, optimal ISI is dependent primarily on the response system (cf. Mackintosh, 1974). Optimal ISI for heart-rate conditioning is said to be 5-20 sec. For conditioning of the nictitating membrane response, the optimal ISI is 200-400 msec, and for conditioned licking, it is 2-4 sec and so on. Thus, even though Stein (1977) reported successful conditioning of an evoked potential and failure to observe heart-rate conditioning, this may have been because the ISI was appropriate for evoked potential conditioning but not for heart-rate conditioning.

An earlier study using dogs demonstrated an increased heart rate CR after pairing a 2-min buzzer CS with a subcutaneous injection of morphine (2 mg/kg) (Rush et al., 1970). Morphine was administered immediately after CS onset. The UR was tachycardia when a low dose of morphine (2 mg/kg) was the US and was tachycardia followed by bradycardia after a high dose of morphine (10 mg/kg). Tachycardia was the CR during a placebo trial when the low dose of morphine had been the US. With the high dose of morphine as the US, the tachycardic CR was inconsistent and variable.

Bykov (1957) reported conditioned bradycardia in dogs that had received repeated pairings of a buzzer CS (duration unspecified) with

subcutaneous morphine (200 mg). Although it is difficult to determine all differences in the experimental protocols of Rush et al. and Bykov, an obvious difference was dose. When the US was 2 mg/kg (e.g. Rush et al.), the CR was tachycardia, opposite in direction to the UR. When the dose was 10-20 mg/kg (e.g., for a 10-20 kg dog; Bykov) the UR was bradycardia (Bykov) or an inconsistent tachycardia (Rush et al., 1970).

Pavlovian Conditioning of Tolerance and Sensitization

The studies described in this section are also experiments in which a CS was paired with a drug US, but the concern of the investigators was not only the evocation of a CR, but more importantly, how a CR that overlaps drug administration can alter the response to the drug. The measured response to the drug may be diminished (tolerance) or enhanced (sensitization). For example, a study by Subkov and Zilov (1937) showed how a conditioned increase in parasympathetic activity underlay attenuation of the tachycardic effect of epinephrine in dogs. After several injections of epinephrine, the dogs were treated exactly as previously, but saline or Ringers solution was injected. The placebo injection resulted in bradycardia and other signs of parasympathetic nervous system activation. Subkov and Zilov postulated that the adaptation to epinephrine was due, at least partly, to an increased ability of the parasympathetic system to respond to activation of pressor receptors. Implied, but not discussed explicitly, is that the dogs may have learned to increase parasympathetic tone in response to cues surrounding epinephrine injection. No control groups were employed to fully distinguish associative from nonassociative changes.

Wikler (1973a, 1973b) developed an hypothesis about conditioning with a drug US. He considered the nervous system to be an integrated

organization of reflex neural circuits composed of an afferent arm, a central processing unit and an efferent arm. Drugs that directly stimulate the afferent arm of the circuit produce a response in the effector arm and this response is considered to be the UR. However, drugs that stimulate the efferent arm of the circuit produce a response which then activates the afferent arm which, in turn, produces a response to counteract the direct drug action on the efferent arm. In this case the observed drug response is the US and the reponse to afferent stimulation is the UR. A CS repeatedly paired with drug administration causes conditioning of central processing activities identical with those elicited by the US. The CR is the same or opposite in direction to the observed drug effect depending on the site of action, e.g., a CR is similar if the drug acts on the afferent arm of "reflex" neural circuits, and the CR is opposite if the drug acts on the efferent arm. Wikler postulated that with continued pairings of CS and drug, the CR changes and becomes a compensatory response or counteradaptation to the drug UR. Wikler thought that these counteradaptions underlay tolerance and physical dependence such that the CR is a manifestation of withdrawal (Wikler, 1973a).

Siegel (1975, 1977, 1978) has also developed an hypothesis of drug tolerance based on Wikler's hypothesis which states that repeated exposure to morphine in the presence of distinctive cues will lead to the development of a compensatory CR, opposite in direction to the UR. Siegel's theory was more specific than Wikler's in assuming that the compensatory CR directly alters the drug UR by summating with it to result in an attenuated drug response. For example, rats that were tested in the presence of cues previously paired with morphine were more

tolerant to the analgesic (Siegel, 1975; Siegel, Hinson & Krank, 1978; Tiffany & Baker, 1981) and hyperthermic (Siegel, 1978) effects of morphine relative to rats tested in the presence of cues not previously paired with morphine. When rats were administered placebo in the presence of drug-paired cues they responded with hyperalgesia (Siegel, 1975) and hypothermia (Siegel, 1978). Siegel postulated it was the summation of conditioned hyperalgesia and hypothermia with morphine-induced analgesia and hyperthermia, respectively, that was responsible for tolerance. Siegel (1975, 1977) and Siegel, Sherman and Mitchell (1980) also showed that presentations of drug-paired cues without drug extinguished morphine tolerance. Also, pre-exposure to morphine without the CS slowed development of tolerance, as did partial reinforcement. In addition, Tiffany and Baker (1981) demonstrated attenuation of tolerance due to latent inhibition by pre-exposure to the distinctive environment. These results lend support to Siegel's Pavlovian model of drug tolerance by showing that development and loss of tolerance are subject to the "rules" of classical conditioning.

Context-specific tolerance to the analgesic effect of morphine has been investigated by others in rats (Dafters & Bach, 1985; LaHoste, Olson, Olson & Kastin, 1980; Paletta & Wagner, 1986; Sherman, 1979; Tiffany & Baker, 1981) and in snails (Kavaliers & Hirst, 1986). All but Sherman (1979) showed context-specific tolerance to the analgesic effect of morphine. Sherman was able to demonstrate context-specific sensitization to morphine's hyperthermic effect (see below) but he observed analgesic tolerance regardless of test environment. During testing for a CR, neither LaHoste et al. nor Paletta and Wagner saw a conditioned hyperalgesic response when placebo was administered in the

presence of drug-paired cues. Dafters and Bach, Tiffany and Baker and Kavaliers and Hirst did not test for compensatory hyperalgesia. In summary, although most have reported context-specific tolerance to morphine analgesia, Siegel has been the only experimenter to observe compensatory hyperalgesia.

Context-specific tolerance to morphine-induced hyperthermia has not been reported by investigators other than Siegel (1978). In fact, Siegel is one of very few investigators to report tolerance to this effect of morphine (see below). Siegel is also one of very few investigators to report a hypothermic CR after pairing morphine with a distinctive environment. Sherman (1979) reported that exposure to morphine (5 mg/kg) in a distinctive room for 2 hrs/trial (ISI = 15-40 min) produced a context-specific increase in hyperthermia. During a placebo test trial, rats who had received repeated pairings of cues with morphine showed a higher body temperature than rats who had received the same amount of morphine in the presence of other cues, suggesting a hyperthermic CR. Sherman's parameters (e.g., dose, ISI, time in distinctive environment) were the same as Siegel's. Miksic, Smith, Numan and Lal (1975) also reported a hyperthermic CR to a 90-min tone that had been repeatedly paired with morphine (20 mg/kg). Zelman, Tiffany and Baker (1985) reported sensitization to morphine's (35 mg/kg) hyperthermic effects, but they found no evidence of context-specific sensitization.

Eikelboom and Stewart (1979) attempted to evaluate these divergent results, that is, whether the thermal CR resembles the UR or is opposite the UR to morphine, by using separate pre-injection and post-injection cues. Rats were transferred from the home cage to the pre-injection

environment and were kept there for 2 hrs. The animals were then moved to the post-injection environment, injected, and kept there for 3 hrs. During conditioning, the morphine groups (5, 25 and 200 mg/kg) all showed a decrease in temperature in the pre-injection environment, while the saline group showed no consistent change suggesting that pre-injection cues were a CS for the elicitation of hypothermia. Temperatures measured in the home cage at the same time of day as measured in the pre-injection room were higher in each group except for the saline group which showed no difference in temperature between the two environments. In the post-injection environment, the morphine groups were hyperthermic relative to the saline group even after placebo injection, indicating a hyperthermic CR. After a period of abstinence from morphine, hypothermia in the pre-injection environment disappeared. Hyperthermia in the post-injection room remained unchanged in each group (Eikelboom & Stewart, 1979). Eikelboom and Stewart suggested that temporal cues may have been acting as conditioned stimuli, and that extinction of the hypothermic CR was occurring during the period of abstinence.

In a subsequent set of experiments, Eikelboom and Stewart (1981) further evaluated how temporal cues might affect the direction of the CR. Results of one experiment in which temporal cues were minimized showed a hyperthermic CR in both the pre-injection and post-injection room. The other experiment, in which temporal cues were maximized and environmental cues were minimized, showed that animals that always received morphine at the same time of day were hypothermic around the time of injection, whereas animals that received morphine at irregular times of day were hypothermic at all times. Eikelboom and Stewart

concluded that when temporal cues are used by the animal, the CR will be opposite the UR, but when environmental cues are used, the CR will be in the same direction as the UR.

In an attempt to replicate the results of Siegel (1978), Zelman, Tiffany and Baker (1985) reported a decreased hyperthermia to morphine (5 mg/kg) after several exposures. However, they also reported a decrease in the hyperthermia produced by rectal probing in saline groups due to habituation to the high level of stress caused by numerous post-injection rectal probings. In additional experiments utilizing fewer rectal probings, and therefore less stress, Zelman et al. found no change or an increase in the hyperthermic response to morphine and a decrease in stress-induced hyperthermia in rats that received saline. Zelman et al. also found evidence of attenuated hyperthermia to morphine in rats that had received a high level of stress in the presence of morphine-paired cues relative to rats for which the stress and cues had been paired with saline. There was no appearance of conditioned hypothermia when saline was administered in the presence of drug-paired cues. The attenuation of hyperthermia was not attributed to tolerance or to the presence of a conditioned hypothermic response because, as stated previously, reduced hyperthermia occurred only in the presence of high levels of stress, and rats that had received morphine under less stressful conditions showed enhanced hyperthermia (Zelman et al., 1985). Perhaps under low stress repeated morphine administration led to greater hyperthermia due to sensitization of the drug effect, while under high stress hyperthermia decreased due to habituation to the rectal probing. These data support Siegel's finding of "tolerance" to the hyperthermic effect of morphine, but they do not support Siegel's finding of a

compensatory hypothermic CR as being responsible for the tolerance because Zelman et al. saw no evidence of a compensatory CR.

Context-specific tolerance has been most extensively studied with respect to the analgesic and thermic effects of morphine. However, learned changes in locomotor activity have also been described (Fanselow & German, 1982; Hinson & Siegel, 1983; Mucha, Volkovskis & Kalant, 1981; Paletta & Wagner, 1986). In the rat, morphine produces hyperactivity at doses of 0.1 to 3.0 mg/kg (Babbini & Davis, 1972; Brady & Holtzman, 1981; Fog, 1970; Vasko & Domino, 1978). Doses of 3.2 to 40 mg/kg have been reported to produce a biphasic response, hypoactivity followed by hyperactivity (Babbini & Davis, 1972; Paletta & Wagner, 1986; Vasko & Domino, 1978). However, there is some discrepancy in the literature concerning the activity effects of a 5 mg/kg dose of morphine as many investigators have reported only a hyperactive effect (Babbini & Davis, 1972; Martin & Papp, 1980).

In general, studies concerning associative tolerance to morphine's activity effects are in agreement with each other. Cues repeatedly paired with a 5 mg/kg (Mucha et al., 1981; Paletta & Wagner, 1986) or 40 mg/kg dose (Hinson & Siegel, 1983) of morphine as the US, elicited a hyperactive CR in rats when presented without the US. All of the studies, including one by Fanselow and German (1982) who failed to see evidence of a compensatory CR, reported context-specific tolerance to the hypoactive effect of morphine. Paletta and Wagner's study shows not only context-specific tolerance to the hypoactivity but context-specific sensitization to the hyperactivity produced by morphine.

Context-specific tolerance also occurs to the hypothermic effect of ethanol in rats (Crowell, Hinson & Siegel, 1981; Lê, Poulos & Cappell,

1979; Mansfield & Cunningham, 1980). Mansfield and Cunningham used a discrimination paradigm in which one CS (CS+) was repeatedly paired with alcohol, and another CS (CS-) was paired with saline. Tolerance to the hypothermic effect was evident only in the presence of the CS+, and a compensatory hyperthermic CR was seen when saline was administered in the presence of the CS+. Further support for Pavlovian conditioned tolerance was obtained by showing that extinction (saline injection in the presence of the CS+) but not rest (saline injection in CS-) was successful in attenuating tolerance. The results of Crowell et al. and Le et al. are in agreement with those of Mansfield and Cunningham, in that environment-specific tolerance to ethanol's hypothermic effect was observed. A hyperthermic CR was measured when saline was administered in the drug-paired environment. Crowell et al. also showed that extinction but not rest resulted in loss of tolerance. Melchior and Tabakoff (1981) attempted to show an effect of learning on tolerance to the hypothermic effect of ethanol in mice, but they conducted tolerance testing in a novel environment. Loss of tolerance in the novel environment may have been due to novelty stress rather than lack of context-specific (i.e., learned) tolerance; however, a novel environment showed little effect on temperature in drug-naive control rats.

Context-specific sensitization to hyperactivity induced by amphetamine (Tilson & Rech, 1973) and cocaine (Hinson & Poulos, 1981) has also been shown to occur in rats. In both studies the CR resembled the UR, that is, the CR was an increase in behavioral activity. The magnitude of the CR was positively related to US magnitude (amphetamine dose) (Tilson & Rech, 1973). Extinction but not rest successfully attenuated sensitization to cocaine (Hinson & Poulos, 1981). Thus, like

tolerance, conditioned sensitization is subject to Pavlovian manipulations.

In summary, context-specific tolerance has been repeatedly demonstrated to the analgesic and hypoactive effects of morphine, as well as to the hypothermic effect of ethanol and to other effects of various drugs. Similarly, environment-specific sensitization has been demonstrated to the hyperthermic effect of morphine and to the hyperactivity effect of morphine, amphetamine and cocaine. Disagreement exists about the presence or absence of the CR as well as the direction of the CR when present, i.e., whether it is similar or opposite the unconditional drug effects. Additionally, it should be noted that in some cases loss of tolerance was not complete when subjects were tested in an environment different from the one paired with drug (e.g., Melchior & Tabakoff, 1981; Tiffany & Baker, 1981) suggesting some degree of non-associative tolerance or stimulus generalization.

Theories of Context-Specific Tolerance

Siegel states that tolerance or sensitization is observed after repeated drug exposure because a CR, either compensatory to or in the same direction as the drug UR develops to the cues surrounding drug administration. This CR is presumed to summate with the drug UR, thereby producing a response that is less (tolerance) or greater (sensitization) than that originally produced. Siegel's theory cannot account for context-specific tolerance or sensitization in the absence of a measurable CR. Siegel (1987) allows for nonassociative mechanisms of tolerance when tolerance is observed in certain preparations such as isolated tissue cultures or in procedures which use continuous drug administration such as inhalation or pellet implantation. He makes no

statement about the presence of nonassociative tolerance that may occur in addition to associative tolerance.

Kesner and Cook (1983) have developed a two-process model of opiate tolerance. They suggest that an habituation process accounts for tolerance observed in a "nondistinctive" environment, and classical conditioning accounts for tolerance seen in a "distinctive" environment. Kesner and Cook performed an experiment in which dose (US magnitude; 5 vs. 15 mg/kg), intertrial interval (ITI; 12 vs. 48 hrs) and environnment (distinctive vs. nondistinctive from the home cage room) were varied. The group of rats given repeated injections of morphine in the nondistinctive environment showed tolerance to the analgesic effect of morphine with massed trials (12-hr ITI) but not with spaced trials (48-hr ITI). There was no difference in tolerance with respect to dose, nor was there a loss of tolerance when environment was changed. A 2-week rest period with no injections or exposure to the environments resulted in loss of tolerance. Kesner and Cook concluded that these results were consistent with a non-associative habituation process of tolerance: animals repeatedly exposed to the US (morphine) show a decrement in analgesic response (tolerance). The group administered morphine repeatedly in the distinctive environment showed a slightly faster development of tolerance with spaced versus massed trials. There was no difference due to US magnitude. Tolerance was sensitive to environmental change, but did not decrease after a 2-week rest period. Kesner and Cook concluded that tolerance acquired in distinctive contexts is governed by a classical conditioning process.

A later experiment conducted by Dafters and Bach (1985) was also designed to assess Kesner and Cook's (1983) dual-process hypothesis. As

in Kesner and Cook's experiment, Dafters and Bach used distinctive and nondistinctive environments. In contrast to Kesner and Cook's experiment, Dafters and Bach used a discrimination design in which rats received morphine in one room and saline in the other room on alternate days. In their first experiment, all animals were pre-exposed to 14 sessions in which they were weighed and injected with saline daily in the home cage room. Following pre-exposure to the injection cues, rats were assigned to one of two groups differing only in which environment was paired with morphine (10 mg/kg) or saline. Analgesia was assessed the same way as in Kesner and Cook's experiment. After 14 sessions, each group was tested for tolerance in the distinctive environment followed by a test in the nondistinctive environment. The results indicated that tolerance was evident in both groups when tested in the environment in which they had been trained. When environment was changed, neither group demonstrated tolerance. Furthermore, after a 14-day rest period, both groups showed tolerance when tested in the drug-paired environment. These results contrast with those of Kesner and Cook, in that rats receiving training in the nondistinctive environment showed tolerance only when tested in the nondistinctive room, and this tolerance was not changed after a 14-day rest period. The major differences in the two experiments was pre-exposure to injection cues and the discrimination required between saline-paired and drug-paired cues. Dafters and Bach suggested that the injection cues overshadowed the nondistinctive environmental cues in Kesner and Cook's experiment, and pre-exposure to the injection ritual allowed for an association to be made between the so-called "nondistinctive" environment and drug effects.

Dafters and Bach's second experiment was nearly identical to the first, except there was no pre-exposure to injection cues. In contrast to Experiment 1, both groups showed tolerance during the two test sessions regardless of test environment indicating a lack of environment-specific tolerance. Following the test sessions, rats were either left undisturbed in their home cage or removed, injected with saline and returned immediately to the home cage (extinction). The subjects were then tested for tolerance in their morphine-paired environment. The group exposed to the extinction procedure showed a significant loss of analgesic tolerance while the group left undisturbed remained tolerant. These results are consistent with Dafters and Bach's proposed hypothesis that injection cues may overshadow environmental cues. Kesner and Cook's nonassociative habituation model was not supported in that a 14-day rest period had no adverse effect on tolerance despite training in the nondistinctive environment. In general, Dafters and Bach's experiments lend additional support to the idea that associative mechanisms are involved in morphine tolerance.

Another theory that can account for tolerance to drug effects is Solomon and Corbit's (1974) opponent-process model of motivation. Basically, the opponent-process theory is a homeostatic model that posits that for a given affective, hedonic or emotional state aroused by any stimulus, there is a CNS mechanism which serves to decrease the magnitude of the hedonic feelings, whether they are aversive or pleasant. Initially, a primary 'a' process is elicited by a stimulus. Next, an opponent loop generates the secondary 'b' process which is opposite to the 'a' process. Once the 'b' process begins, the two processes summate. Initially, the 'a' process is larger than 'b';

therefore, the direction of 'a' predominates. The 'b' process has a long latency and decays slowly so that upon termination of the 'a' process, the 'b' process is still activated, thus, the direction of the response changes. With repeated exposure to the stimulus, 'b' shows a shorter latency response to 'a', a quicker rise, higher asymptote and longer decay time. In other words, the opponent process is a homeostatic counteradjustment to the 'a' process and is strengthened by use and weakened by disuse, but the primary process is not affected by use. Therefore, the sum of the 'a' and 'b' process results in a net affective change that is smaller in magnitude and shorter in duration. Solomon and Corbit point out that these changes are due to repeated exposure to the stimulus and are generally nonassociative. However, Solomon and Corbit assume that either the 'a' or 'b' processes or both could be conditioned. A CS paired with the 'a' process will result in a biphasic CR, whereas a CS paired with the peak of the 'b' process would result in a CR resembling the 'b' process.

In terms of drug use, Solomon and Corbit assume that pleasurable drug effects correspond to the 'a' process and aversive withdrawal-like effects correspond to the 'b' process. If drug use is repeated, the opponent process strengthens and withdrawal symptoms intensify; therefore, the user will seek more drug. This will result in further strengthening of the 'b' process. Tolerance is represented by the requirement of increased dose in order for the net affective change to be greater than zero. The increase in dose further strengthens the 'b' process which results in addiction. According to Solomon and Corbit, Pavlovian conditioning of previously neutral stimuli can result in conditioning of the 'a' process which results in initial conditioned

euphoria followed by withdrawal. Conditioning of the 'b' process results in conditioned withdrawal. Therefore, the behavior of an addict is determined by both conditioned and unconditioned processes. Thus, Solomon and Corbit's opponent-process theory allows associative and nonassociative changes to occur in drug responses leading to addiction. One difficulty in applying their theory is that it requires assumptions be made about the relationship between positive or negative affective states and overt physiological responses. Also, their theory does not seem to account for context-specific sensitization of a monophasic drug response (e.g., amphetamine-induced hyperactivity).

Wagner (Donegan & Wagner, 1987; Wagner, 1981) has developed a priming model of habituation that in some ways is similar to Solomon and Corbit's opponent-process model. Wagner asserts that presentation of a stimulus causes activation of elements contained within a "memory node" from an inactive (I) state to a primary A1 state. This is followed by transfer of activated elements to a secondary A2 state followed by decay of the elements back to the I state. The A2 state may produce a response that is similar (i.e., in the same direction) or opposite to that produced by the A1 state. That is, when the UR is monophasic, the response of the A2 state resembles that of the A1 state, and when the UR is biphasic, the response of the A2 state is opposite to that of the A1 state. Because of this, Wagner has named his theory Sometimes Opponent-Process (SOP) in reference to the occasional similarity of Solomon and Corbit's opponent-process theory.

Wagner's model views an organism's memory as a diffuse structure in which nodes are representations of environmental events. A node consists of a set of informational elements. Variation in nodal

activation is described in terms of the proportion of "like" elements that are in one of three theoretically discriminable states: A1, A2 and I. When the US is presented, any nodal element of the US in the I state will transfer to the A1 state with a probability of p_1. Once an element is in the A1 state, it will decay to the A2 state with a probability of p_{A1}, and from the A2 state, it will decay back to the I state with a probability of p_{A2}. The value of p_1 increases with an increase in US intensity. At any given moment, the activity state of a representational node can be described by the proportion of its elements distributed among the three states. The magnitude of the response will be a function of the weighted proportion of nodal elements in the A1 state plus the weighted proportion of nodal elements in the A2 state. This theory predicts that a US will result in greater response when a greater proportion of elements is in the I state at the moment of US onset. A CS can influence the same response by altering US nodal activity by way of associative linkages between the CS node and US node. The CS does not merely substitute for the US, i.e., by transferring elements in the I state to the A1 state, but by transferring inactive elements directly to the A2 state. Because of this, a CR will resemble the secondary response to the US since both reflect the A2 state of US representation (Wagner, 1981).

From these predictions, it follows that presentation of the US soon after a previous presentation would result in a diminished response because not all of the elements have decayed back to the I state; therefore, since there are fewer elements in the I state, fewer elements would be activated to the A1 state. This is called self-generated priming due to the fact that the US was primed in memory by a previous

presentation of itself. A signalled US presentation results in an attenuated UR relative to unsignalled US presentation due to CS-induced activation of elements from the I state directly to the A2 state, thereby leaving fewer elements in the I state to be activated by the US. This is called associatively-generated priming because it is through prior association of the CS with the US that the CS can activate elements in the US node to the A2 state. This theory, therefore, accounts for both non-associative and associative mechanisms of habituation. Wagner's model has been favored by some (e.g., Baker & Tiffany, 1985) over a compensatory response model of tolerance due to the assertion that tolerance is more analogous to habituation than is classical conditioning alone. Baker and Tiffany also assert that the compensatory response model cannot account for nonassociative tolerance or for tolerance in the absence of a CR, whereas Wagner's model can. Finally, all of the learning-related phenomena (e.g., latent inhibition, extinction, partial reinforcement) can be accounted for by Wagner's associatively-generated priming mechanism.

Wagner's model also allows for priming-produced facilitation of the UR when the US is signalled relative to US-alone presentation. In general, the UR is added to, or subtracted from, by the CR, i.e., the response measured on a CS+US trial will be a combination of a CR and a diminished UR. If the CR is opposite the UR or if no CR is observed, then diminution of the UR is observed. If the CR mimics the UR, then either diminution or facilitation of the UR is seen depending on the weighted proportions of elements in the A1 and A2 states. Priming, whether self- or associatively-generated, always results in diminution of the UR. Facilitation is seen only when the effect of the CR

overwhelms the priming effect, that is, when the ratio of the weighted proportion of elements in the A2 state to those in the A1 state is greater than that determined by US intensity (Donegan & Wagner, 1987).

Wagner's theory, therefore predicts that a compensatory CR would only be expected for those responses that are biphasic, i.e., where the response produced by the A2 state is opposite to that of the A1 state. For example, pairing of environmental cues with morphine's biphasic activity response (hypoactivity (A1) followed by hyperactivity (A2)) resulted in a hyperactive CR. Changes in the UR were characterized as a diminution of the hypoactivity and facilitation of hyperactivity (Paletta & Wagner, 1986). In contrast, a CR that resembles the UR should occur in cases of a monophasic response, i.e., where the response of the A2 state is similar to that of the A1 state. The resulting change in the UR can be either facilitation or diminution (see above). For an example of facilitation, amphetamine induces a monophasic hyperactivity. As a result of conditioning, context-specific facilitation (i.e., sensitization) of activity occurred in the presence of drug-paired cues. Likewise, placebo administered in the presence of amphetamine-paired cues resulted in a hyperactive CR (Tilson & Rech, 1973). Finally, the absence of a CR when placebo is administered in the presence of drug-paired cues, should result when conditioned diminution of a monophasic UR is observed. For example, morphine produces a monophasic analgesic response to which context-specific tolerance develops, but often there is no measurable compensatory CR (e.g., LaHoste et al., 1980; Paletta & Wagner, 1986). In fact, Siegel (e.g., 1975) and Siegel et al. (1978) are the only investigators known to have observed compensatory analgesic CRs. Siegel and Siegel et al. measured

analgesia as the latency to lift a paw off a hot plate. One possibility for this discrepancy, suggested by Paletta and Wagner (1986), is that the conditioned hyperalgesia observed by Siegel may have been a secondary result of conditioned hyperactivity, that is, the greater the animal's level of activity, the greater the probability it will lift its paw regardless of its sensitivity to the painful stimulus. Paletta and Wagner reported the lack of a compensatory hyperalgesic CR, and presence of context-specific analgesic tolerance in their study. Analgesia was measured using the tail-flick response, and therefore, presumably was not confounded by increased levels of activity.

In summary, of the various theories concerning context-specific tolerance, i.e., Siegel's compensatory conditioned response theory, Kesner and Cook's two-process model, Solomon and Corbit's opponent-process theory and Wagner's SOP memory model, all theories predict context-specific tolerance. Kesner and Cook's habituation theory cannot account for sensitization to drug effects. Siegel's theory predicts a CR whenever a CS+ is presented without drug, and this CR sums with the UR to produce tolerance or sensization. Solomon's theory is not specific regarding the presence or absence of the CR, the direction of the CR relative to the UR, nor does it make a prediction for context-specific sensitization. Only Wagner's SOP model can account for context-specific tolerance and sensitization when there is a measurable CR and when there is no evidence of a CR.

Temperature Effects of Morphine

The present experiments are concerned with stimulus control of the thermal response to morphine. Morphine's unconditioned effect on body temperature in the rat is dose- and time-dependent. In general, low

doses of morphine elicit hyperthermia, and intermediate to high doses elicit a time-dependent biphasic response: hypothermia followed by hyperthermia (Clark, 1979). The range of doses that elicits a monophasic hyperthermic response is generally 1 to 20 mg/kg when administered intraperitoneally (Cox, Ary, Chesarek & Lomax, 1976; Eikelboom & Stewart, 1981; Miksic, Smith, Numan & Lal, 1975; Mucha, Kalant & Linseman, 1979; Rudy & Yaksh, 1977; Sloan, Brooks, Eisenman & Martin, 1962; Stewart & Eikelboom, 1981) or subcutaneously (Appelbaum & Holtzman, 1984; Geller, Hawk, Keinath, Tallarida & Adler, 1983; Gunne, 1960; Oka, Nozaki & Hosoya, 1972; Siegel, 1978; Thornhill, Hirst & Gowdy, 1978; Ushijima, Tanaka, Tsuda, Koga & Nagasaki, 1985). Intravenous administration has shown that a slightly smaller dose range is necessary to produce differential effects relative to subcutaneous or intraperitoneal administration. Lotti (1973) measured temperature for 9 hrs after i.v. morphine administration and showed that doses of 1 to 5 mg/kg produced hyperthermia, and doses of 10 and 15 mg/kg produced a biphasic response. Doses of 35 and 50 mg/kg produced only hypothermia (Lotti, 1973).

In general, it is thought that morphine's hyperthermic effect is due to an increase in the level at which body temperature is regulated (e.g., Clark, 1979). For example, rats administered a 4 mg/kg dose of morphine i.p. showed an increase in core temperature with a decrease in tail skin temperature (Cox et al., 1976). When the rats were placed under a heat lamp, core temperature increased further with a slight increase in tail skin temperature, while the saline control rats showed no change in core temperature, but a very large increase in skin temperature, illustrating the importance of the tail as a regulator of

body temperature in the rat. Further evidence suggesting altered set point as a mechanism for morphine hyperthermia is that rats given morphine (4 mg/kg, i.p.) showed a longer escape time from under the heat lamp relative to saline-treated controls. The morphine-treated rats did not exhibit sedation or catatonia at this dose, thus, delayed escape could not be explained in these terms (Cox et al., 1976). Similar studies have been performed in cats, and those data also suggest an increase in thermoregulatory set-point by morphine (Clark & Cumby, 1978). An alternative explanation would be stimulation of the pathway between cold sensors and effectors which increase heat production (e.g., increase activity or secretion of catecholamines).

The magnitude of hypothermia produced by higher doses of morphine is dependent on ambient temperature (Clark, 1979). Lower ambient temperatures augment the hypothermic response, and higher ambient temperatures attenuate the hypothermia or even reverse the response to hyperthermia. Therefore, morphine's hypothermic effect is thought to be due to a depression of thermoregulatory control.

Chronic exposure to morphine usually results in no change or sensitization to the hyperthermic effect. Chronic exposure to small doses that produce hyperthermia produce either no change in hyperthermia (Eikelboom & Stewart, 1981) or a greater magnitude of hyperthermia often with a shorter latency (Gunne, 1960; Mucha et al., 1979; Oka et al., 1972; Sherman, 1979; Stewart & Eikelboom, 1981; Thornhill et al., 1978; Zelman et al., 1985). Doses that produce hypothermia followed by hyperthermia have been shown to produce a smaller magnitude hypothermia or only hyperthermia after repeated administration (Cox et al., 1976; Gunne, 1960; Lotti, 1973; Oka et al., 1972).

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Although most experiments concerning chronic administration of morphine report sensitization to morphine's hyperthermic effect in rats, there are a few reports of tolerance. Siegel (1978) reported tolerance to the hyperthermic effect of morphine, but as Zelman et al. (1985) pointed out, this decrease in hyperthermia may have been due to habituation to the stress of the large number of post-injection rectal probings. Rudy and Yaksh (1977) also reported tolerance to the hyperthermic effect of morphine. However, rats were restrained in their study. Therefore, as in Siegel's study, this attenuation of morphine hyperthermia may have been due to habituation to the stress caused by restraint. Fernandes, Kluwe and Coper (1977) also reported tolerance to morphine-induced hyperthermia. Their dose-response curves showed a very small shift to the right after 20 days exposure to one 16 mg/kg dose per day. However, they found the same size shift to the left after 20 days exposure to two 32 mg/kg doses per day. It was not stated whether these small shifts were statistically significant.

Mucha, Kalant and Kim (1987) showed that after chronic exposure to 20 or 200 mg/kg morphine, there was a significantly shorter latency to reach peak hyperthermia, due to tolerance to the hypothermic effect. Mucha et al. also reported a decreased area under the hyperthermic curve suggesting tolerance to the hyperthermic effect. In their study, peak hyperthermia did not decrease in the morphine-experienced rats.

In summary, morphine produces hyperthermia at low doses and hypothermia followed by hyperthermia at higher doses. With repeated exposure to morphine, most investigators report that tolerance develops to the hypothermia and either no change or sensitization develops to the hyperthermia (determined using peak hyperthermia). The few reports of

tolerance to morphine hyperthermia, observed as a lower peak hyperthermia, are most likely the result of habituation to stress.

EXPERIMENT 1

Rationale

This experiment was designed to study the effects of repeatedly pairing an explicit CS with morphine administration. The studies described previously used "diffuse" contextual cues to demonstrate an association between the context and drug effects. Often the cues included a sequence of events such as transport, handling, injection and so on. The present study differed from those already described, in that an explicit CS was paired with automatic i.v. drug infusion. It was presumed that this CS would be the most reliable predictor for drug effects to the rat. The purpose of this experiment was to demonstrate successful conditioning of the thermic effect of morphine without the presence of unauthorized cues such as transport from room to room, prick of the i.p. injection or rectal probe to assess temperature.

Two groups were used in this experiment, a group which received paired presentations of the CS and US (Group P), and a control group which received explicitly unpaired presentations of the CS and US (Group U). The explicitly-unpaired control procedure equated both groups for total exposure to the CS and US, but the two stimuli never occurred close together in time. After several pairings of the CS and US, the responses of Group P on test trials were compared with those of Group U to evaluate the effect of learning on the response to the CS as well as how learning changed the UR to morphine.

The present study involved housing the rats continuously in the experimental chambers. By doing this, the chamber became a minimal cue, at best, for drug administration in Group P. Also, this eliminated the stress and cue properties of transport from the home cage room to the experimental chamber.

Generally, previous studies of the influence of learning on the thermic effects of morphine involved some kind of stress, that is, stress of the i.p. injection, repeated rectal probings to assess temperature and the handling involved in transport of the subjects to and from the distinct environment. All of these stressors serve to increase body temperature. Habituation to these stressors potentially confounds interpretation of results (e.g., Tiffany & Baker, 1985). Furthermore, a change in the thermic response to stress over the course of the experiment may mislead the experimenter into concluding learning and/or tolerance to drug-induced thermal effects. The present experiment was designed to automatically monitor temperature continously throughout the trials by biotelemetry devices implanted surgically, thus eliminating the stress of rectal probing. Morphine was infused automatically through jugular vein cannulas, minimizing the stress and cue-property of the injection. Previous research has shown that the i.p. injection itself can act as a cue and overshadow environmental cues (Dafters & Bach, 1985).

The interstimulus interval (ISI) for Group P was 30 sec with a 14.5 min CS-US overlap. This relatively long CS duration was chosen because the thermoregulatory system requires several minutes to show a change in body temperature due to its ability to maintain a constant core temperature even in fairly extreme conditions (Guyton, 1986). A previous experiment conducted in our lab showed no learning when the ISI was 15 min with no CS-US overlap. It was thought that perhaps, as suggested by Wagner (1981, 1986), this lack of learning was due to lack of sufficient overlap of the A1 state of the CS with the A1 state of the US.

The ISI for the unpaired group was 90 min so that when the US was administered, all of the CS nodal elements would be in the I state.

Therefore, Group U should show no evidence of an association between the CS and US.

The intertrial interval was 24 hours. It was assumed, based on the pharmacokinetics of morphine, that 24 hrs was a sufficient amount of time for the morphine and its metabolites to be nearly completely removed from the body. Iwamoto and Klaassen (1977) measured plasma levels of unchanged morphine and determined the half-life to be 1 hr 53 min after i.v. administration of a 5 mg/kg dose. The volume of distribution (Vd) was found to be 10.8 l/kg, and the total plasma clearance was 66.1 ml/min/kg. Iwamoto and Klaassen did not mention whether their measurements included free morphine plus albumin-bound morphine or free morphine only. This is an important distinction because only the free morphine will bind to target tissues, and the value of Vd might be low if bound morphine were included in the measurement. Morphine is metabolized primarily in the liver to an inactive glucuronide conjugate. A small percentage of morphine is metabolized to an active N-demethylated compound, normorphine, and a small percentage is excreted unchange in the urine.

During the course of the experiment, a CR was periodically assessed by delaying the US for the paired group until the end of CS presentation. This was done because the 30 sec ISI did not allow enough time for a measurable change in temperature to occur.

Learned changes in the UR due to an association with the CS were assessed after 22 trials by exposing all subjects to morphine administered in the presence of the CS (i.e., a 'paired' CS+US trial) or without the CS (i.e., a US-alone trial). By doing this, the influence of learning on the drug response could be determined by a between-group comparison of the paired versus unpaired groups' response to morphine with and without the CS. Also, a

within-group comparison allowed assessment of how learning affected the drug response by comparing the paired group's response to the US in the presence versus absence of the CS. A comparison of the unpaired group's responses allowed for assessment of any non-associative effect the CS may have on the UR.

At the end of the experiment, a placebo test was given in which all subjects received saline instead of morphine paired with the CS. Unlike the delayed-US test, the placebo test allowed for determining the duration of the conditioned temperature response after CS offset.

It was hypothesized that sensitization would occur to the hyperthermic effect of morphine. This would be in agreement with most of the literature (cf. previous section on temperature effects of morphine). Sensitization was predicted in the Group P relative to Group U due to the summation of a hyperthermic CR with the hyperthermic UR. Within the paired group, it was hypothesized that sensitization would be evident in the presence of the CS relative to its absence.

Method

Subjects

The subjects were 16 male albino rats (Harlan/Holtzman Co., Madison, WI) which were approximately 80 days old and weighed an average of 391 g at the start of the experiment. The subjects were individually housed in sound- and light-attenuating experimental chambers (see below). Food and water were available ad lib throughout the experiment, but intake was not measured during the experiment.

Surgical Procedure

Four days before the start of the experiment, the rats were fully anesthetized with halothane gas (Loading Dose = 5% in oxygen; Maintenance Dose = 2% in oxygen) while a jugular vein cannula and a biotelemetric temperature monitoring device were surgically implanted under antiseptic conditions.

After the animal was anesthetized, 0.1 ml Crysticillin (penicillin in procaine suspension) was injected i.m. into the left hind leg to prevent post-surgical infection. The rat was then shaved below and left of the navel, on the right side of the chest, and on the back just above the clavicle. Betadine antiseptic solution was rubbed on the shaved areas to cleanse the skin and wipe away loose hair.

Jugular Cannula. The design of the cannula was modeled after Weeks (1972). A complete description of the cannula construction can be found elsewhere (Schwarz, 1986). Basically, the intravascular portion was silastic tubing (0.51 mm i.d. x 0.94 mm o.d.) and the subcutaneous portion consisted of polyethylene tubing (Intramedic, PE10 and PE20).

A 1.5 cm incision was made rostral to the clavicle and to the right of the midline. The right external jugular vein was isolated and cleared of fascia. The beveled tip of the cannula was inserted through a 1-mm incision

in the vascular wall and slid toward the heart. Suture (000 silk) was tied around the cannulated portion of the vein. The cannula was tunneled subcutaneously to an incision in the skin at the back of the neck. Additional sutures anchored the cannula ventrally to the midline muscle and dorsally to the superficial muscles. The external end of the cannula was attached to a blunt hypodermic needle which was plugged. After surgery, each rat was fitted with a harness made of foam padding and velcro strips (Weeks, 1972) to which the cannula was attached. The harness served to protect the cannula and to provide a convenient way of attaching the cannula to the fluid swivel.

The cannulas were flushed daily with 0.2 ml saline. Neither heparin nor ethylenediamine tetraacetate were needed to maintain cannula patency.

Biotelemetric Device. Core body temperature was monitored by an implanted Mini-Mitter (Model M, Mini-Mitter Co., Sunriver, OR), a small AM-band transmitter that emits a signal pulse at a rate proportional to the surrounding temperature. The Mini-Mitter consists of two thermistors and a battery-powered transmitter encased in a small, nontoxic waterproof capsule. Each unit was protected from fluid corrosion by a coating of Parafin/Elvax. Each Mini-Mitter was calibrated in a water bath at 34, 37 and 40 °C before surgery. The time intervals between signal pulses at each temperature were used to create a calibration curve for each Mini-Mitter. This device enables detection of temperature changes as small as 0.1 °C. The Mini-Mitter was inserted into a 1.5 cm incision through the skin and peritoneum in the lower left quadrant of the abdomen.

Apparatus

The animals were housed and tested in clear plexiglas cylinders (25.4 cm diameter x 30.5 cm height) with a stainless-steel grid floor. This cage was placed inside a ventilated light- and sound-attenuating chamber (70 x 70 x 60

cm). A light bulb (30 V, 6 W, powered by 24 VDC) and speaker (8.9 cm diameter) were mounted on the back wall of the chamber approximately 44.5 and 53 cm, respectively, above the floor of the chamber. The white noise component of the CS was provided by a Grason-Stadler noise generator (Model 901B). The white noise averaged 74.5 dBA, and the ambient noise level averaged 57.7 dBA (Scott Instruments ANSI Type 2, Model 452 sound level meter positioned in the cage at the level of the rat's head). A fluid swivel (Brown, Amit & Weeks, 1976; Ledger Technical Services, Kalamazoo, MI) placed above the cage was connected to the rat's cannula via polyethylene tubing (Intramedic, PE50) which was protected by a wire spring. The fluid swivel was connected to a syringe by PE20 tubing. Morphine sulphate (5 mg/kg) was automatically administered by an infusion pump (Model A, Razel Scientific Instruments, Stamford, CT) at a rate of 0.5 ml/30 sec. The infusion pump was placed outside the experimental chambers.

A modified transistor radio was used to receive the signal transmitted from each Mini-Mitter. An Apple II+ computer controlled CS and US presentations and timed and recorded interpulse intervals (IPIs) from the Mini-Mitters (accurate to 10 msec). A complete description of the hardware and software used for biotelemetry can be found elsewhere (Cunningham & Peris, 1983).

Procedure

Following recovery from surgery, rats were randomly assigned (coin flip) to either the paired (Group P) or unpaired (Group U) group for classical conditioning. The CS was a 15-min presentation of the compound stimulus composed of light and white noise. The US was intravenous administration of morphine (5 mg/kg in a 0.5 ml volume). Rats in Group P received presentation of the US 30 sec after CS onset. The CS remained on for an additional 14.5

min after US presentation. Group U received explicitly unpaired presentations of both the CS and US, i.e., the US was presented 75 min after offset of the 15-min CS. The subjects were given one trial per 24-hr period. The training phase consisted of 21 trials. Table 1 outlines the experimental procedure. On every seventh day (D7, D14, D21) during training, a Delayed-US test was given in which the US presentation for Group P was delayed until the end of the CS period. This was done in order to see if a thermic CR, which would not be apparent during the 30-sec ISI, had developed to the CS

Following the training phase, the rats were each given two drug tests in which all subjects received the US paired with CS (CS+US test) or alone (US-alone test). The purpose of these tests was to assess context-specific differences in the drug response. The order of these two tests was counterbalanced, and these test trials were alternated with conditioning trials in order to maintain the CR in Group P. Following the drug tests, all subjects were given one placebo test trial in which saline was presented with the CS. The purpose of this test was to measure the groups' response to the CS in the absence of drug. The total number of trials (conditioning and test) was 27.

Each trial began at approximately 0800 hrs and lasted 3 hrs 5 min. At approximately 1700 hrs each day, all rats were weighed, and fresh morphine solutions were prepared. The chambers were provided with fresh food, water and wood shavings.

At the end of the experiment, each rat was infused i.v. with 0.1 ml Methohexital sodium (10 mg/ml concentration in distilled water), an ultrashort, ultrafast acting barbituate to verify patency of the cannula. Patency was determined by ataxia, which occurred within seconds after infusion

Table 1. The procedure in Experiment 1 for all subjects. COND refers to trials during the training phase in which Group P received paired presentations of the CS and US, and Group U received unpaired CS and US presentations. DEL-US refers to the Delayed-US trials in which Group P received the US at the end of the CS.

DAY	TREATMENT
1-6	COND
7	DEL-US
8-13	COND
14	DEL-US
15-20	COND
21	DEL-US
22	COND
23	DRUG TEST
24	COND
25	DRUG TEST
26	COND
27	PLACEBO TEST

through a good cannula. Ataxia did not occur at all if the cannula was leaky or clogged.

Data Analysis

The mean IPI from each Mini-Mitter was computed for one 30-sec sample period before and each one during the CS presentation. Following the CS period, mean IPI was computed for each 5-min sample period for the rest of the session. In order to eliminate electrical noise, all IPIs that were different by more than 20 msec from the previous IPI were ignored. In addition, all IPIs greater than 600 msec or less than 200 msec were ignored. For the Model M Mini-Mitter, intervals outside this range were assumed to be missing signals or noise.

The mean IPI for each sample period was converted to body temperature using calibration values obtained before surgery. If signal errors required the data for a whole sample period to be discarded, an average score computed from adjacent periods was inserted in place of the discarded data. Inserted means represented less than 2% of the data reported. During the training phase, the data were averaged across 2-day blocks for Analysis of Variance (ANOVA). All p values less than .05 were considered significant.

Results

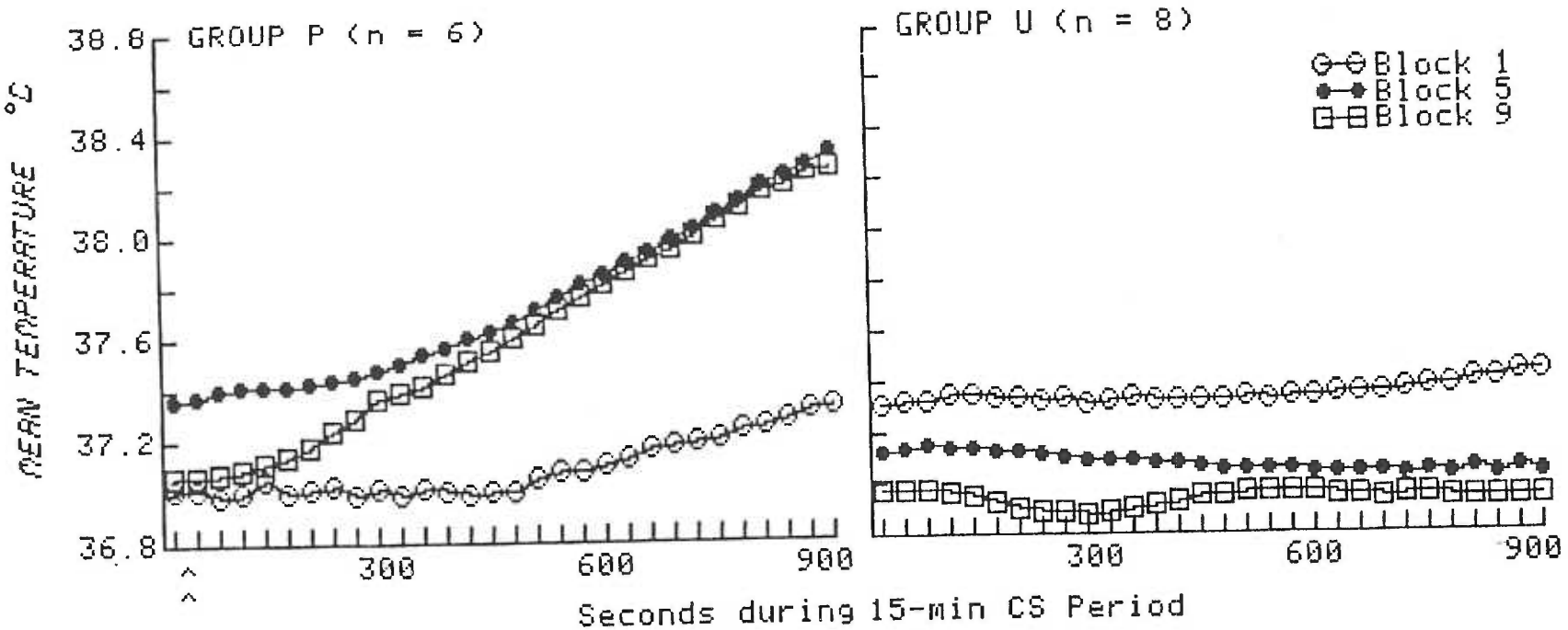
The data for two subjects in Group P were discarded. One rat showed no response to Methohexital Sodium indicating an obstructed or damaged cannula. The other rat's Mini-Mitter stopped emitting a signal during the training phase. Therefore, the number of subjects in Groups P and U were 6 and 8, respectively. In addition, the data for one rat in Group P during the drug tests were omitted due to a leaky cannula which was successfully repaired for the remainder of the experiment. Thus, group sizes for Groups P and U during the Drug tests were 5 and 8, respectively.

Training Phase. Figure 1 shows the body temperature response during the CS period in Blocks 1, 5 and 9 during training (i.e., Days 1-2, 9-10 and 19-20). For Group P (left panel) the first sample period is CS alone, and the US was presented during the second sample period. Group P responded with a small increase in temperature on Block 1. Over the training phase, morphine-induced hyperthermia became more apparent within the 15-min CS period. Generally, Group U's (right panel) body temperature in later blocks showed no change or a slight decrease during the CS period.

These observations were supported by a three-way ANOVA (Groups x Blocks x Sample Periods) which revealed a Groups x Blocks x Sample Periods interaction, $F(232,2784) = 4.21$. Follow-up analyses revealed a Blocks x Sample Periods interaction in Group P, $F(232,1160) = 4.59$ but not in Group U. The Blocks x Sample Periods interaction in Group P was due to a Blocks effect found in Sample Period 15 and 30, $Fs(8,40) = 2.46$ and 7.31, respectively, but not in Sample Period 1. In the overall analysis, Group P also showed significant main effects of Blocks, $F(8,40) = 2.82$ and Sample Periods, $F(29,145) = 62.77$.

The right panel in Figure 1 indicates that Group U's temperature decreased over blocks during the CS period, presumably due to habituation.

Figure 1. Mean temperature response during the CS period is plotted over 30-sec sample periods in Blocks 1, 5 and 9 during training. Group P is shown in the left panel, and Group U is shown in the right. Morphine delivery is denoted by the ^.


GROUP P (n = 6)
GROUP U (n = 8)
Block 1
Block 5
Block 9
MEAN TEMPERATURE °C
38.8
38.4
38.0
37.6
37.2
36.8
300
600
900
Seconds during 15-min CS Period

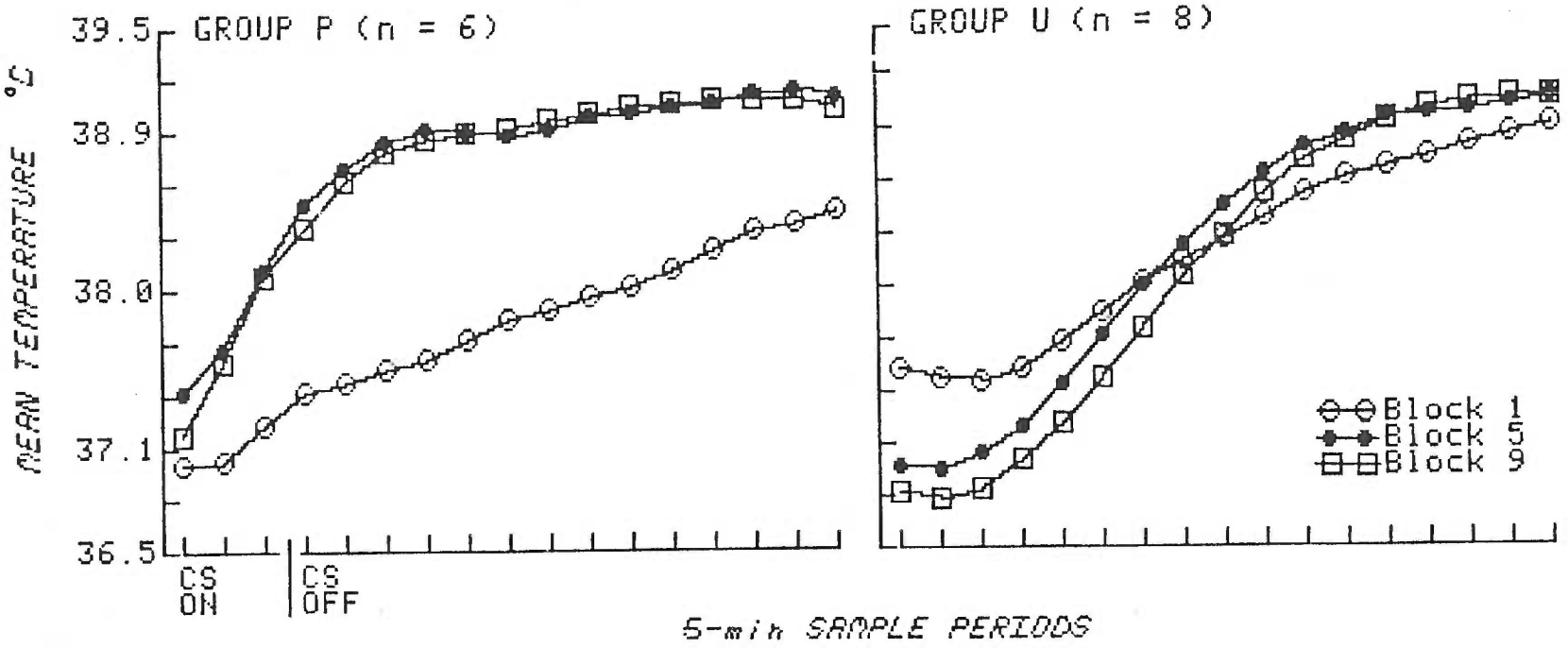
This observation was supported by a significant Blocks effect, $F(8,56) = 3.95$. A significant effect of Sample Periods, $F(29,203) = 4.84$ was also revealed for Group U.

Figure 2 shows the UR to morphine on Blocks 1, 5 and 9 in Group P (left panel) and Group U (right panel). Plotted are 5-min sample periods for 85 min after morphine administration. The first three sample periods for Group P occurred during the CS. In Block 1, both Group P and Group U showed a slower rate of temperature increase relative to Blocks 5 and 9. In addition, the shape of the response differed between groups, especially in Blocks 5 and 9 in which Group U showed a very slight decrease in temperature before showing an increase, while Group P showed a quicker rate of temperature increase relative to Group U.

These observations are supported by a three-way ANOVA (Groups x Blocks x Sample Periods) which revealed a significant Groups x Blocks x Sample Periods interaction, $F(128,1536) = 2.05$. This three-way interaction was due to a significant Groups x Sample Periods interaction in Blocks 5 and 9, $Fs(16,192) = 13.95$ and 39.62, respectively, but not in Block 1. A main effect of Groups was also significant in Blocks 5 and 9, $Fs(1,12) = 5.95$ and 13.03, respectively, but not in Block 1. The lack of a Groups x Sample Periods interaction or main effect of Groups in Block 1 suggests no difference between groups in their response to morphine initially.

Figure 2 also indicates that the temporal pattern of each group's response to morphine changed differently over blocks. Group P showed a steeper rise to maximal hyperthermia as well as an increase in magnitude of hyperthermia. These observations are supported in an analysis of Group P which revealed a Blocks x Sample Periods interaction, $F(128,640) = 2.39$. Follow-up analyses of Sample Periods 1, 5 and 17 revealed a significant Blocks

Figure 2. Mean temperature response to morphine US is plotted for Group P (left panel) and Group U (right panel) in Blocks 1, 5 and 9 during training. The data points are 5-min sample periods. The first three sample periods for Group P occur during the CS period.


GROUP P (n = 6)
GROUP U (n = 8)
MEAN TEMPERATURE °C
39.5
38.9
38.0
37.1
36.5
CS ON
CS OFF
Block 1
Block 5
Block 9
5-min SAMPLE PERIODS

effect in Sample Periods 5 and 17, $Fs(8,40) = 11.76$ and 4.72, respectively, but not in Sample Period 1. Sample Periods 1 and 17 were chosen in all UR analyses because they were the first and last sample periods, respectively after drug administration. Sample Period 5 was chosen because significant effects were more likely to be seen in this sample period relative to other intermediate sample periods. Main effects of Blocks, $F(8,40) = 9.97$ and Sample Periods, $F(16,80) = 204.80$ were also revealed in Group P's analysis.

The change in response to morphine in Group U appeared to be primarily due to a decrease in initial temperature over blocks (see Figure 2, right panel). The maximal hyperthermia did not change over blocks. These observations were supported by an analysis of Group U which revealed a significant Blocks x Sample Periods interaction, $F(128,896) = 3.34$. This interaction was due to a significant effect of Blocks in Sample Period 1, $F(8,56) = 5.26$, but not in Sample Periods 5 or 17.

Pre-CS Baseline. Figure 3 shows the 30-sec sample period just before CS onset plotted over 2-day blocks during training. In general, Group P showed an increase in baseline temperature during Blocks 4 and 5, then temperature declined over the remaining blocks back to where it was on Blocks 1-3. Group U, however, showed a general decline in baseline temperature over the training phase. This observation was supported by a two-way ANOVA (Groups x Blocks) which revealed a Groups x Blocks interaction, $F(8,95) = 2.34$. Neither main effect was significant. The Groups x Blocks interaction was due to a significant effect of Blocks in Group U, $F(8,55) = 2.17$ but not in Group P. Differences between groups in Baseline temperature were attributed to sampling error.

Pre-US Period--Group U. Figure 4 shows the temperature response in Group U during the 1-hr period preceding US presentation during the training phase.

Figure 3. Mean temperature is graphed for Group P and Group U in the 30-sec sample period just before CS-onset (Baseline). The data are plotted over 2-day Blocks during training.

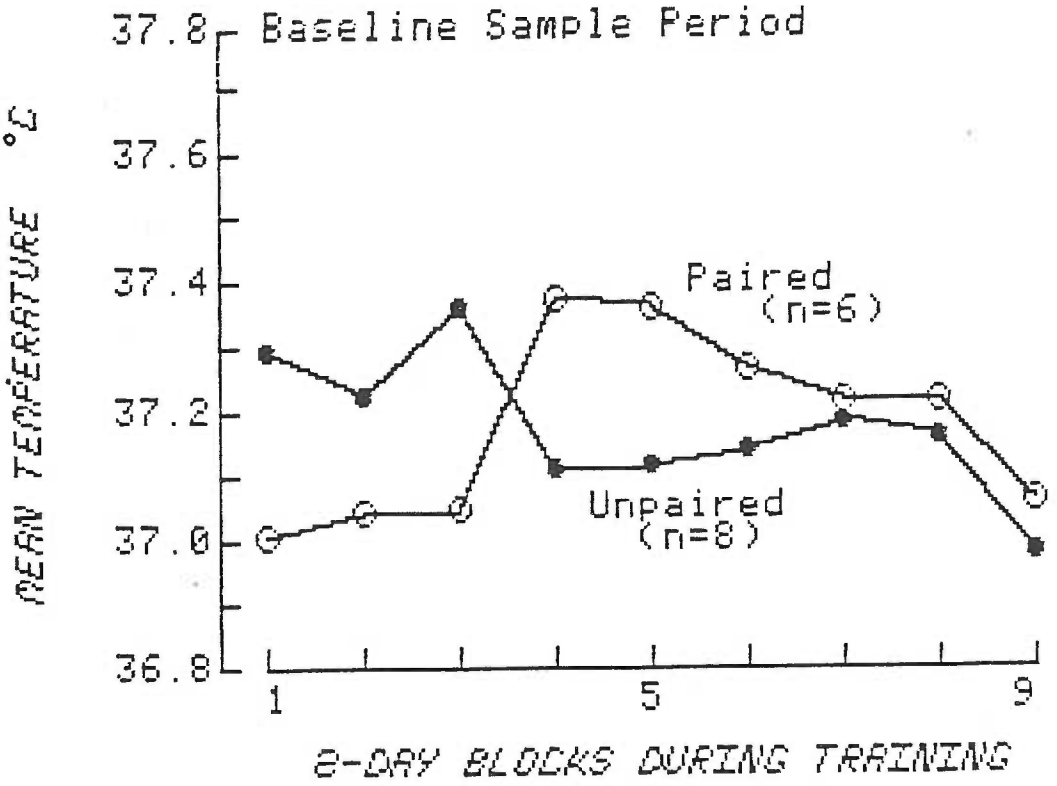
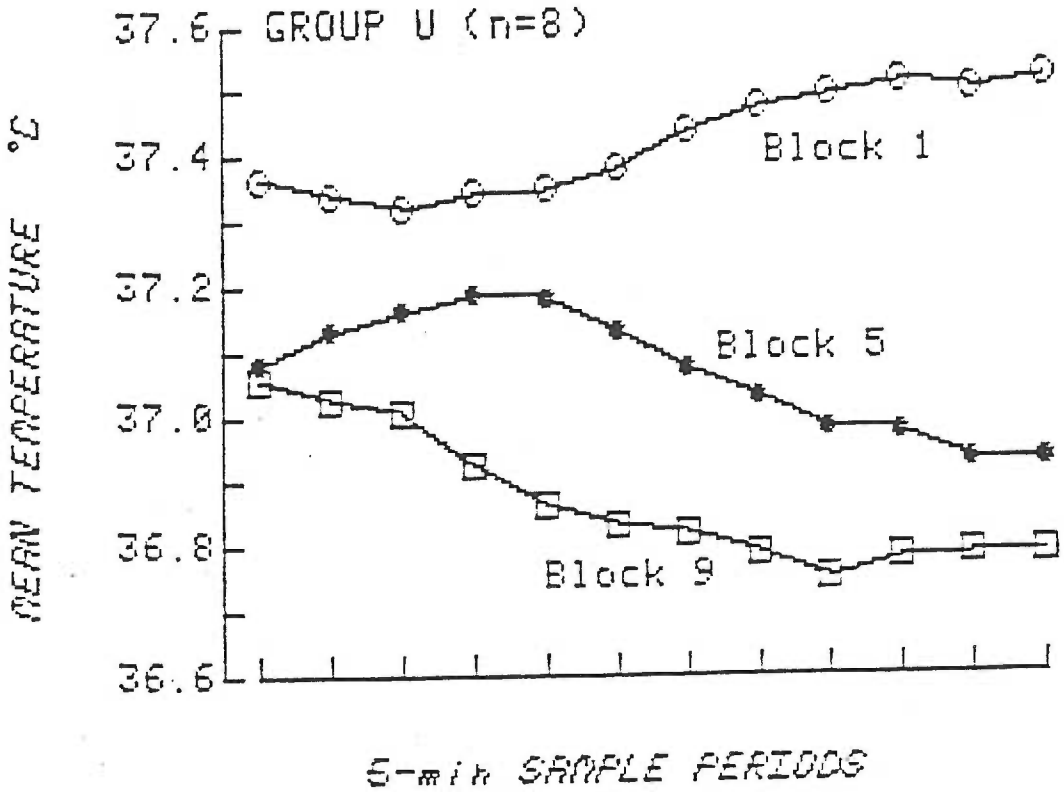

Baseline Sample Period
37.8
37.6
37.4
37.2
37.0
36.8
MEAN TEMPERATURE °C
Paired
(n=6)
Unpaired
(n=8)
1
5
9
8-DAY BLOCKS DURING TRAINING

Figure 4. Mean temperature in Group U during the 1-hr period before morphine delivery is plotted for Blocks 1, 5 and 9 during training. The data are collapsed over 5-min sample periods.


GROUP U (n=8)
37.6
37.4
37.2
37.0
36.8
36.6
MEAN TEMPERATURE °C
Block 1
Block 5
Block 9
5-min SAMPLE PERIODS

This figure illustrates that in the first block there was a slight increase in temperature over the 1-hr period (+.12°C), while in Blocks 5 and 9, there was a decrease in temperature (-.15°C and -.26°C, respectively). This observation was supported by a two-way ANOVA (Blocks x Sample Periods) which revealed a Blocks x Sample Periods interaction, $F(88,616) = 2.26$. Main effects of Blocks, $F(8,56) = 6.36$ and Sample Periods, $F(11,77) = 4.62$ were also significant. The two-way interaction was caused by the presence of a significant Sample Periods effect in Blocks 1, 5 and 9, $Fs(11,77) = 5.93$, 11.75 and 8.16, respectively but not in Block 6.

Delayed-US tests. Figure 5 shows the mean temperature response (±SEM) during the 15-min CS period collapsed over all three test sessions. Neither group received morphine during the CS. As is illustrated, Group P's temperature increased during the 15-min CS (+.38°C) and Group U showed no change in temperature. This observation was supported by a three-way ANOVA (Groups x Test Sessions x Sample Periods) which revealed a significant Groups x Sample Periods interaction, $F(29,348) = 4.54$. Main effects of Groups, $F(1,12) = 4.80$ and Sample Periods, $F(29,348) = 3.74$ were also significant. The Groups x Sample Periods interaction was due to a Sample Periods effect in Group P, $F(29,145) = 4.44$ but not in Group U. No effect involving Test Sessions was significant.

Drug Tests. Figure 6 shows the temperature response to morphine of both groups in the CS+US test (left panel) and US-alone test (right panel). As can be seen in Figure 6, Group P showed a greater amount of hyperthermia relative to Group U in the CS+US test, but there was no difference between groups in the US-alone test. This observation was supported by a four-way ANOVA (Groups x Order x Tests x Sample Periods) which revealed a significant Groups x Tests x Sample Periods interaction, $F(29,261) = 1.70$. Follow-up analyses revealed a

Figure 5. Mean temperature (±SEM) is plotted for Group P and Group U during the Delayed-US test. Data are plotted in 30-sec sample periods during the CS period and are collapsed across the three tests from Days 7, 14 and 21.

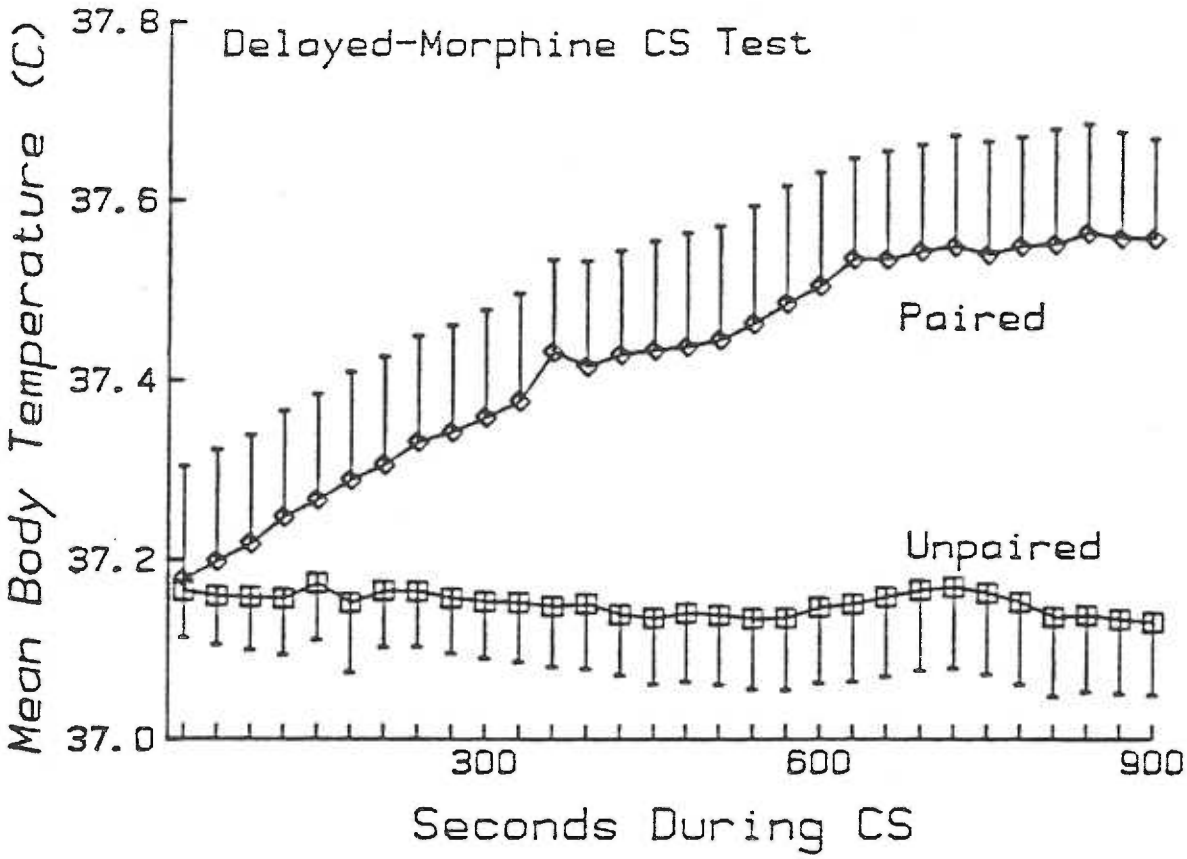

Delayed-Morphine CS Test
Mean Body Temperature (C)
37.8
37.6
37.4
37.2
37.0
Paired
Unpaired
300
600
900
Seconds During CS

Figure 6. Mean temperature for Group P and Group U is plotted during the CS period on the CS+US test (left panel) and US-alone test (right panel). The data are 30-sec sample periods. Morphine was administered just after the first sample period.

significant Groups x Sample Periods interaction in the CS+US test, $F(29,261)$ = 6.27 but not in the US-alone test. This interaction was due to a significant effect of Groups in Sample Periods 15 and 30 of the CS+US test, $Fs(1,9)$ = 10.76 and 18.99, respectively, but not in Sample Period 1.

Figure 6 also shows that both groups showed a greater increase in temperature during the CS+US test relative to the US-alone test. This observation was supported by a Tests x Sample Periods interaction in both groups, $F(29,87)$ = 13.86 for Group P, and $F(29,174)$ = 5.05 for Group U. Follow-up analyses in Sample Periods 1, 15 and 30 revealed a significant Tests effect in Group P in Sample Periods 15 and 30, $Fs(1,3)$ = 24.70 and 146.13, respectively, but not in Sample Period 1. The overall analysis for Group P also revealed main effects of Tests, $F(1,3)$ = 51.98 and Sample Periods, $F(29,87)$ = 47.12.

The Tests x Sample Periods interaction in Group U was also due to a divergence in responding over time across the two tests. However, the magnitude of the test effect was much smaller in Group U than in Group P. In fact, follow-up analyses at various sample periods failed to yield significant effects of Tests in Group U. The overall analysis for Group U revealed a main effect of Sample Periods, $F(29,174)$ = 67.58, but no main effect of Tests.

In addition, an Order x Sample Periods interaction, $F(29,261)$ = 4.18, was revealed in the overall ANOVA. In general, groups given the CS+US test before the US-alone test showed a greater increase in body temperature than the group given the opposite order (data not shown). Because the Order factor was not involved in any interactions involving Groups or Tests, the interaction may have been due to a sampling error.

Figure 7 shows the response to morphine in 5-min sample periods for both drug tests in Group P (left panel) and Group U (right panel). As previously

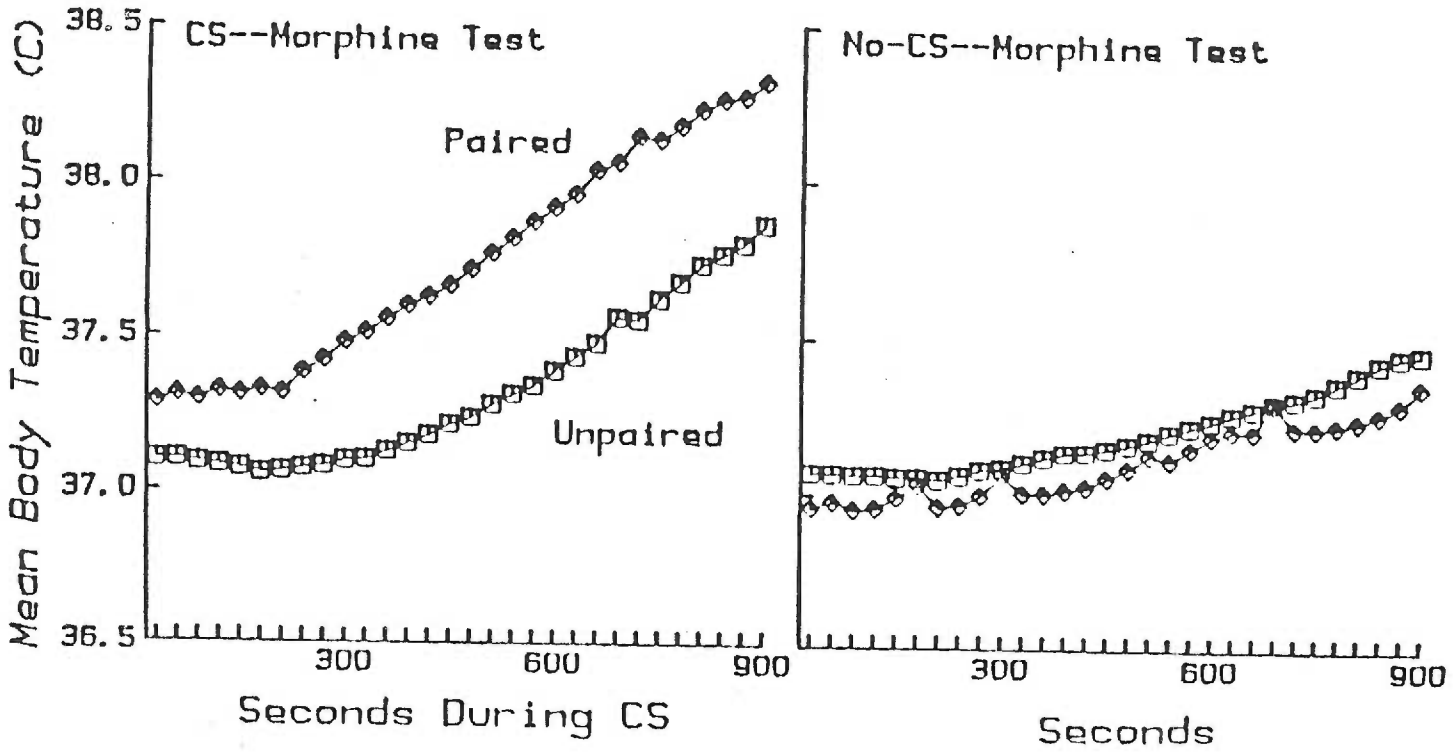
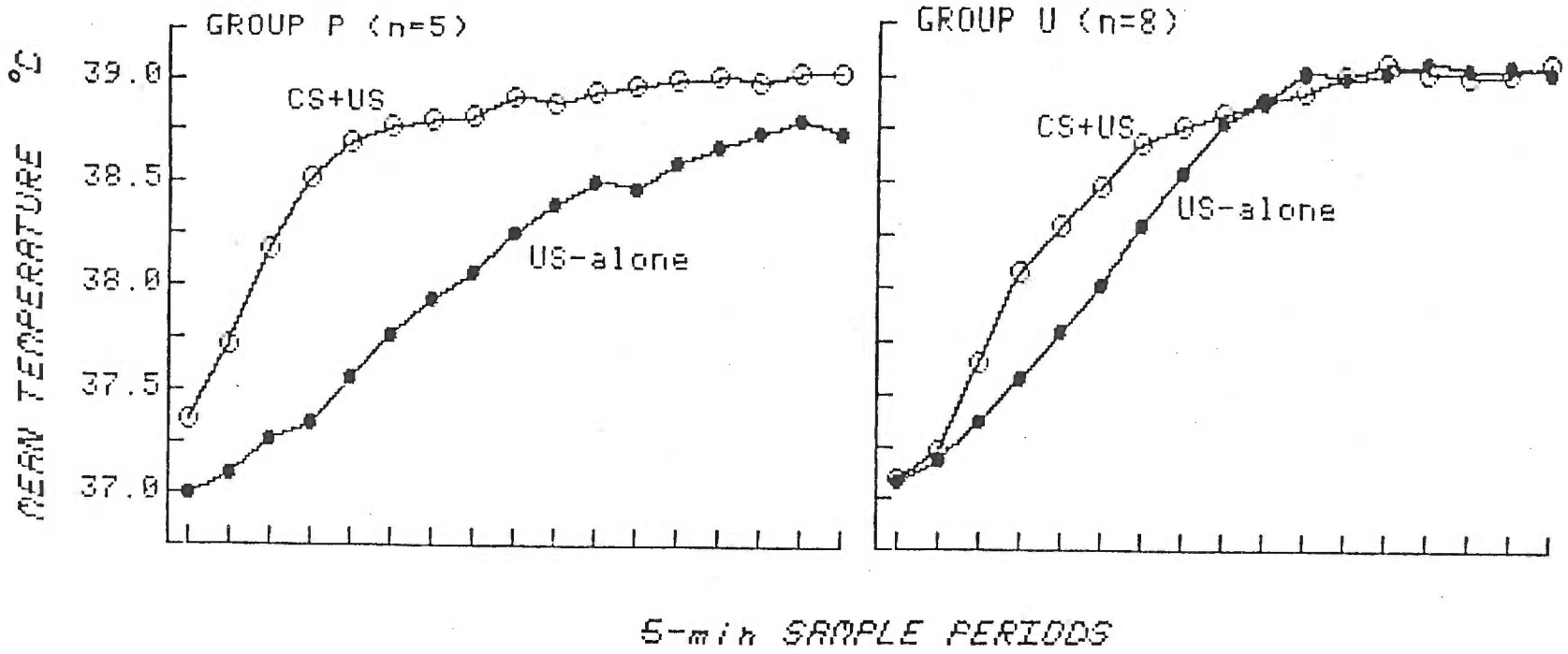

Mean Body Temperature (C)
38.5
38.0
37.5
37.0
36.5
CS--Morphine Test
Paired
Unpaired
300
600
900
Seconds During CS
No-CS--Morphine Test
300
600
900
Seconds

Figure 7. Mean temperature is plotted in 5-min sample periods for the CS+US test and US-alone tests for Group P (left panel) and Group U (right panel). The first three sample periods of the CS+US test in both groups occur during the CS.


GROUP P (n=5)
GROUP U (n=8)
CS+US
US-alone
CS+US
US-alone
MEAN TEMPERATURE °C
39.0
38.5
38.0
37.5
37.0
5-min SAMPLE PERIODS

mentioned, the UR was observed as a faster rate of temperature increase in the CS+US test relative to the US-alone test in each group. The three-way ANOVA (Groups x Tests x Sample Periods) revealed a Tests x Sample Periods interaction, $F(16, 176) = 6.46$ as well as main effects of Tests, $F(1,11) = 6.79$ and Sample Periods, $F(16,176) = 81.18$. Follow-up analyses on the Tests x Sample Periods interaction revealed a significant Tests effect in Sample Period 5, $F(1,11) = 21.00$ but not Sample Period 1 or 17. No effects involving Groups were significant.

Unconditioned Response: Day 22 vs. US-alone test--Group U. Figure 8 shows Group U's response to morphine on Day 22 and in the US-alone drug test. The purpose of this figure is to illustrate the difference in Group U's response to drug when morphine was given at different times during the trial. The difference in response on the two days was apparent in the early post-drug sample periods. Maximal hyperthermia was not different. These observations were supported by a two-way ANOVA (Days x Sample Periods) which revealed a significant Days x Sample Periods interaction, $F(16,96) = 4.00$. Follow-up analyses in Sample Periods 1, 3, 5 and 17 revealed a significant effect of Days in Sample Period 3, $F(1,6) = 7.76$ but not in Sample Period 1, 5 or 17.

Placebo Test. Figure 9 shows the response during the 15-min CS in both Group P and Group U when saline rather than morphine was administered. Similar to the Delayed-US test, Group P responded with an increase in temperature (+.64 ^{o}C) and Group U showed no change in temperature to the CS. A two-way ANOVA (Groups x Sample Periods) supported these observations with a Groups x Sample Periods interaction, $F(29,348) = 9.40$ and a main effect of Sample Periods, $F(29,348) = 6.25$. The interaction was due to a significant effect of Sample Periods in Group P, $F(29,145) = 13.27$ but not in Group U.

Figure 8. Mean temperature in response to morphine is plotted in 5-min sample periods for Group U. These data are from Day 22 and from the US-alone drug test (Day 23 or 25).

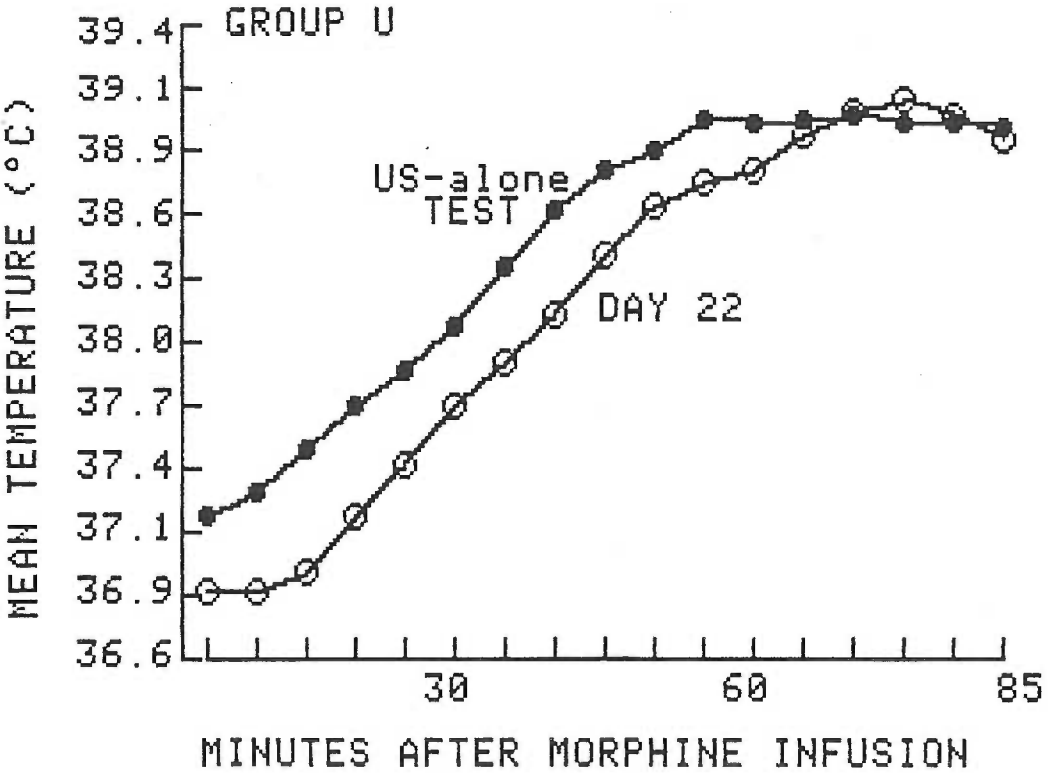

GROUP U
39.4
39.1
38.9
38.6
38.3
38.0
37.7
37.4
37.1
36.9
36.6
MEAN TEMPERATURE (°C)
US-alone TEST
DAY 22
30
60
85
MINUTES AFTER MORPHINE INFUSION

Figure 9. Mean temperature is plotted in 30-sec sample periods during the CS period in Group P and Group U. These data are from the Placebo test when saline was administered rather than morphine. Saline administration is denoted by the ≪.

As can be seen in Figure 10, temperature in Group P remained elevated after CS offset and did not begin to decrease until approximately 30 min after CS offset. Group U showed a decrease in temperature over the post-CS period. A two-way ANOVA revealed a significant Groups x Sample Periods interaction, $F(16,192) = 2.17$ and a main effect of Sample Periods, $F(16,192) = 5.98$. A significant effect of Sample Periods was present within each group, $F(16,80) = 4.38$ for Group P, and $F(16,112) = 2.99$ for Group U. Follow-up analyses on the Groups x Sample Periods interaction revealed no Groups effect in Sample Periods 1, 5, 6, 7, 8 or 17. An analysis comparing the mean score of Sample Periods 3-8 also failed to reveal a Groups effect. Although the interaction was not explained by the follow-up analyses chosen, it appears to be due to the different pattern of each group's response.

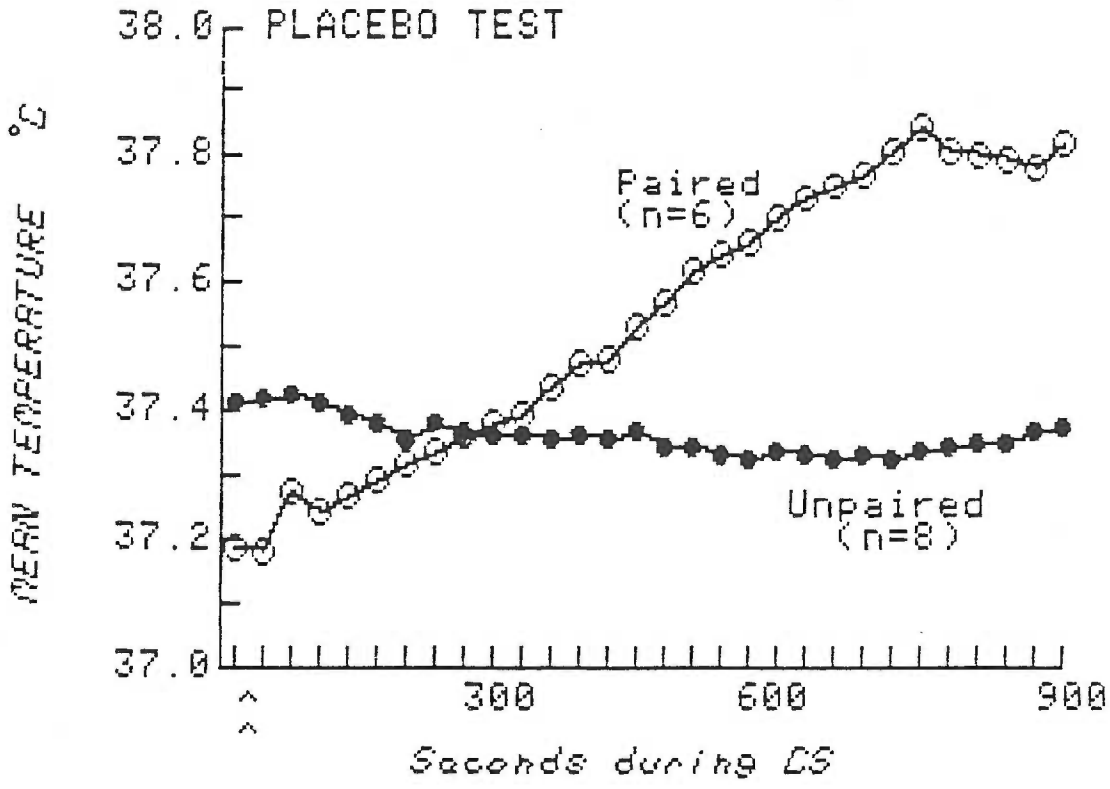
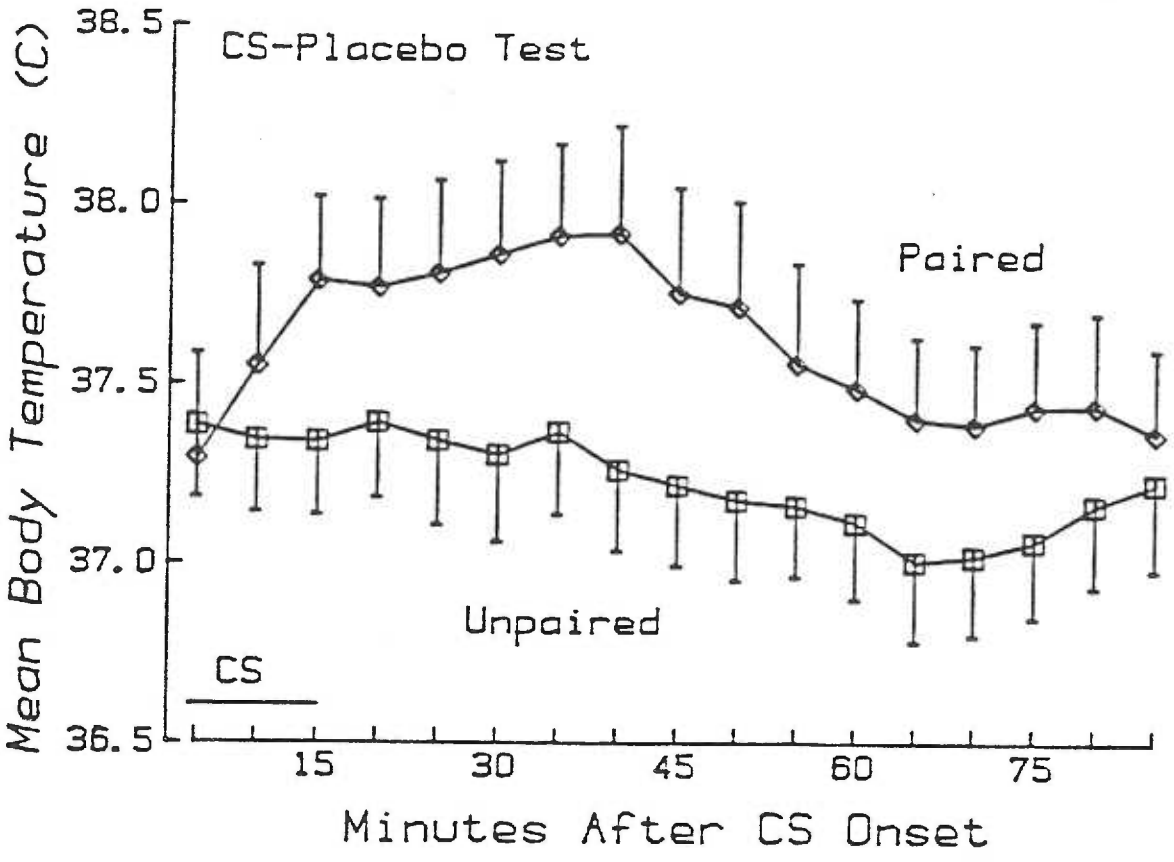

PLACEBO TEST
38.0
37.8
37.6
37.4
37.2
37.0
°C
MEAN TEMPERATURE
Paired
(n=6)
Unpaired
(n=8)
300
600
900
Seconds during CS

Figure 10. Mean temperature (±SEM) during the Placebo Test is plotted in 5-min sample periods for Group P and Group U. The first three sample periods for both groups occur during the CS period.


CS-Placebo Test
Mean Body Temperature (C)
38.5
38.0
37.5
37.0
36.5
Paired
Unpaired
CS
15
30
45
60
75
Minutes After CS Onset

DISCUSSION

Experiment 1 demonstrated cue-specific sensitization to the thermic effect of morphine in rats who received an i.v. infusion of morphine paired with an explicit light/noise CS. The presence of a hyperthermic CR to the CS was also evident in Group P when the US was delayed (see figure 5) as well as when placebo was infused instead of morphine (see figure 9). The hyperthermic CR extended beyond CS offset about 30 min during the placebo test (see figure 10).

The change in Group P's response to morphine during training can be characterized as an increase in the magnitude of hyperthermia observed within 85 min of infusion as well as an increase in the rate of temperature change (see figure 2, left panel). This sensitized response was presumably due to the summation of the hyperthermic CR and hyperthermic UR. This conclusion was supported by the fact that there was no difference between Groups P and U in response to drug on Block 1, but by Block 5, Group P showed a faster rate of temperature increase relative to Group U. This conclusion was also supported by the fact that Group P showed a greater hyperthermic response to morphine in the presence of the CS versus its absence (see figures 6 and 7). Group U also responded differently on the CS+US versus US-alone tests, but a key finding was the difference between Groups P and U in the CS+US test such that Group P was more hyperthermic than Group U.

In general, the results of Group P are in agreement with some of the studies of the context-specific thermic response to morphine (Eikelboom & Stewart, 1979, 1981; Miksic et al., 1975; Sherman, 1979). These results are also in agreement with those studies of context-specific sensitization to morphine's hyperactive effect (Fanselow & German, 1982; Hinson & Siegel, 1983; Kamat et al., 1974; Mucha et al., 1981). In most of the studies of

temperature and all of those of activity, a hyperthermic or hyperactive CR was elicited by environmental cues and thus led to cue-specific tolerance to the initial hypothermia or hypoactivity (when observed) and sensitization to the hyperthermia and hyperactivity due to summation of the CR with the UR. These results are in direct disagreement with Siegel's (1978) study. In his study, a hypothermic CR summed with morphine's hyperthermic UR and resulted in tolerance to the hyperthermia. The hypothermic CR was induced by environmental cues and was extinguished only by exposure to the environmental cues paired with saline.

The results of Group U during the drug tests imply some kind of interaction between the CS and US, in that Group U showed a shorter latency to peak hyperthermia on the CS+US test versus the US-alone test. One possibility could be that repeated exposure to the CS explicitly unpaired with morphine resulted in inhibitory conditioning to the CS; however, conditioned inhibition should have attenuated rather than augmented responding in the presence of the CS relative to the US-alone test (Konorski, 1948). Therefore, conditioned inhibition probably did not occur in Group U.

A second possible explanation is that the i.v. infusion of room-temperature fluid was a CS for morphine's effects in Group U. When infusion occurred in the presence of the light/noise CS during testing, the CS may have acted as a distractor, augmenting the response to morphine relative to that from the US-alone test. According to this account, infusion cues should alter the drug response regardless of time of infusion. However, Group U's response differed on the US-alone test relative to Day 22 of the training phase (see figure 8). Thus, it seems unlikely that infusion acted as a CS.

A third possibility is that Group U's orienting response to the CS was habituated and morphine presentation following CS onset in testing disrupted

this habituation. That is, morphine may have acted as the distractor, dishabituating the response to the CS. Therefore, the greater response during the CS+US test relative to the US-alone test might have been due to the summation of the dishabituated response to the CS and the UR to morphine. Although this account handles Group U's different responses on the CS+US and US-alone tests, it does not explain the difference in UR during the US-alone test and Day 22.

A possible explanation for Group U's different responses on the US-alone test versus Day 22 is that it learned to use temporal cues as the predictor for drug effects. This is possible because the US for Group U was always administered at the same time of day (0945 hrs) in the absence of an explicit signalling stimulus. It is also possible that the rats were not learning about time of day, per se, but rather a temporal relationship within the trial. In other words, the rats may have learned that the US always occurred 90 min after CS onset. This interpretation is consistent with the fact that Group U showed a faster rate of temperature change on the US-alone test relative to Day 22 (see figure 8). This difference may have been due to the lack of temporal cues or lack of the CS or both. Eikelboom and Stewart (1981) have demonstrated that rats show a hypothermic CR at the same time of day that morphine was regularly injected (1030 hrs). Figure 4 shows that Group U's temperature during the 1-hr pre-injection period of the training phase changed from a gradual increase to a gradual decrease over the hour. This decrease in temperature may have occurred in anticipation of morphine, similar to the hypothermic CR seen in Eikelboom and Stewart's study. The implication is that the CR to a CS paired with a drug US varies depending on whether temporal cues are part of the CS. The hypothermic CR in Group U summating with the

hyperthermic UR may have slowed the rate of temperature change in Group U relative to Group P.

Assessment of Group U's learning about temporal cues (either time of day or a CS-US relationship) would have been possible with the addition of various control groups. For example, a group receiving CS presentations at the same time as Groups P and U but receiving the US at random times within the trial would presumably have no temporal cues to predict drug effects. Thus, a comparison between Day 22 versus the US-alone test should show no difference in the form of the UR. Another possible control group would be a a no-drug control group, i.e., a group receiving the same number of CS presentations but receiving saline rather than morphine. This group could have allowed comparison of temperature changes during the trial with those in Group U to determine which changes are due solely to circadian rhythm in the absence of drug. This group, however, would not receive equal exposure to drug US which can be a problem in interpreting strength of association (cf. Rescorla, 1967).

Additional drug tests might provide information regarding temporal conditioning in Group U in the absence of extra control groups. For example, giving Groups P and U a test in which the CS and US are presented in the same temporal relationship as on explicitly unpaired trials (i.e., ISI = 90 min) would allow for a between-groups comparison to be made of the response to drug at that time of day. The other test would involve presenting the US at the same time as on explicitly unpaired trials, but without prior presentation of the CS. A within-group comparison of Group U's response on these two tests might not show a difference if time of day is serving as a cue for the US. However, a difference in response on these two tests might indicate that Group U is learning that there is a temporal relationship between CS onset and morphine administration or that whenever the CS occurs, morphine effects are

eventually experienced. A within-group comparison of Group P's response on these two tests may show no difference due to lack of cues. However, a difference in response might be expected due to either sensitization of the UR caused by prior CS presentation or inhibition of the UR due to negative induction (cf. Mackintosh, 1974). Basically, negative induction is the inhibition of a response due to previous presentation (within a certain time limit) of a CS+. That is, prior presentation of the CS could result in either a sensitized or inhibited UR in Group P.

In summary, the results of the present experiment demonstrate that conditioned sensitization can be observed in an experimental setting that involves an explicit CS paired with morphine. The advantage to this type of design is that it allows for further research into the parameters important for excitatory conditioning to occur. Experiment 2 examined the effect of varying one parameter on the strength of conditioning, CS-US overlap duration.

EXPERIMENT 2

Experiment 2 was designed to assess the effects of varying CS duration on conditioned changes in the body temperature and heart-rate responses to morphine. Experiment 1 showed that successful conditioning of a hyperthermic CR could be achieved with an ISI of 30 sec and CS-US overlap duration of 14.5 min.

According to Wagner's (1981) and Donegan and Wagner's (1987) SOP theory, strength of the excitatory connection between the CS and US increases when elements of both nodes are in the A1 state. Strengthening of the inhibitory connection between the CS and US nodes is assumed to occur when elements of the CS node are in the A1 state while elements in the US node are in the A2 state. The net associative change in any trial is produced by subtracting the amount of inhibitory learning from the amount of excitatory learning.

According to SOP theory (Donegan & Wagner, 1987; Paletta & Wagner, 1986; Wagner, 1981), associative strength plotted as a function of length of CS-US interval is an inverse U-shaped function when the CS and US are punctate stimuli. In forward conditioning where the CS is presented before the US at an ideal interval, there is a large increment in excitatory learning due to CS elements in the A1 state overlapping with US elements in the A1 state. There is also a small increment in inhibitory strength due to overlap of CS elements in the A1 state overlapping with US elements in the A2 state. A net positive association occurs. As the forward CS-US interval is increased, there is less associative learning expected because there is less overlap of CS elements in the A1 state with those of the US. In backward conditioning where the CS is presented after the US, there is a small proportion of CS elements in the A1 state overlapping with US elements in the A1 state and a relatively large proportion of CS elements in the A1 state overlapping with US elements in the

A2 state. Therefore, a net negative associative tendency develops unless the ISI is very short. Short backward ISIs will lead to net positive association because US elements will still be in the A1 state when CS onset occurs, thus there will be overlap of CS and US elements each in the A1 state. With longer intervals the net associative strength will be negative, and with very long backward ISIs, less inhibitory conditioning will develop (Wagner, 1981).

Wagner's SOP theory offers a rather unique prediction with respect to CS duration in forward conditioning. Specifically, SOP theory predicts that an "intermediate" duration CS should provide better conditioning relative to very long or short durations. An ideal, intermediate CS duration would allow a maximal number of CS elements to be recruited to the A1 state at the moment of US presentation. With very short CSs there should be a decrease in conditioning because fewer CS elements would be in A1 state at US onset. Long CSs would lead to less conditioning because many CS elements would have transferred to the A2 state by the time of US presentation. When the CS lasts for very long durations after the US or before the US, there will be no net associative effect, either inhibitory or excitatory (Wagner, 1981).

Studies of context-specific tolerance and sensitization vary with respect to how long the animal is exposed to the CS+ environment before and after the drug US is injected. Generally, the CS-US interval ranges from 0 min (e.g., Fanselow & German, 1982) to 30 min (e.g., Hinson & Siegel, 1983), except for two studies in which rats were injected in the colony room and then transported to the CS environment (Dafters & Bach, 1985; Miksic et al., 1975). The amount of time spent in the environment (CS) after the US presentation ranged from about 2 min (Mucha et al., 1981) to 5.5 hrs (Hinson & Siegel, 1983). In all of these studies, context-specific tolerance or sensitization, or the presence of a CR was reported. From these studies it would not appear

that ISI or CS duration are important. However, these studies usually involved a set of several cues probably acting as the CS, i.e., the CS was more like a compound CS with successive, overlapping components. Relative intensity of the components, their temporal relationship to each other and to the US influence the separate associative strengths of the components in a compound CS (Baker, 1968). In these studies, it is not known which components were most important. Therefore, the ISI or duration of the most salient CS component cannot be determined. Wagner's SOP theory is based primarily on conditioning studies using punctate stimuli, whereas in drug studies the stimuli are more "diffuse." It is assumed, however, that the effects of ISI and CS duration are essentially the same in drug studies as in studies using punctate stimuli, although the effective range of temporal intervals may be different, i.e., minutes versus seconds.

Another major difference between conditioning studies using punctate stimuli and those that use drugs as the US is the UR. Drug studies involve URs that are not instantaneous as is leg flexion or eye blink. Drug URs require time to develop and decay, due to pharmacokinetic factors (i.e., absorption, distribution, metabolism, elimination), which may be the reason for successful excitatory conditioning in studies involving relatively long CS-US intervals and overlaps.

Paletta and Wagner (1986) performed an experiment to examine the effects of CS-US overlap duration on context-specific sensitization to morphine's (5 mg/kg) activating effect. After injection, they left the subjects in the CS context for 10, 30 or 90 min. Paletta and Wagner observed a hyperactive CR and context-specific tolerance to morphine's hypoactive effect with a corresponding context-specific sensitization to the hyperactive effect in the 10- and 30-min groups, but not in the 90-min group. Paletta and Wagner

concluded that in the 90-min group, processing of the CS environment in conjunction with the secondary (A2) processing of the US led to inhibition and decreased net associative tendencies. They had expected to find evidence of less conditioning in the 10-min group relative to the 30-min group due to less overlap of CS elements in the A1 state with those of the US. The results of the analgesia measurement supported their prediction, in that neither the 10-min nor the 90-min group showed context-specific tolerance to the analgesic effect of morphine, but the 30-min group did.

There are few studies from the traditional conditioning literature on the effects of CS-US overlap duration. Barnes (1956) measured leg flexion response to shock (US) in dogs and showed that strength of conditioning was inversely related to CS-US overlap duration. Barnes kept ISI constant (0.9 sec) for all groups, but the CS-US overlap duration was varied. Durations of 0, 5, 15 or 30 sec were used. The results indicated that the longer the CS-US overlap, the less conditioning was evident in terms of number and proportion of CRs. Barnes' results suggest a linear inverse relationship between overlap duration and excitatory strength rather than the inverse U-shaped function predicted by Wagner's SOP theory (1981).

Studies of conditioned suppression generally show that suppression is less when the CS is extended beyond US offset by more than 1 min (Ayres, Albert & Bombace, 1987; Burkhardt & Ayres, 1978). In the study by Burkhardt and Ayres, rats were given one simultaneous conditioning trial in which an auditory CS was paired with shock US. The US duration was 4 sec, and the CS durations were 0, 1, 4, 64 and 128 sec. Thus, the 0 sec group was a US-alone control group, and in the 1-sec group, CS offset occurred 3 sec before US offset. For the 64- and 128-sec groups, the CS was extended 60 and 124 sec beyond US offset, respectively. The 4-sec group, for which CS and US onset

and offset were both simultaneous, suppressed responding significantly more than Group 0. None of the other groups differed from the control group. In another experiment, Burkhardt and Ayres showed significant suppression when the CS was extended 4 sec beyond US offset.

The conditioned suppression study by Ayres et al. (1987) showed that rats receiving three trials of a noise CS with an ISI of 2 min and no CS-US overlap showed greater suppression than rats receiving the same ISI but with the CS extended 10 min beyond US offset.

In summary, the results of the traditional conditioning experiments (Ayres et al., 1987; Barnes, 1956; Burkhardt & Ayres, 1978) and the conditioning study of morphine's activity response by Paletta and Wagner (1986) are in agreement, in that the longer the CS-US overlap duration, the weaker the strength of excitatory conditioning. However, the traditional conditioning experiments showed that no CS-US overlap or very short CS-US overlap durations were ideal for conditioning, whereas Paletta and Wagner showed that short overlaps were detrimental for conditioning morphine analgesic tolerance. Generally, it is thought that in drug studies, ISIs and CS-US overlap duration can be longer than those in studies using punctate stimuli because the nature of the UR requires some time to occur and dissipate due to pharmacokinetic factors. It may be that the inverted U-shaped relationship of CS-US overlap duration to learning may be unique to drug USs.

Heart-rate effects of morphine

Experiment 2 is concerned with the stimulus control of the heart rate and thermal responses to morphine. Because the temperature effects of morphine have already been described, this section only offers a brief summary of the drug's effect on heart rate.

Morphine's effect on heart rate in rats is similar to its effect on body temperature and activity, in that it produces a depressant effect followed by a stimulant effect. Bradycardia followed by tachycardia is reported to occur in conscious rats receiving i.v. administration of morphine at a dose of 1 to 10 mg/kg (Gomes, Svenson and Trolin, 1976; Schwarz, Peris & Cunningham, 1987). Most studies have reported only bradycardia after s.c. or i.v. morphine injection in rats (Fennessey & Rattray, 1971; Hine, 1985; Kiang, Dewey & Wei, 1983; Stein, 1976). The length of the post-injection recording period appears to be particularly important for observing a biphasic response. The studies reporting only a bradycardic effect measured heart rate for 30 sec (Stein, 1976), 2 min (Hine, 1985) or 3 min after injection (Kiang et al., 1983). The studies reporting biphasic effects measured heart rate for 30 min (Gomes et al., 1976) and 2 hrs after injection (Schwarz et al., 1987).

Anesthesia and restraint both augment the bradycardic portion of the response (Gomes et al., 1976; Schwarz et al., 1987). Gomes et al. showed that a 10 mg/kg dose produced a biphasic response in conscious rats but only bradycardia in anesthetized rats. However, a 5 mg/kg dose produced a biphasic response in anesthetized rats. Schwarz et al. showed that restraint increased both the magnitude and duration of bradycardia, particularly at doses of 5 and 10 mg/kg.

It has been suggested that morphine's bradycardic effect is vagally-mediated (Holaday, 1983; Fennessey & Rattray, 1971). Fennessey and Rattray showed that, in rats, the bradycardia evoked by doses of morphine from 0.1 mg/kg and higher (i.v.) was abolished by pretreatment with atropine, and significantly attenuated by bilateral vagotomy. Feldberg and Wei (1978) showed that morphpine administered intracisternally to cats produced

bradycardia presumably due to stimulation of the opioid receptors in the brainstem areas responsible for parasympathic control of the heart.

Morphine's tachycardic effect may be due to an increase in sympathetic outflow which is masked initially by the dominance of parasympathetic influence over sympathetic influence (cf. Berne & Levy, 1981). Feldberg and Wei (1978) showed that morphine injected into the lateral ventricle of cats produced tachycardia presumably by stimulation of opioid receptors in and around the areas of the brain responsible for sympathetic control of the heart. An increase in plasma catecholamines has been reported in rats treated with morphine (e.g., Mansfield, Wenger, Benedict, Halter & Woods, 1981). On the basis of this, one might argue that the excitatory action of morphine on heart rate is indirect, i.e., morphine elicits an increase in plasma catecholamines which cause an increase in heart rate.

Chronic administration of morphine leads to development of tolerance to the bradycardic effect (Hine, 1985; Kiang et al., 1983; Schwarz & Cunningham, in press; Stein, 1977). Either no change or sensitization develops to the tachycardic effect (Schwarz & Cunningham, in press).

In summary, morphine produces bradycardia followed by tachycardia in rats. This biphasic response is qualitatively similar to morphine's effect on body temperature and activity. Heart rate may actually be a more sensitive index of morphine's biphasic effect because it has been observed to occur at doses (2-5 mg/kg) that are usually reported as producing only increases in body temperature and activity (Babbini & Davis, 1972; Gunne, 1960; Schwarz et al., 1987). Repeated exposure to morphine results in tolerance to the bradycardic effect and either no change or sensitization to the tachycardic effect.

Rationale

Experiment 1 showed that context-specific sensitization can occur when morphine is repeatedly experienced in the presence of an explicit CS. The conditioning parameters used in Experiment 1 were an ISI of 30 sec and a CS-US overlap duration of 14.5 min. The purpose of Experiment 2 was to determine how CS-US overlap duration affected conditioning and to evaluate the predictions derived from Wagner's SOP theory of conditioning.

Wagner (1981) and Paletta and Wagner (1986) suggest that the relationship between strength of excitatory conditioning and CS-US overlap duration is an inverted U-shaped function. That is, very short and very long overlap durations are detrimental to the formation of associative links between the CS and US nodes. Paletta and Wagner (1986) found that a 90-min CS-US overlap resulted in no conditioning of morphine hyperactivity while the 10- and 30-min CS-US overlap durations did. They also measured analgesia, and found that neither the 10- nor the 90-min CS-US overlap resulted in conditioned tolerance to morphine's analgesic effect. Barne's (1957) study showed that in leg flexion conditioning, strength of excitatory conditioning is inversely related to CS-US overlap, such that the longer the CS-US overlap, the fewer the number or proportion of CRs measured. In addition, studies of conditioned suppression have also indicated a detrimental effect of extending the CS beyond US offset (Ayres et al., 1987; Burkhardt & Ayres, 1978). The detrimental effect was seen as reduced suppression of operant responding in the presence of the CS. Only Paletta and Wagner have shown that an intermediate CS duration produces better conditioning than short or long durations.

In Experiment 2, there were four groups: three paired and one explicitly unpaired. The three paired groups received the CS paired with i.v. morphine

infusion. As in Experiment 1, the ISI for all paired groups was 30 sec, but in this experiment, the groups differed with respect to CS duration. One group received a 5-min CS (Group P5), another group received a 15-min CS (Group P15), and the third group received a 60-min CS (Group P60). Group P15 was treated the same as Group P in Experiment 1, and served to replicate those results.

It is assumed that the A1 and A2 states of the US, as proposed by Wagner (1981) control morphine's depressant and stimulant effects, respectively. The 5-min CS duration was chosen based on the heart-rate response to an acute exposure of 5 mg/kg morphine (Schwarz et al., 1987). Schwarz et al. showed that maximum bradycardia occurred about 5 min after morphine administration; therefore, it was thought that this duration was long enough for the subjects to experience only the initial depressant effect of morphine in the presence of the CS. Therefore, there would be some overlap of the A1 processing of the CS and US nodes, but not sufficient overlap (cf. Paletta & Wagner, 1986). The 60-min duration was chosen because the rats experienced the excitatory effect of morphine while the CS was still on (Schwarz et al., 1987). Therefore, this overlap duration resulted in the CS extending into A2 processing of the US node. With the 15-min CS duration, there was presumably sufficient overlap of A1 processing of the CS with that of the US.

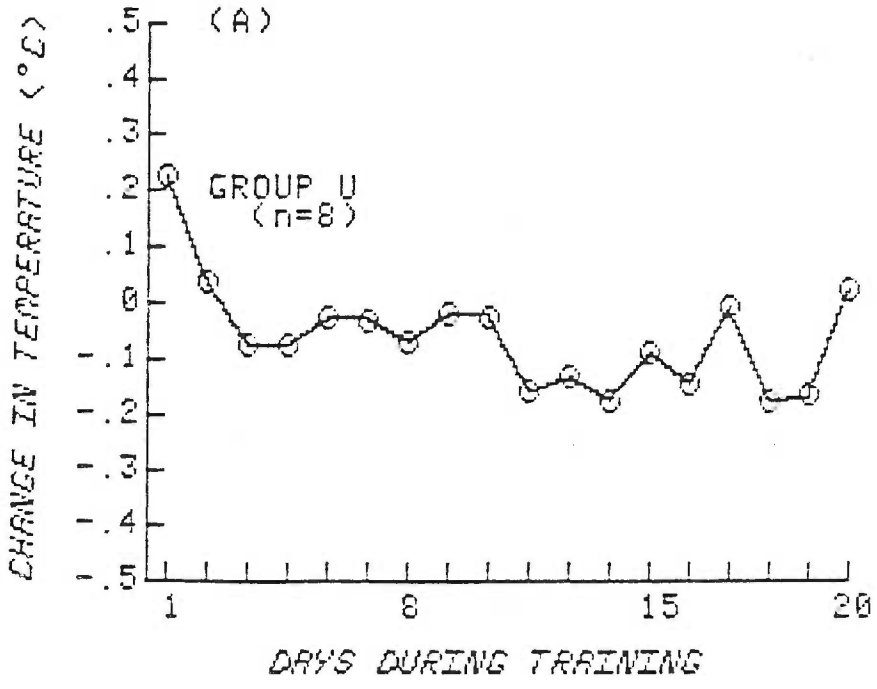
Group U received explicitly unpaired presentations of the CS and US. It received a quasi-random order of presentations of each CS duration such that it always received the 60-min CS duration on the test days. By getting unpaired presentations of the CS and US, this group controlled for nonassociative effects of repeated exposure to both stimuli. This group received the same number of CS and US presentations as Group P, but not the same total amount of exposure to the CS as the paired groups. Thus, the

unpaired group might have differed from the paired groups with respect to habituation to the CS. However, data collected from Group U during Experiment 1 suggested this would not be a problem. Specifically, Figure 11 shows Group U's change in temperature on the last sample period of the CS plotted over days of the training phase in Experiment 1. On the first exposure, Group U's temperature increased in response to the CS, but by the third exposure, its change in temperature was negligible. These data indicate that habituation occurred within the first 60 min of cumulative exposure to the CS. Therefore, it was expected that Group U would be completely habituated before the test phase began. ISI for Group U was 120 min so that in trials in which the 60-min CS was presented, all of the CS elements would presumably be in the A2 or I state at the time of US presentation.

In addition to body temperature, heart rate was also measured in Experiment 2. Heart rate is thought to be a very sensitive measure of morphine's biphasic effect in rats because the effect is produced at doses lower than those that elicit a measurable biphasic temperature and activity response (Schwarz et al., 1987). Unlike temperature, heart rate is a response that can change dramatically within a few seconds. Therefore, learned changes in heart rate can be observed within the 30-sec ISI. The addition of heart-rate data provided information on the generality of the previous results, i.e., whether heart-rate and temperature can both be conditioned, and if so, how parameter manipulations such as CS-US overlap duration affected the conditioning of each.

Learned changes in heart rate have been demonstrated using a morphine US in dogs (Bykov, 1957; Rush et al., 1970) but not in rats (Stein, 1976). Rush et al. showed a tachycardic CR in dogs that had received morphine (2 mg/kg) paired with a buzzer CS. Results are less clear with a high dose of morphine,

Figure 11. Mean change in temperature from pre-CS baseline is plotted over days during training for Group U (n = 8) from Experiment 1.


(A)
GROUP U
(n=8)
.5
.4
.3
.2
.1
0
-.1
-.2
-.3
-.4
-.5
1
8
15
20
CHANGE IN TEMPERATURE (°C)
DAYS DURING TRAINING

in that, Rush et al. reported an inconsistent tachycardic CR when the US was a 10 mg/kg dose, and Bykov reported a bradycardic CR in dogs when the US was a 10-20 mg/kg dose.

Although it was not specifically designed to evaluate the role of conditioning, a study by Schwarz and Cunningham (in press) suggested that the heart rate response of rats to morphine can also be affected by learning. In this study, restrained or freely-moving rats that received repeated injections of either a 4 or 8 mg/kg dose of morphine showed a significant increase in preinfusion baseline heart rate after nine exposures. Those results suggested some effect of previous days' exposure to morphine which could have been due to anticipation of the current day's treatment inasmuch as each animal was tested at the same time of day and exposed to the same cues everyday.

In the present experiment, a CR was assessed by administering saline rather than morphine on Day 11 and Day 22. These two placebo tests were given because the 30 sec ISI was not a long enough time for a measurable change in body temperature to occur.

Post-training testing procedures in Experiment 2 were identical to those in Experiment 1 with the addition of two more drug tests. As in Experiment 1, all rats received the morphine US with (CS+US test) and without the CS (US-alone test) to assess context-specific differences in drug response. In the US-alone test, morphine was administered at the same time in the trial as it was when paired with the CS. This procedure appeared to affect Group U's response to morphine in Experiment 1, in that Group U's response was different on the US-alone test relative to its response on the Day 22. To determine whether this difference might have been due to learning a temporal relationship, all rats were given two additional drug tests in which the US

was presented at the time normal for unpaired presentations. In one test, the CS was presented at its usual time and morphine was administered as in the explicitly unpaired trials (CS//US test). In the other test, morphine was administered at the same time as for the explicitly unpaired group, but no CS occurred earlier (NOCS//US). A within-group comparison of Group U's responses on these tests allowed assessment of whether Group U's response to the US at that time of day is dependent on previous presentation of the CS within the trial. A comparison of Group U's response on the US-alone and NOCS//US tests established whether or not time of day per se was important.

Based on the results of Experiment 1, it was hypothesized that the temperature CR would be hyperthermia. Due to summation of the hyperthermic CR with the hyperthermic UR in the paired groups, the UR will change after several morphine exposures and result in a faster rate of temperature change relative to the first exposure. It was also predicted that the heart rate CR would be tachycardia. This would be in agreement with the activity data showing that the CR resembles the stimulant component of the response (Hinson & Siegel, 1983; Mucha et al., 1981; Paletta & Wagner, 1986) and with the data reported by Rush et al. (1970) and Schwarz and Cunningham (in press). Due to summation of the tachycardic CR with the biphasic UR in the paired groups, it was predicted that the UR would change so that the magnitude and duration of the bradycardia would be shorter (i.e., tolerance) and there would be a faster rate of heart-rate increase relative to the first exposure (i.e., sensitization; Schwarz & Cunningham, in press). Proper assessment of tolerance or sensitization was made by comparing the responses of Group U with those of the three paired groups. Due to the lack of a hyperthermic or tachycardic CR in Group U, and due to the possible presence of a hypothermic CR, Group U was predicted to show a slower rate of temperature increase. It

was expected that Group U would also show a longer duration bradycardia with a corresponding slower rate of heart-rate increase relative to the paired groups.

In terms of CS-US overlap duration, it was predicted that the results would agree with Wagner's SOP theory. That is, the optimal overlap would be 15 min due to overlap of CS and US nodal elements in the A1 state. As suggested by Paletta and Wagner (1986), the 5-min CS-US overlap was predicted to be too short for sufficient overlap of A1 processing. The 60-min CS-US overlap was expected to be too long and extend into A2 processing of the US node. Both of these situations should have resulted in less evidence of excitatory conditioning. It was predicted that differences between the three paired groups' responses would not necessarily be in magnitude of hyperthermia but in response rate, with the faster rate of heart-rate and temperature changes corresponding to a stronger association between the CS and UR. This prediction was based on the data from Experiment 1 which showed that Group P's UR differed from Group U's UR primarily in rate of temperature change but not in magnitude of hyperthermia (see Figure 2).

Method

Subjects

The subjects were 32 adult, male albino rats (Harlan/Holtzman Co., Madison, WI) which were approximately 82 days old and weighed an average of 445 g (± 15 g) at the start of testing. The subjects were housed and maintained as in Experiment 1.

Surgical Procedure

Anesthesia and the MiniMitter and cannulation surgeries were identical to those in Experiment 1. Two heart-rate electrodes were also implanted.

Heart-rate electrodes. The heart-rate electrodes consisted of four strands of stainless-steel wire (diameter = 0.36 mm) wound together and covered to the tip with PE100 (1.2 mm i.d. x 1.7 mm o.d.) tubing for insulation. A molex pin was crimped onto the ends of each electrode and inserted into a nylon housing.

A 0.5 cm incision was made to the left of the midline just below the left forearm, and the superficial muscle was exposed. A 40 cm length of wire was loosely looped twice through the muscle such that there were four strands of wire, each 10 cm in length, projecting from the muscle. These four strands were then wound together and covered with PE100. A dorsal incision was made 3 cm below the skull, between the scapulae, and a second electrode was looped twice through the muscle and also covered with PE100. The ventral electrode was then tunneled subcutaneously to the dorsal incision and looped once with the dorsal electrode. The loop was anchored to the dorsal superficial muscle, and the incisions were closed with 000 silk suture. Molex connector pins

were crimped onto the ends of each electrode and protected with nylon housing plugs.

Apparatus

Experimental chambers, drug infusion apparatus and temperature monitoring equipment were the same as those used in Experiment 1. A miniature 3-channel fluid swivel with five electrical circuits (Model 211, Spalding Medical Products, Arroyo Grande, CA) was mounted above the cage. The rats' electrodes were attached to the swivel wires via the Molex pin connectors housed in nylon plugs. A spring leash from the swivel to the rat, protected the fluid and electrical lines. Wires led from the swivel to an amplifier. After amplification, the ECG signal was sent to a signal detector and converted into a digital signal. An Apple II+ computer calculated and recorded interbeat intervals (IBI) from the digital signal.

Procedure

Seven days after surgery, rats were randomly assigned to one of the three paired groups (Group P5, P15 and P60) or to the unpaired group (Group U) for classical conditioning. The CS was the same compound stimulus composed of light and white noise as in Experiment 1. The US was 5 mg/kg morphine in a 0.5 ml volume. Rats in the paired groups received presentation of the US 30 sec after CS onset. The CS remained on for an additional 4.5 min (Group P5), 14.5 min (Group P15) or 59.5 min (Group P60). Group U received explicitly unpaired presentations of both the CS and US, i.e., the US was administered 120 min after CS onset. Group U received presentations of each CS duration in a quasi-random order, such that it always received presentations of the 60-min CS on test days. This was done so that Group U's data can be

compared with those from each of the paired groups regardless of CS duration.

In this experiment, the subjects were given one trial per 24-hr period, and the training phase consisted of 22 trials. Table 2 outlines the experimental procedure. On every eleventh day (D11 and D22) during training, a Placebo trial was given in which all subjects received saline rather than morphine. The purpose of these tests was to measure the groups' response to the CS in the absence of drug. Two conditioning trials occurred after the second Placebo test and before the first post-training drug test in order to maintain the CR in the paired groups.

Following the training phase, the rats were given four drug tests. Two of the tests were the same as the post-training drug tests in Experiment 1. That is, all rats received the US paired with the CS (CS+US test) or alone (US-alone test). The purpose of these tests was to assess context-specific differences in the drug response. The order of these two tests was counterbalanced and alternated with conditioning trials to maintain the CR in the paired groups. The subjects were then given two additional drug tests in which they received the US explicitly unpaired with the CS (CS//US test) or the US without the CS but at the same time within the trial as the unpaired presentation (NOCS//US). The purpose of these two tests was to assess temporal-specific differences in the presence or absence of a previous CS presentation in Group U. The order of these two tests were counterbalanced, and these trials were also alternated with conditioning trials in order to maintain the CR in the paired groups. The total number of trials (conditioning and test)

Table 2. The procedure in Experiment 2 for all subjects. COND refers to trials during the training phase in which the paired groups receive the CS paired with the US, and Group U receives unpaired CS and US presentations.

DAY	TREATMENT
1-10	COND
11	PLACEBO
11-21	COND
22	PLACEBO
23-24	COND
25	DRUG TEST
26	COND
27	DRUG TEST
28	COND
29	DRUG TEST
30	COND
31	DRUG TEST

was 31. At the end of the experiment, each rat received an i.v. infusion of 0.1 ml Methohexital sodium to verify patency of the cannula.

As in Experiment 1, each trial began at approximately 0800 hrs. The trials lasted 65 min longer than in Experiment 1, a duration of 4 hrs 10 min. The extra time was added so that there was 120 min of recording after Group U received the US. At approximately 1700 hrs each day, all rats were weighed, and fresh morphine solutions were prepared. The chambers were provided with fresh food, water and wood shavings.

Eight rats were run at one time, thus, four replications were required obtain a group size of eight animals. All groups were represented by two subjects per replication.

Data Analysis

Temperature data was computed as in Experiment 1. For heart rate data, the mean IBI from each rat was computed for six 5-sec sample periods before and six 5-sec sample periods during the first 30 sec of CS presentation. For the remainder of the maximum CS duration (59.5 min), IBI was computed in 30-sec sample periods. After CS offset, IBI was computed in 1-min sample periods to the end of the trial. As a method of eliminating noise, all IBIs that were different by more than 20 msec from the previous IBI were ignored. In addition, all IBIs greater than 300 msec (<200 bpm) or less than 80 msec (>750 bpm) were ignored.

The mean IBI recorded during each sample period was then translated into an average heart-rate (bpm) and stored on a floppy disk by the Apple II+ computer. As with the temperature data, if signal errors required data for a whole sample period to be discarded, an average score was computed from adjacent sample periods and inserted in place of

the discarded data. As in Experiment 1, the data from the training phase were averaged over 2-day blocks. The data were analyzed using ANOVA. The degrees of freedom were properly adjusted according to the method of Linton and Gallo (1975). As in Experiment 1, all p values less than .05 were considered significant.

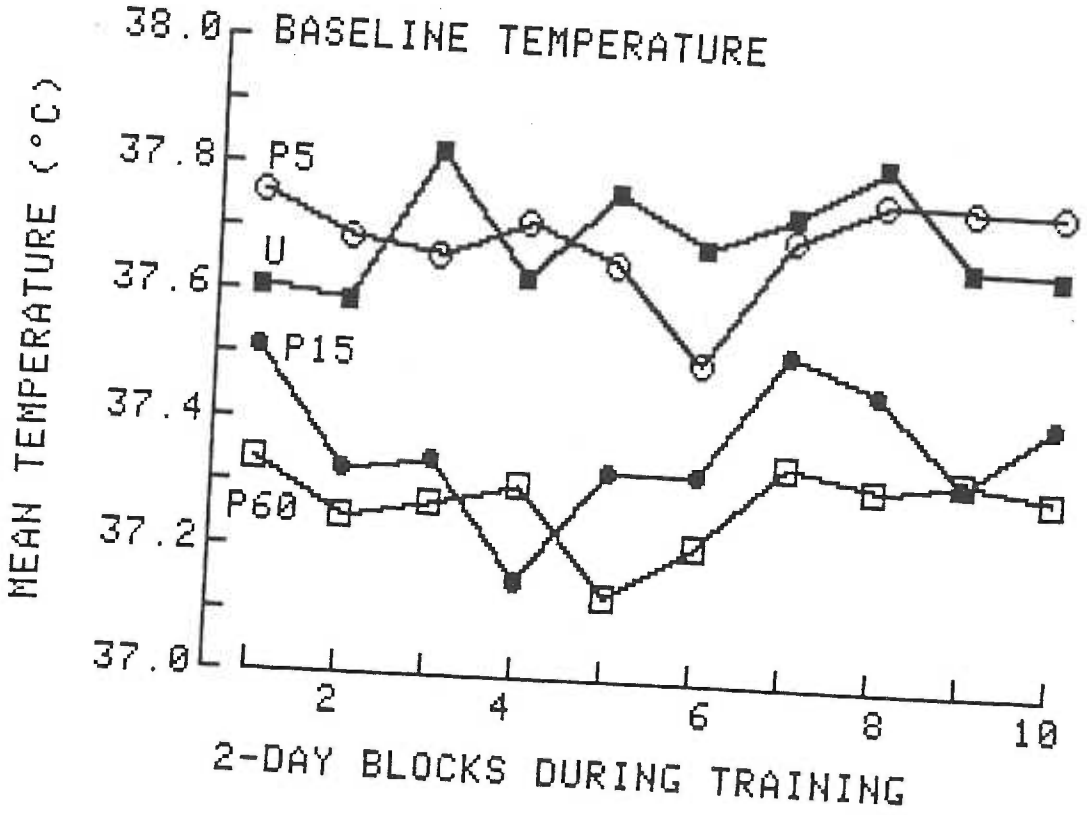
Results

For the analyses of body temperature, the data from eight subjects were discarded. For three of the eight rats, the MiniMitters stopped emitting a signal during the training phase (one rat each was in Group P5, P15 and U). Of the remaining five rats, both their temperature and heart rate data were discarded. Three rats showed no response to the Methohexital Sodium indicating an obstructed or damaged cannula (two were in Group P60 and one was in Group P5). Two rats died before the end of the training phase (one was in Group P15 and one was in Group P60). Therefore, for the temperature analyses during training, the numbers of subjects in Groups P5, P15, P60 and U were 6, 6, 5 and 7, respectively. For the heart rate analyses, the numbers of subjects were 7, 6, 5 and 8. In addition, one rat in Group P60 died after the Placebo tests, but before the Drug Tests, leaving group sizes for temperature analyses of 6, 6, 4 and 7 for Groups P5, P15, P60 and U, respectively. A heart-rate electrode of another rat in Group P60 was irreparably damaged leaving group sizes for heart rate analyses during the drug tests of 7, 6, 3 and 8 for groups P5, P15, P60 and U, respectively.

Body Temperature Data

Training Phase. Figure 12 shows Baseline temperature for each group plotted over 2-day blocks of the Training Phase. In general, Groups P5 and U showed higher baseline temperatures relative to Groups P15 and P60. This observation was supported by a two-way ANOVA (Groups x Blocks) which revealed a significant Groups effect, $F(3,20) = 3.71$. Because the Groups effect did not interact with Blocks, which would have suggested group differences in habituation to the procedure, the differences in baseline temperature were attributed to sampling error.

Figure 12. Mean baseline temperature for each group is plotted as a function of 2-day blocks during the training phase. The first sample period at the beginning of each trial, before either CS or US onset, was used as the Baseline.

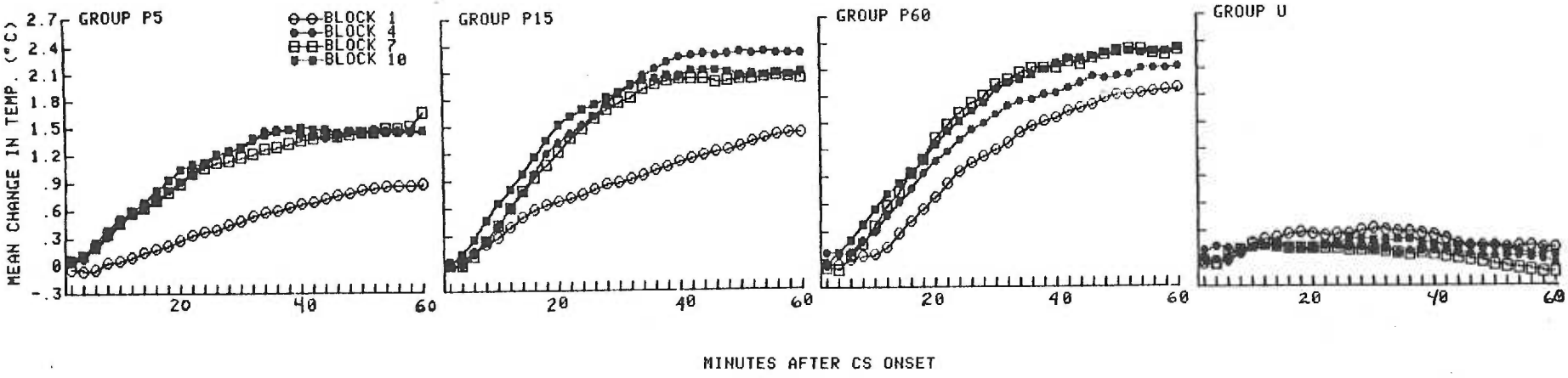

BASELINE TEMPERATURE
MEAN TEMPERATURE (°C)
38.0
37.8
37.6
37.4
37.2
37.0
P5
U
P15
P60
2
4
6
8
10
2-DAY BLOCKS DURING TRAINING

Because there were differences in baseline, remaining analyses of the temperature data were performed on change scores.

Figure 13 shows the change in body temperature during the 60-min period after CS onset in the first, fourth, seventh and tenth 2-day blocks during training. For Groups P5 and P15, the CS is only present during the first three and eight sample periods, respectively. For Group P60, the CS was present the entire time depicted in this figure. For Group U, the CS duration varied. For all Paired groups, morphine was infused 30 sec after CS onset (i.e., during the first sample period). Group U was not infused during the CS period. In the Paired Groups, the major change in response to morphine was an increase in hyperthermia with an increased rate of temperature change. This change took place early during training, since there is little difference in the responses in Blocks 4, 7 and 10. Group U showed little change in response to the CS alone during training.

These observations were supported by a three-way ANOVA (Groups x Blocks x Sample Periods) which revealed a significant Groups x Blocks x Sample Periods interaction, $F(783,4920) = 2.23$. Follow-up analyses indicated this three-way interaction was due to a significant Blocks x Sample Periods interaction in all three Paired groups, $F(261,1250) = 1.97$ for P5, $F(261,1212) = 7.01$ for P15, and $F(261,990) = 2.64$ for P60, but no interaction was found in Group U. Follow-up analyses of individual sample periods within each Paired group revealed a significant Blocks effect in Sample Period 15 and 30 in both Groups P5 and P15, $Fs(9,45) = 4.84$ and 3.49 in P5, and 12.56 and 5.86 in P15. In Group P60 a significant Blocks effect was observed in Sample Period 15, $F(9,36) = 6.35$ but not in Sample Period 1 or 30. The analysis of Group

Figure 13. Mean change in body temperature is plotted in 2-min sample periods after CS onset in Blocks 1, 4, 7 and 10. Each panel depicts the response of each group. For Groups P5 and P15 the CS was present during the first 3 and 8 sample periods, respectively. For Group U, the CS duration varied.


GROUP P5
BLOCK 1
BLOCK 4
BLOCK 7
BLOCK 10
GROUP P15
GROUP P60
GROUP U
MEAN CHANGE IN TEMP. (°C)
2.7
2.4
2.1
1.8
1.5
1.2
.9
.6
.3
0
-.3
20
40
60
MINUTES AFTER CS ONSET

U revealed a main effect of Sample Periods, $F(29,174) = 12.06$, but no effect of Blocks.

Follow-up analyses comparing the three Paired groups in Blocks 1 and 10 were also performed in order to assess any differences in the UR due to CS duration. The analyses of Blocks 1 and 10 both revealed a Groups x Sample Periods interaction, $F(58,406) = 3.19$ and 3.59, respectively, suggesting an effect of CS-US overlap on morphine's thermic response within 1-hr after infusion. Follow-up analyses in Block 1 revealed a significant Groups effect in Sample Period 30, $F(2,14) = 4.59$, but not in Sample Period 1 or 15. Contrast analyses of pairwise comparisons among the Paired groups revealed a significant difference between Groups P5 and P60, $F(1,14) = 8.87$. No differences were found between Groups P15 and P5 nor between Groups P15 and P60. In Block 10, none of the sample periods analyzed revealed a significant Groups effect; therefore, interaction contrast analyses were performed on the difference between Sample Periods 1 and 30. These analyses revealed a significantly smaller increase in temperature in Group P5 relative to both Groups P15 and P60, $F(1,406) = 27.27$ and 14.28, respectively. No difference between Groups P15 and P60 was found.

Figure 14 shows the change in body temperature in response to morphine in all four groups collapsed across the training phase. This figure includes the data for the P groups presented in Figure 13. The advantage of Figure 14 is that it extends the time period to 2 hrs after morphine infusion and includes Group U's response to morphine. Morphine produced hyperthermia in all groups. In general, the rate of temperature change was faster in the P groups relative to Group U.

Figure 14. Mean change in body temperature is plotted in 5-min sample periods during a 2-hr period after morphine infusion. The group means are collapsed across Blocks.

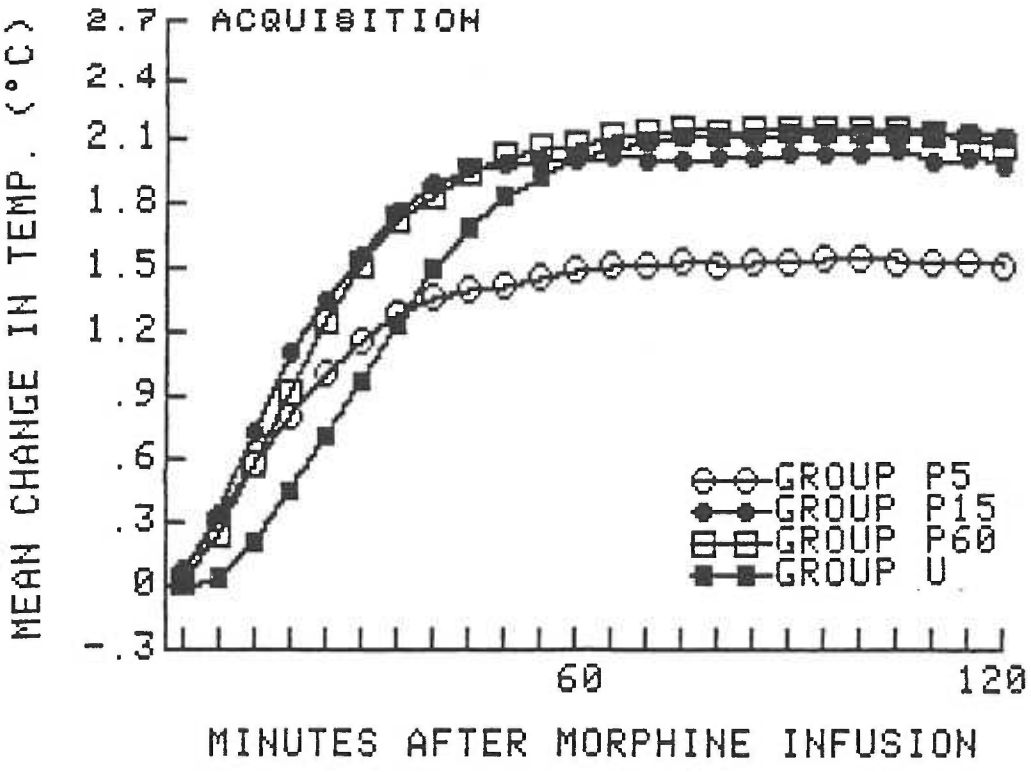

ACQUISITION
MEAN CHANGE IN TEMP. (°C)
2.7
2.4
2.1
1.8
1.5
1.2
.9
.6
.3
0
-.3
60
120
MINUTES AFTER MORPHINE INFUSION
GROUP P5
GROUP P15
GROUP P60
GROUP U

Figure 15 shows the response to morphine in Blocks 1, 4, 7 and 10, collapsed across groups. As observed previously, the rate of temperature change in response to morphine increases over blocks.

A three-way ANOVA (Groups x Blocks x Sample Periods) ANOVA supported the observations made in Figures 14 and 15, in that it revealed a significant Groups x Sample Periods interaction, $F(69,460) = 3.27$ and a Blocks x Sample Periods interaction, $F(207,4005) = 7.33$. Main effects of Blocks, $F(9,175) = 15.00$ and Sample Periods, $F(23,460) = 160.54$ were also significant. Follow-up analyses of individual sample periods explained the Groups x Sample Periods interaction, in that, a significant effect of Groups was observed in Sample Periods 1-5, $Fs(3,20) = 4.17$, 11.58, 11.08, 6.98 and 3.93, but not in later sample periods. Contrast analyses comparing all three Paired groups with Group U showed that in each of the first five sample periods, the Groups effect could be attributed to a significant difference between the Paired and Unpaired conditions, $Fs(1,20) = 15.89$, 33.45, 32.67, 18.96 and 9.95 for Sample Periods 1-5, respectively. Separate pairwise comparisons between individual Paired groups in these sample periods revealed no differences.

The Blocks x Sample Periods interaction was due to a significant effect of Blocks in Sample Periods 12 and 24, $F(9,180) = 13.10$ and 4.38, respectively, but not in Sample Period 1. Since the Groups factor was not involved in an interaction with Blocks and Sample Periods, it was assumed that a similar change in the UR occurred in all groups.

Figure 16 shows the change in temperature in response to each CS duration in Group U. The purpose of this figure is to see if the higher peak hyperthermia in Groups P15 and P60 might be due to some

Figure 15. Mean change in body temperature is plotted for 2 hrs after morphine infusion on Blocks 1, 4, 7 and 10. The data are collapsed across Groups.

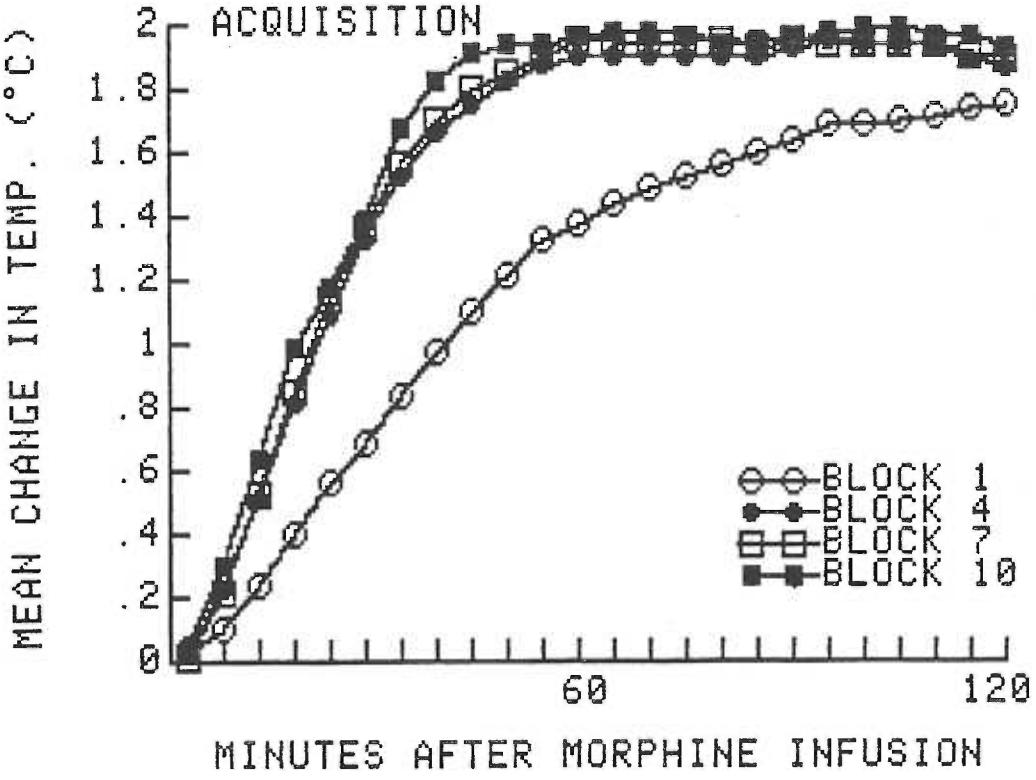
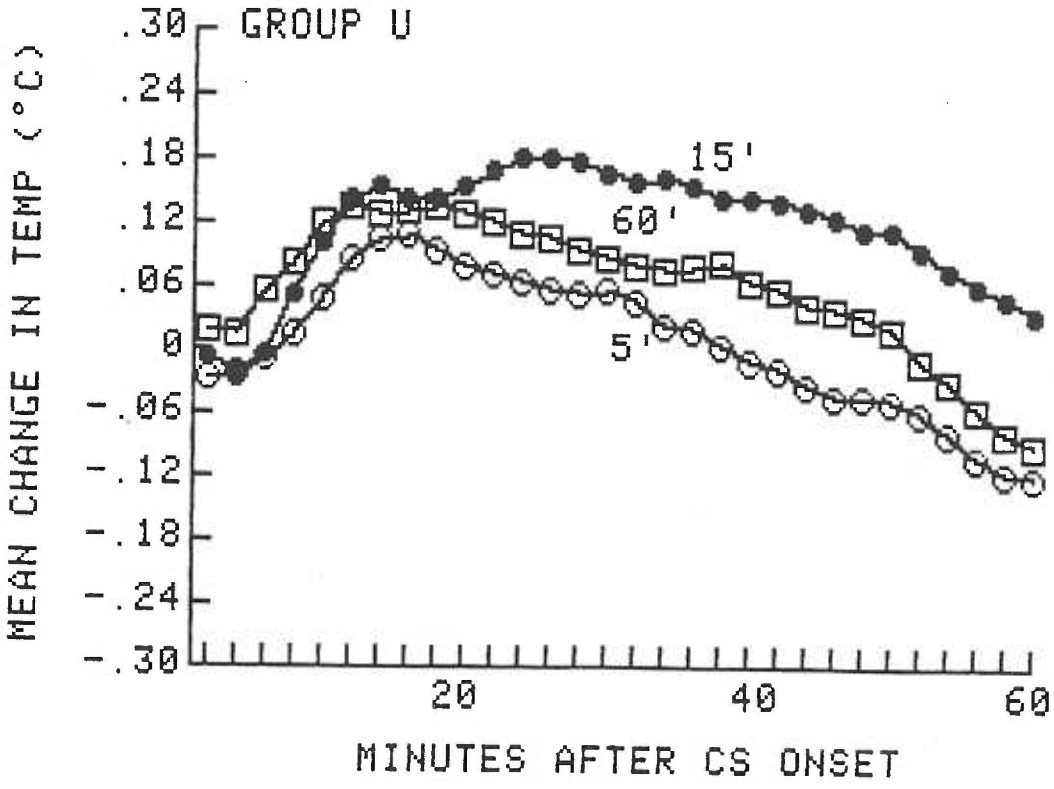

ACQUISITION
MEAN CHANGE IN TEMP. (°C)
2
1.8
1.6
1.4
1.2
1
.8
.6
.4
.2
0
60
120
MINUTES AFTER MORPHINE INFUSION
BLOCK 1
BLOCK 4
BLOCK 7
BLOCK 10

Figure 16. Mean change in temperature in Group U for 1-hr after onset of each CS duration. For the 5-min line, the CS is on only in the first 3 sample periods and for the 15-min line, the CS is on for the first 8 sample periods. The data are collapsed across trials.


GROUP U
.30
.24
.18
.12
.06
0
-.06
-.12
-.18
-.24
-.30
MEAN CHANGE IN TEMP (°C)
15'
60'
5'
20
40
60
MINUTES AFTER CS ONSET

nonassociative facilitative effect of the CS on the response to morphine. This figure shows that within the first 16 min after CS onset, Group U showed a similar increase in temperature to all three CS durations. However, decline back toward baseline differed with respect to CS duration such that temperature remained elevated after the 15-min CS relative to the 5- and 60-min CSs.

These observations were supported by a two-way ANOVA which revealed a CS Duration x Sample Periods interaction, $F(58,348) = 1.66$. A main effect of Sample Periods was also significant, $F(29,174) = 14.08$. Individual sample periods were analyzed to assess the source of the interaction; however, none of the analyses revealed an effect of CS Duration. Separate follow-up analyses of each CS duration revealed a significant effect of Sample Periods in each Duration, $F(29,174) = 8.75$, 3.94 and 7.55, for the 5-, 15- and 60-min durations, respectively. An interaction contrast analysis of the difference between Sample Periods 8 and 30 indicated that after the increase in temperature to the CS, temperature declined more after the 5- and 60-min CSs relative to the 15-min CS, $Fs(1,348) = 7.20$ (5- vs. 15-min), and 6.05 (60- vs. 15-min). No other comparisons revealed a difference.

An analysis of the 1-hr pre-infusion period for Group U (data not shown) revealed only an effect of Sample Periods, $F(11,66) = 2.21$. In general, temperature decreased over the 1-hr period in all blocks including Block 1. Neither the Blocks effect nor interactions involving Blocks were significant.

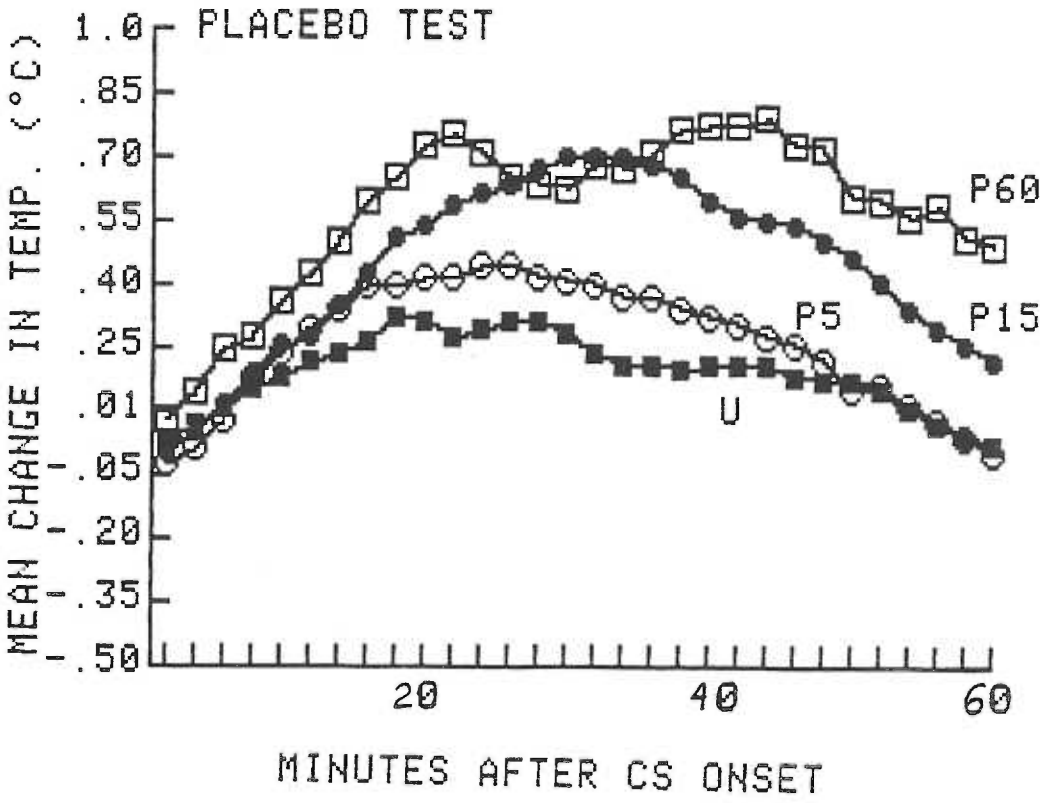
In summary, during the training phase, rats developed sensitization to the hyperthermic effect of morphine. Rats in the Paired groups showed a faster rate of temperature change than rats in Group U. There

was an effect of CS duration on the UR when measured within 1-hr of infusion in the first block. This difference was due to Group P5 showing a smaller magnitude of morphine hyperthermia. In Group U, the temperature increase to all three CS durations was the same, but it declined faster after onset of the 5- and 60-min CSs, relative to the 15-min CS.

Placebo Tests. On Days 11 and 22, saline rather than morphine was infused in order to assess the presence of a CR. Figure 17 shows the change in body temperature to the CS after saline administration collapsed across the two tests. In general, all groups showed an increase in temperature to the CS. The Paired groups, however, showed a greater increase than Group U. The three-way ANOVA (Groups x Tests x Sample Periods) performed on these data revealed a significant Groups x Sample Periods interaction, $F(87,551) = 1.40$. A main effect of Sample Periods was also found, $F(29,551) = 15.30$. The Groups x Sample Periods interaction was due to a significant Groups effect found in Sample Period 11, $F(3,19) = 3.22$ but not in any other sample periods tested.

In order to evaluate the source of the group differences, separate ANOVAs (Groups x Tests x Sample Periods) were performed on the data comparing each paired group individually with Group U. The comparison of Groups P5 and U revealed a main effect of Sample Periods, $F(29,290) = 6.74$, but no effect of Groups or interaction with Groups. The comparison between Group P15 and Group U revealed a significant Groups x Sample Periods interaction, $F(29,319) = 2.58$ and a main effect of Sample Periods, $F(29,319) = 9.04$. The comparison of Group P60 and Group U revealed a Groups x Sample Periods interaction, $F(29,290) = 2.32$ as well as main effects of Groups, $F(1,10) = 6.20$ and Sample Periods, $F(29,290)$

Figure 17. Mean change in body temperature is plotted in 2-min sample periods after CS onset during the Placebo test. For Group P5, the CS is present during the first 3 sample periods. For Group P15, the CS is present during the first 8 sample periods. For Groups P60 and U, the CS is present the entire period shown. The data are collapsed across the two tests on Days 11 and 22.


PLACEBO TEST
MEAN CHANGE IN TEMP. (°C)
1.0
.85
.70
.55
.40
.25
.01
-.05
-.20
-.35
-.50
20
40
60
MINUTES AFTER CS ONSET
P60
P15
P5
U

= 6.27. None of the analyses performed on the Placebo test data revealed a main effect of Tests or interaction involving Tests suggesting the hyperthermic CR had reached asymptote by Day 11 of training.

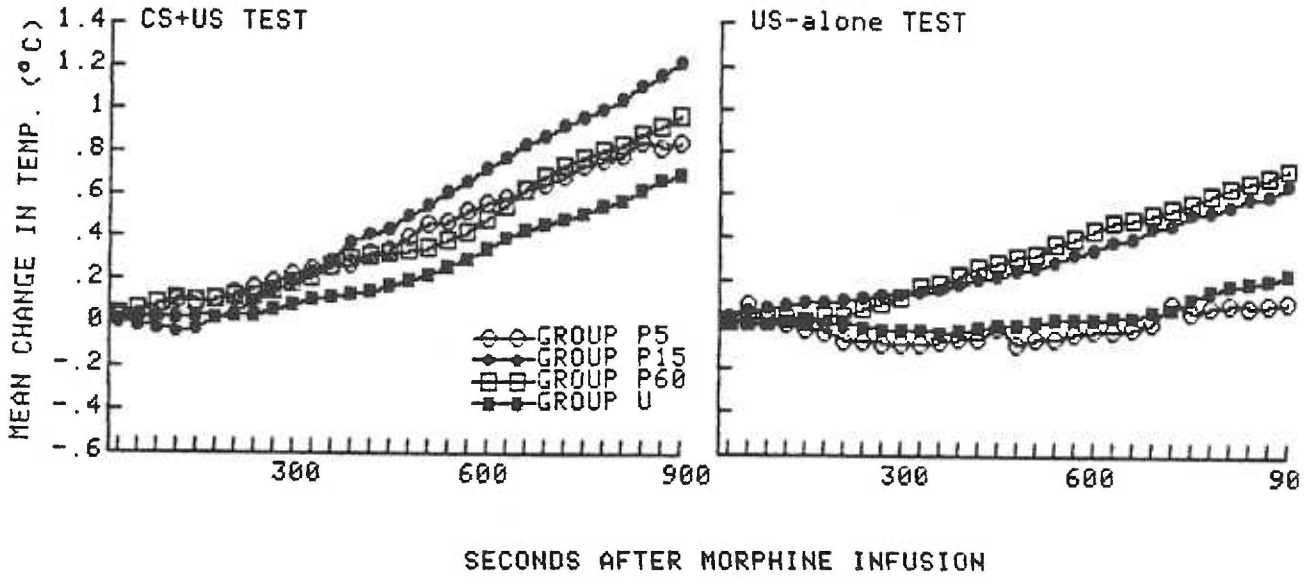
In summary, the data from the Placebo tests showed evidence of a hyperthermic CR in Groups P15 and P60. Group P5 also showed an increase in temperature to the CS, but its response was not significantly different from that of Group U.

Drug Tests. Following training, two drug tests were given in which all subjects received morphine paired with the CS and without the CS. The order of these tests was counterbalanced among the subjects. Analyses of body temperature revealed no effect of Order or interactions involving Order. Therefore, Order was omitted as a factor in the analyses of change scores.

Figure 18 shows the change in temperature during the 15-min period following morphine infusion. In general, the response to morphine was greater in the presence of the CS relative to its absence.

A three-way ANOVA (Groups x Tests x Sample Periods) revealed a significant Groups x Tests x Sample Periods interaction, $F(87,551) = 2.52$. Follow-up analyses within each group revealed a significant Tests x Sample Periods interaction in Groups P5 and P15, $F(29,145) = 17.21$ and 14.26, respectively, and Group U, $F(29,174) = 10.69$, but not in Group P60. Separate analyses of each test revealed a significant Groups x Sample Periods interaction in each test, $F(87,551) = 2.52$ for the CS+US test, and 2.03 for the US-alone test. The analysis of the US-alone test also revealed significant main effects of Groups, $F(3,19) = 4.18$, and

Figure 18. Mean change in body temperature is plotted in 30-sec sample periods in a 15-min period after morphine infusion. The responses on the CS+US (left panel) and US-alone tests (right panel) for each group are depicted.


CS+US TEST
US-alone TEST
MEAN CHANGE IN TEMP. (°C)
1.4
1.2
1
.8
.6
.4
.2
0
-.2
-.4
-.6
300
600
900
GROUP P5
GROUP P15
GROUP P60
GROUP U
SECONDS AFTER MORPHINE INFUSION

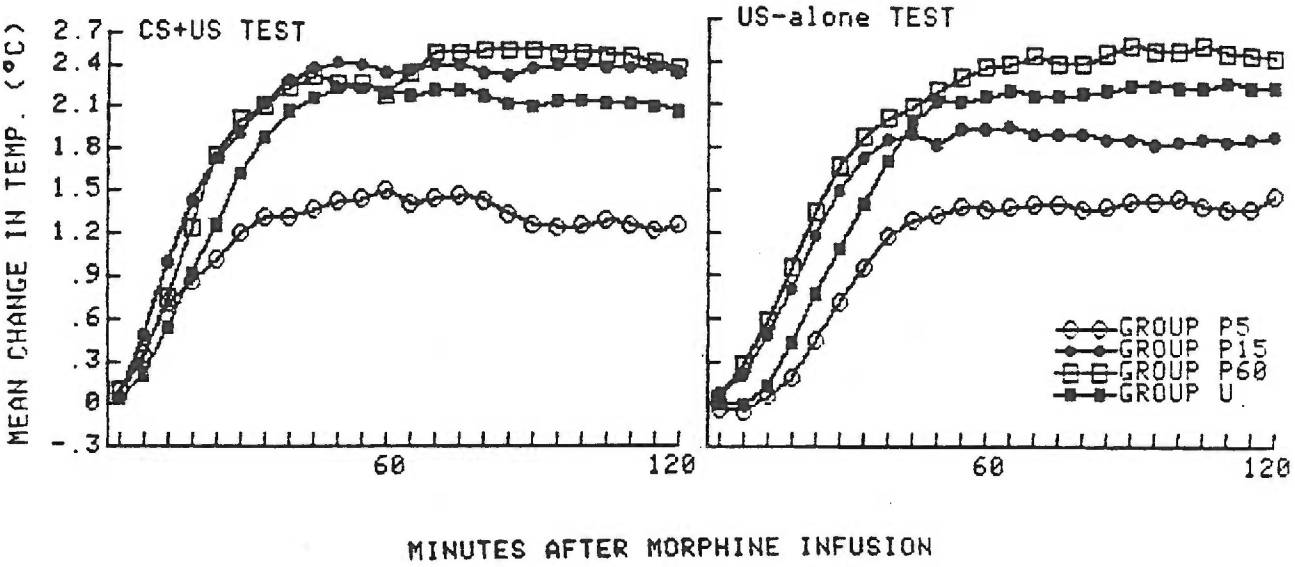
Sample Periods, $F(29,551) = 15.67$. Separate analyses of individual sample periods in each test revealed no effect of Groups.

In the CS+US test, separate pairwise comparisons of the difference between Sample Period 1 and 30 revealed significant differences between Group U and each of the Paired groups, $Fs(1,551) = 6.33$ for the comparison with P5, 87.36 for comparison with P15, and 14.66 for the comparison with P60. A significant difference was also found between Group P15 and each of the other paired groups, $Fs(1,551) = 43.23$ for the comparison with P5, and 18.85 for the comparison with P60. No difference was found between Groups P5 and P60.

In the US-alone test, contrast analyses were performed to assess the source of the Groups difference. Separate pairwise comparisons of the group means showed significant differences between Groups U and P15, $F(1,19) = 5.32$, and between Groups U and P60, $F(1,19) = 5.30$. Differences were also detected between Groups P5 and P15, $F(1,19) = 7.21$, and between Groups P5 and P60, $F(1,19) = 7.02$. No differences were found between Groups P5 and U and Groups P15 and P60.

Figure 19 shows the change in temperature in response to morphine plotted for 2 hrs after infusion. The first three sample periods in each panel are the same data as shown in Figure 18. In general, the rate of change in temperature was faster when morphine was given in the presence of the CS relative to its absence. This observation was supported by a Groups x Tests x Sample Periods interaction, $F(69,437) = 1.69$. Analyses of each group revealed significant Tests x Sample Periods interactions in Groups P5 and P15, $F(23,115) = 5.44$ and 1.76, respectively, and in Group U, $F(23,138) = 3.75$, but not in Group P60. Separate analyses of each test revealed a significant Groups x Sample

Figure 19. Mean change in body temperature in response to morphine in the CS+US (left panel) and US-alone (right panel) tests is plotted over a 2-hr period after infusion for each group.


MEAN CHANGE IN TEMP. (°C)
2.7
2.4
2.1
1.8
1.5
1.2
.9
.6
.3
0
-.3
CS+US TEST
US-alone TEST
60
120
60
120
GROUP P5
GROUP P15
GROUP P60
GROUP U
MINUTES AFTER MORPHINE INFUSION

Periods interaction in each test, $F(69,437) = 2.46$ (CS+US test), and 1.43 (US-alone test). A significant main effect of Sample Periods was also evident in each test, $F(23,437) = 95.96$ (CS+US test) and 93.14 (US-alone test). A main effect of Groups was significant in the CS+US test, $F(3,19) = 3.83$, but not in the US-alone test.

In order to assess the source of the Groups x Sample Periods interactions in each test, follow-up analyses of individual sample periods were conducted for each test. In the CS+US test, a significant effect of Groups was revealed in Sample Period 24, $F(3,19) = 3.19$, but not in any other sample periods tested. Separate pairwise comparisons of the groups in Sample Period 24 showed that Group P5 differed from all of the other groups, $Fs(1,19) = 7.56$ (Group P5 vs. P15), 6.38 (P5 vs. P60) and 5.26 (P5 vs. U) suggesting that in the CS+US test, Group P5 responded to morphine with significantly less maximal hyperthermia relative to all the other groups. No other comparisons revealed any difference.

In the US-alone test, follow-up analyses at individual sample periods revealed no effect of Groups at the sample periods tested. In order to evaluate the source of the Groups x Sample Periods interaction, contrast analyses of the difference between Sample Periods 1 and 24 were conducted in pairwise comparisons. Group P5 differed from all other groups, $Fs(1,437) = 5.04$ (P5 vs. P15), 37.2 (P5 vs. P60) and 25.70 (P5 vs. U). Group P15 also differed significantly from Groups P60 and U, $Fs(1,437) = 16.74$ (P15 vs. P60) and 11.40 (P15 vs. U). There was no difference between Groups P60 and U.

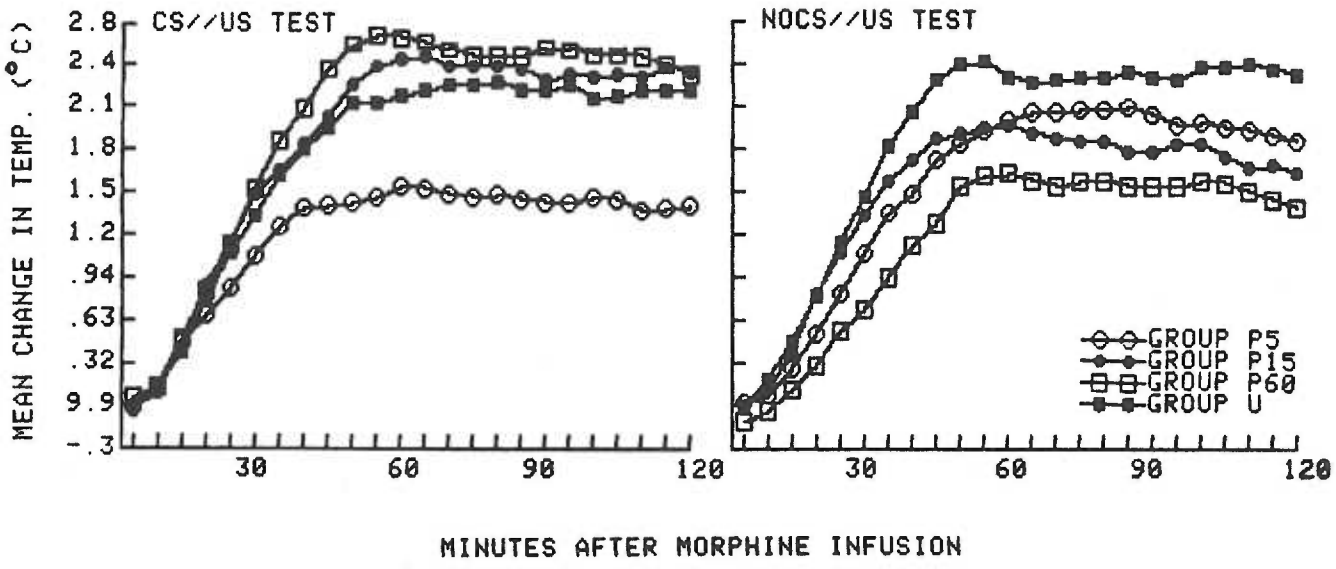
Following the CS+US and US-alone tests, two additional drug tests were given in which all rats received morphine at the time normal for

Group U. In one test, the CS//US test, morphine infusion was preceded by the CS 120-min earlier. In the NOCS//US, morphine was not preceded by the CS. The primary purpose for these tests was to evaluate whether Group U's response to morphine was dependent on previous occurrence of the CS. Figure 20 shows the change in temperature to morphine on each test for all groups. This figure shows that there was no difference in Group U's response on these two tests, suggesting no effect of the CS on their drug response. However, the responses of the Paired groups differed between the two tests, with Group P5 showing more hyperthermia in the NOCS//US test relative to the CS//US test. The effect of tests was reversed in Groups P15 and P60.

A three-way ANOVA revealed a Groups x Tests x Sample Periods interaction, $F(69,414) = 2.64$. Separate analyses of each test revealed a Groups x Sample Periods interaction in the CS//US test, $F(69,414) = 2.20$, but not in the NOCS//US test. A significant effect of Sample Periods was found in both tests, $F(23,414) = 127.51$ and 98.90 for the CS//US and NOCS//US tests, respectively. Individual Sample Periods in the CS//US test were analyzed, and a significant effect of Groups was found in Sample Period 12, $F(3,18) = 3.69$, but not in Sample Period 1 or 24. A contrast analysis at Sample Period 12 indicated that peak hyperthermia was lower in Group P5 relative to the other groups, $F(1,18) = 9.09$. Separate pairwise comparisons suggested this difference was primarily due to the differences between Groups P5 and P15, $F(1,18) = 9.71$, and between Groups P5 and P60, $F(1,18) = 7.91$. No other comparisons revealed any differences.

The differences in response to morphine in the Paired groups may have been due to associative effects of prior CS onset; however, these

Figure 20. Mean change in body temperature in response to morphine in the CS//US test (left panel) and NOCS//US test (right panel) is plotted over a 2-hr period after infusion.


MEAN CHANGE IN TEMP. (°C)
2.8
2.4
2.1
1.8
1.5
1.2
.94
.63
.32
9.9
-.3
CS//US TEST
NOCS//US TEST
30
60
90
120
GROUP P5
GROUP P15
GROUP P60
GROUP U
MINUTES AFTER MORPHINE INFUSION

differences might have merely been due to differences in pre-infusion temperature. In general, pre-infusion temperature was higher in the tests showing lower peak hyperthermia suggesting a ceiling effect with respect to the magnitude increase elicited by morphine. For Group P5, pre-infusion temperatures (C) were 37.77 (CS//US test) and 37.55 (NOCS//US test). For Group P15, pre-infusion temperatures were 37.12 (CS//US test) and 37.60 (NOCS//US test). For Group U, pre-infusion temperatures were 37.29 (CS//US test) and 37.20 (NOCS//US test).

In summary, all of the Paired groups showed greater morphine hyperthermia within 15 min of CS onset relative to Group U in the CS+US test. Groups P5 and P15 also evidenced cue-specific sensitization when their responses in the CS+US test were compared to those in the US-alone test. Group P60 showed no difference in their response in these two tests. When temperature measurement was extended to 2 hrs after infusion, Group P5 showed less hyperthermia in the CS+US test relative to all other groups. In the US-alone test, both Groups P5 and P15 were less hyperthermic relative to Groups P60 and U. In the CS//US and NOCS//US tests, Group U did not respond differently suggesting no effect of prior CS occurrence on their thermal response to morphine. The Paired groups did show differences in peak hyperthermia which may have been due to different preinfusion temperatures suggesting an inverse relationship between preinfusion temperature and magnitude of peak hyperthermia.

Heart Rate Data

Training Phase. Figure 21 shows baseline heart rate for each group plotted over 2-day blocks of the training phase. In general, there were greater differences between the groups at the beginning relative to the end of the training phase. This observation was supported by a two-way ANOVA (Groups x Blocks) which revealed a significant Groups x Blocks interaction, $F(27,197) = 2.36$. Neither a main effect of Groups nor Blocks was significant. Follow-up analyses revealed a main effect of Groups in Block 1, $F(3,22) = 3.79$, but not in Block 10. Pairwise comparisons of group means in Block 1 indicated that baseline heart rate in Group P15 was significantly higher than Groups P5 and P60, $F(1,22) = 4.42$ and 9.62, respectively. No other differences were found. Analysis of each group revealed a significant effect of Blocks in Group P5, $F(9,45) = 3.55$, and Group U, $F(9,63) = 2.03$. In Group P15, the Blocks effect was due to a decrease in baseline heart rate during training, while in Group U, there was an increase in baseline. The Groups x Blocks interaction suggests differences in habituation to the experimental procedure which may have been due to either a sampling error or treatment received. Because there were differences in Baseline heart rate, all subsequent analyses were performed on change scores.

Figure 22 shows the change in heart rate during the first 30 sec of the CS in Block 1 (left panel) and Block 10 (right panel). The increase in heart rate in Block 1 is presumably the orienting response to the CS. By Block 10, Group U's orienting response had habituated, as evidenced by little change in heart rate in response to the CS. However, the Paired groups still showed an increase in heart rate to the CS in Block 10 and this increase was presumably a CR. A three-way ANOVA (Groups x

Figure 21. Mean Baseline heart rate is plotted for each group as a function of 2-day Blocks during the Training Phase. Each data point represents the first sample period of each trial, before either CS or US onset.

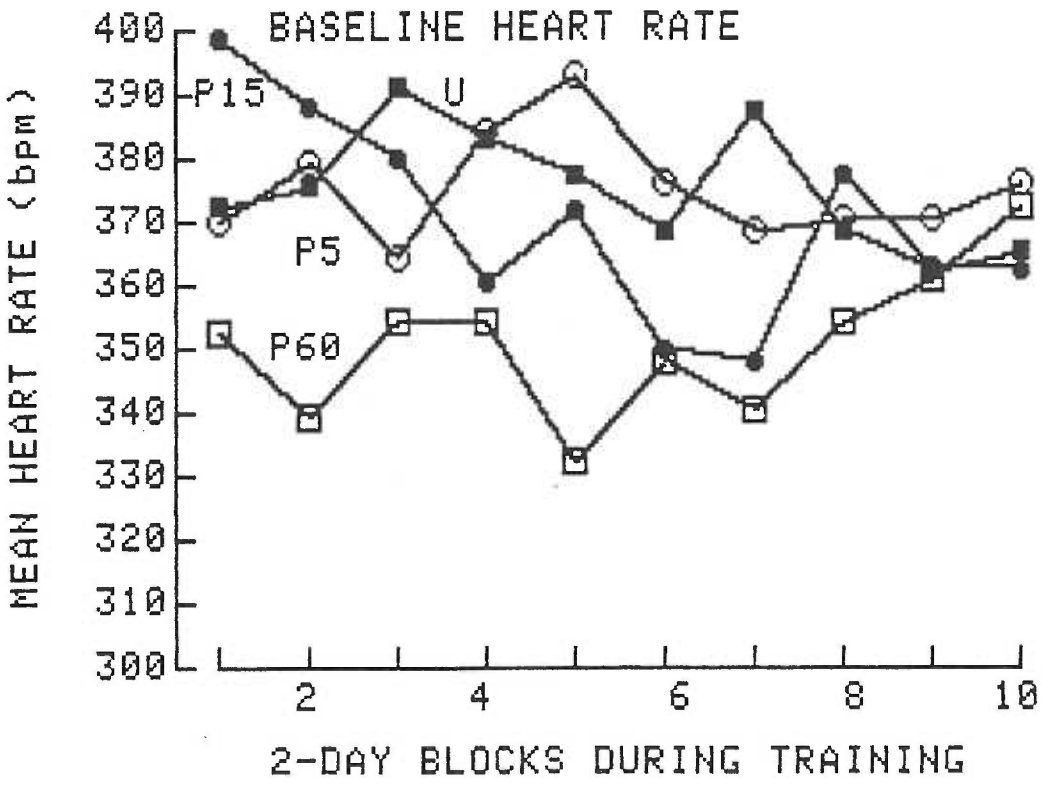
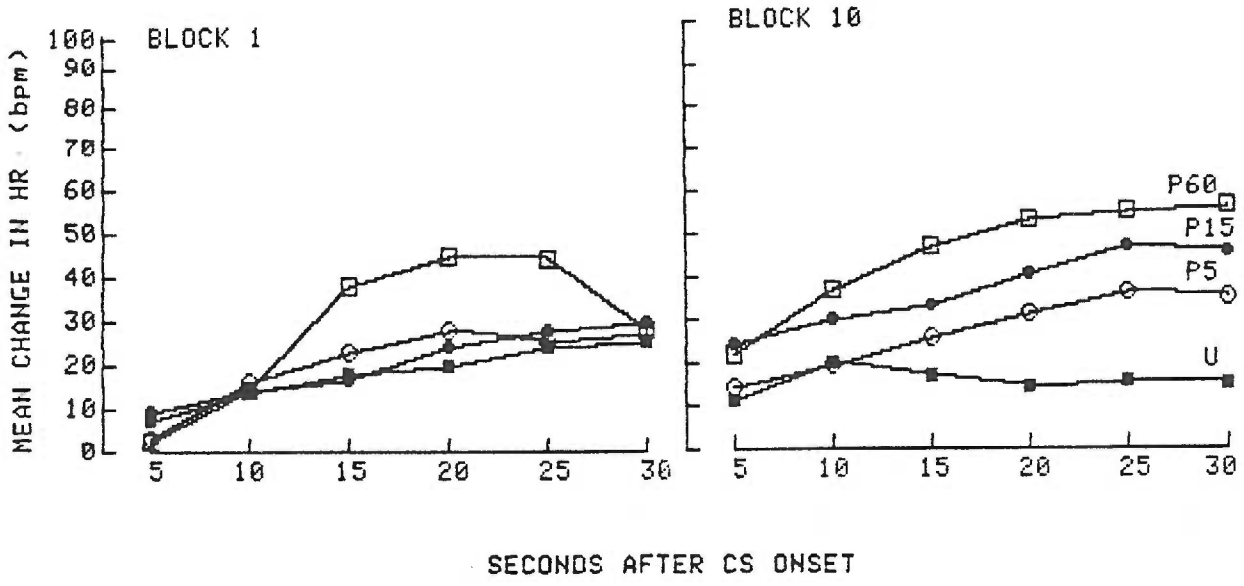

BASELINE HEART RATE
MEAN HEART RATE (bpm)
400
390
380
370
360
350
340
330
320
310
300
P15
U
P5
P60
2
4
6
8
10
2-DAY BLOCKS DURING TRAINING

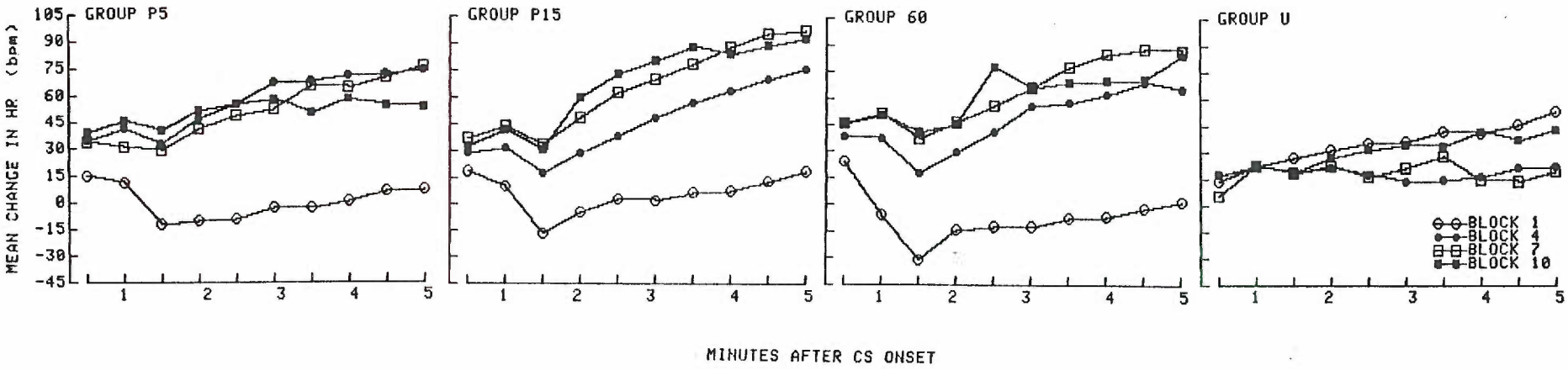
Figure 22. Mean change in heart rate during the first 30-sec after CS onset is plotted from Block 1 (left panel) and Block 10 (right panel) for each group.


BLOCK 1
BLOCK 10
MEAN CHANGE IN HR (bpm)
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
30
P60
P15
P5
U
SECONDS AFTER CS ONSET

Blocks x Sample Periods) performed on the data revealed a significant Groups x Blocks x Sample Periods interaction, $F(135,925) = 1.23$. Analyses in each group revealed a significant Blocks x Sample Periods interaction in Group P60, $F(45,180) = 1.60$, but not in any other group. A significant main effect of Blocks was found in Group P5, $F(9,54) = 3.11$, but not in Groups P15 or U. All groups showed a significant effect of Sample Periods, $F(5,30) = 31.02$ for Group P5, $F(5,25) = 17.34$ for Group P15, $F(5,20) = 16.15$ for Group P60, and $F(5,35) = 5.58$ for Group U. Additional follow-up analyses in Blocks 1 and 10 revealed a Groups x Sample Periods interaction in both blocks, $Fs(15,110) = 2.41$ for Block 1 and 2.29 for Block 10. Both analyses also revealed a main effect of Sample Periods, $F(5,110) = 24.69$ and 16.89, for Blocks 1 and 10, respectively. In Block 1, the Groups x Sample Periods interaction was probably due to Group P60's greater increase in heart rate during the middle portion of the 30-sec period; however, none of the sample periods tested revealed a significant Groups effect. In Block 10, the Groups x Sample Periods interaction was due to the divergence in response over the 30-sec period, particularly between the Paired and Unpaired Groups. Analyses of individual sample periods revealed a significant effect of Groups in Sample Period 6, $F(3,22) = 3.08$, but not in Sample Period 1. A contrast analysis of the data in Sample Period 6 revealed a significant difference between the Paired and Unpaired conditions, $F(1,22) = 8.17$. None of the individual pairwise comparisons among the Paired groups revealed significant differences.

Figure 23 shows the change in heart rate during the first 5 min of the CS on Blocks 1, 4, 7 and 10 for each group. The data depicted in the first sample period in this figure are the mean of the sample

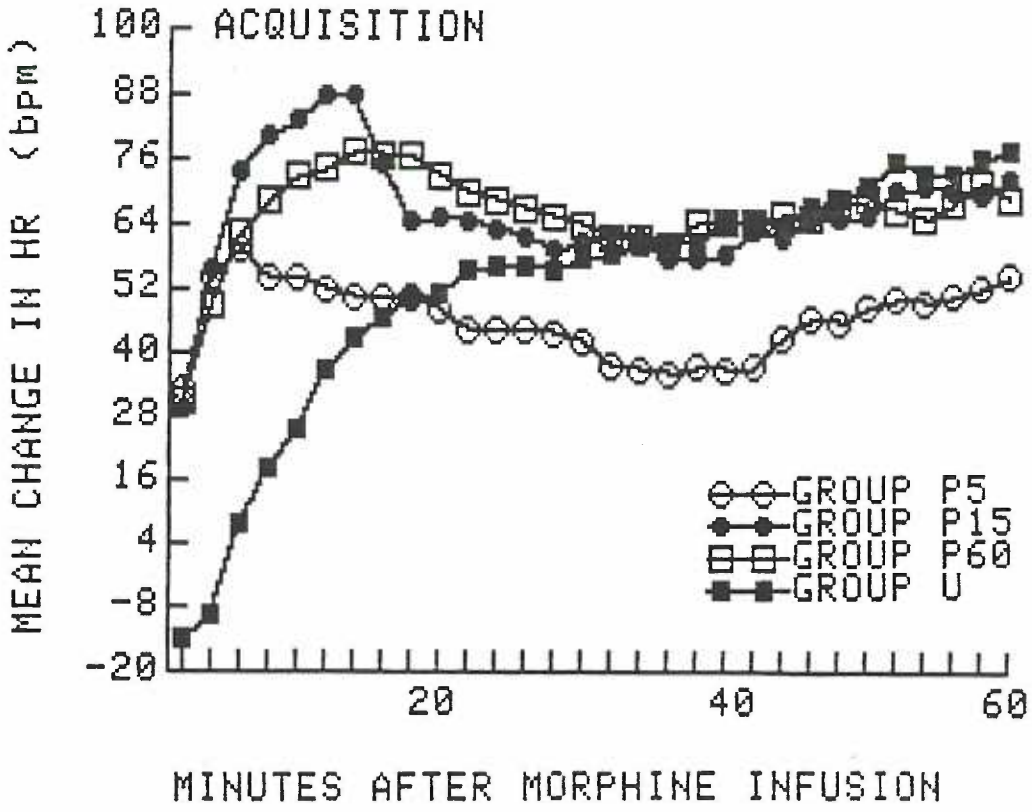
Figure 23. Mean change in heart rate during the 5-min period after CS onset during the Training Phase. All three Paired groups received morphine (5 mg/kg) after the first sample period. Group U received morphine explicitly unpaired with the CS.


GROUP P5
GROUP P15
GROUP 60
GROUP U
MEAN CHANGE IN HR (bpm)
105
90
75
60
45
30
15
0
-15
-30
-45
1
2
3
4
5
BLOCK 1
BLOCK 4
BLOCK 7
BLOCK 10
MINUTES AFTER CS ONSET

periods from Figure 22. This figure was included because it extends the measurement period after CS onset and overlaps with morphine infusion in the Paired groups. Figure 23 shows that all of the Paired groups responded to morphine with bradycardia in the first block. By Block 4, the bradycardic response was attenuated and a greater increase in heart rate was observed. Group U, which did not receive morphine, showed a slight increase in heart rate to the CS. A three-way ANOVA performed on these data revealed a significant Groups x Blocks x Sample Periods interaction, $F(243,1782) = 1.79$. A significant Blocks x Sample Periods interaction was found in every group: $F(81,486) = 2.38$ for Group P5, $F(81,405) = 3.39$ for Group P15, $F(81,324) = 2.20$ for Group P60, and $F(81,567) = 1.37$ for Group U. Analyses of individual blocks revealed a significant Groups x Sample Periods interaction in Blocks 4, 7 and 10, $Fs(27,198) = 2.73, 3.49, 1.82$, respectively, but not in Block 1. Individual Sample Periods in Block 10 were analyzed to determine the source of the Groups x Sample Periods interaction. A significant effect of Groups was revealed in Sample Period 10, $F(3,22) = 3.38$, but not in Sample Period 1 or 5. Follow-up contrast analyses revealed a significant difference between the Paired and Unpaired condition, $F(1,22) = 5.90$. Separate pairwise comparisons revealed differences between Groups P60 and U, $F(1,22) = 4.88$ and between Groups P15 and U, $F(1,22) = 8.04$. Group P5 did not differ from Group P60 or Group U, but did differ from Group P15, $F(1,22) = 4.72$. No other comparisons were significant.

Figure 24 shows the change in response to morphine in all groups collapsed across Blocks during training. This figure extends the measurement period relative to Figure 23 and also includes Group U's

Figure 24. Mean change in heart rate in response to morphine in each group. The data are plotted in 2-min sample periods for 1 hr after infusion, collapsed across Blocks during the Training Phase. In Group P5, the CS was present during the first 3 sample periods. In Group P15, the CS was present during the first 8 sample periods. In Group P60, the CS was present during all of the sample periods. Group U received morphine 2 hrs after CS onset.


ACQUISITION
MEAN CHANGE IN HR (bpm)
100
88
76
64
52
40
28
16
4
-8
-20
20
40
60
MINUTES AFTER MORPHINE INFUSION
GROUP P5
GROUP P15
GROUP P60
GROUP U

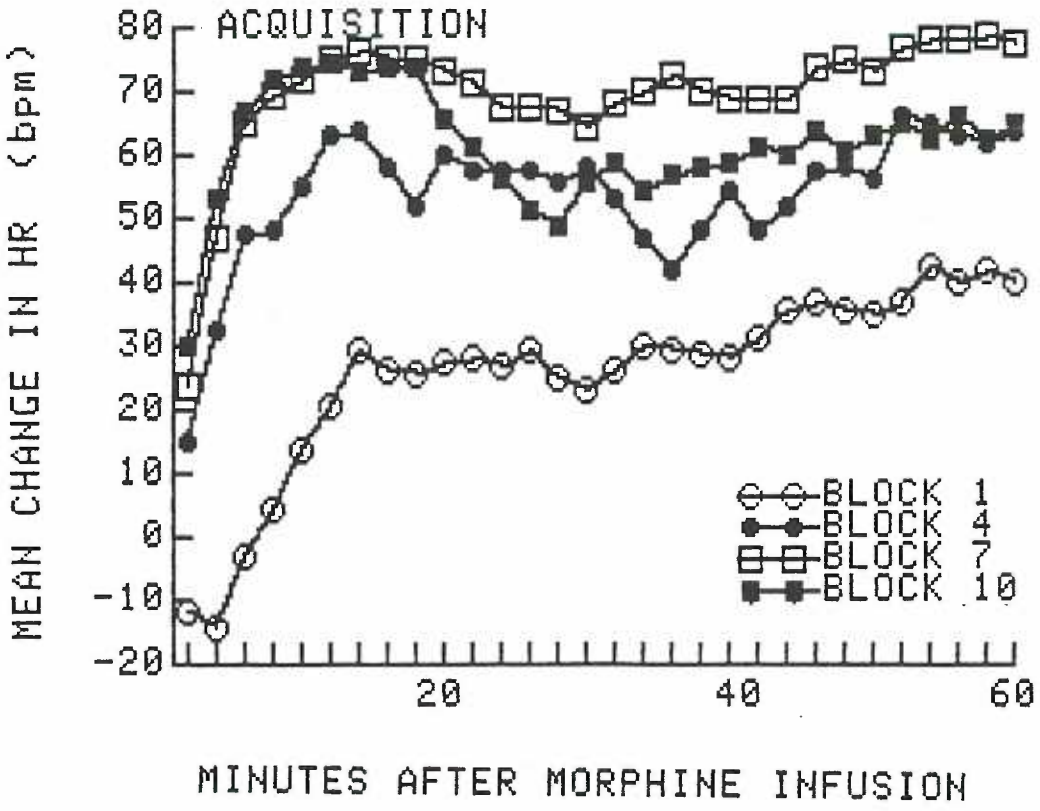
response to morphine. For Group P60, the CS was present during the entire period depicted in this figure. For Groups P5 and P15, the CS was on only during the first three and eight sample periods, respectively. For Group U, the CS was not present at all. In general, all Paired groups responded to morphine with an increase in heart rate. Bradycardia is not apparent in the Paired groups because each response is a mean of the entire training phase. Group U, however, does show initial bradycardia followed by tachycardia.

Figure 25 shows the heart rate response to morphine in Blocks 1, 4, 7 and 10, collapsed across Groups. This figure shows how the form of the response changed from a biphasic bradycardia followed by tachycardia to a monophasic tachycardia.

A three-way ANOVA (Groups x Blocks x Sample Periods) performed on these data revealed a significant Groups x Sample Periods interaction, $F(87,638) = 6.14$, a Blocks x Sample Periods interaction, $F(261,5742) = 2.31$, and a Groups x Blocks interaction, $F(27,198) = 1.56$. Main effects of Blocks, $F(9,198) = 12.49$, and Sample Periods, $F(29,638) = 13.60$, were also significant. The Groups x Sample Periods interaction supports the observation made in Figure 24. Follow-up analyses in individual sample periods revealed a significant Groups effect in Sample Periods 1 and 2, $Fs(3,22) = 8.59$ and 6.86, respectively, but not in Sample Periods 10 and 30. A contrast analysis of the group means revealed a significant difference between Paired and Unpaired conditions in Sample Period 1, $F(1,22) = 29.83$. Pairwise comparisons among Paired groups revealed no differences.

The lack of a three-way interaction suggests that the changes over blocks observed in Figure 25 varied little among the groups. The Groups

Figure 25. Mean change in heart rate in response to morphine is plotted during Blocks 1, 4, 7 and 10 during the Training Phase. The data are collapsed across Groups.

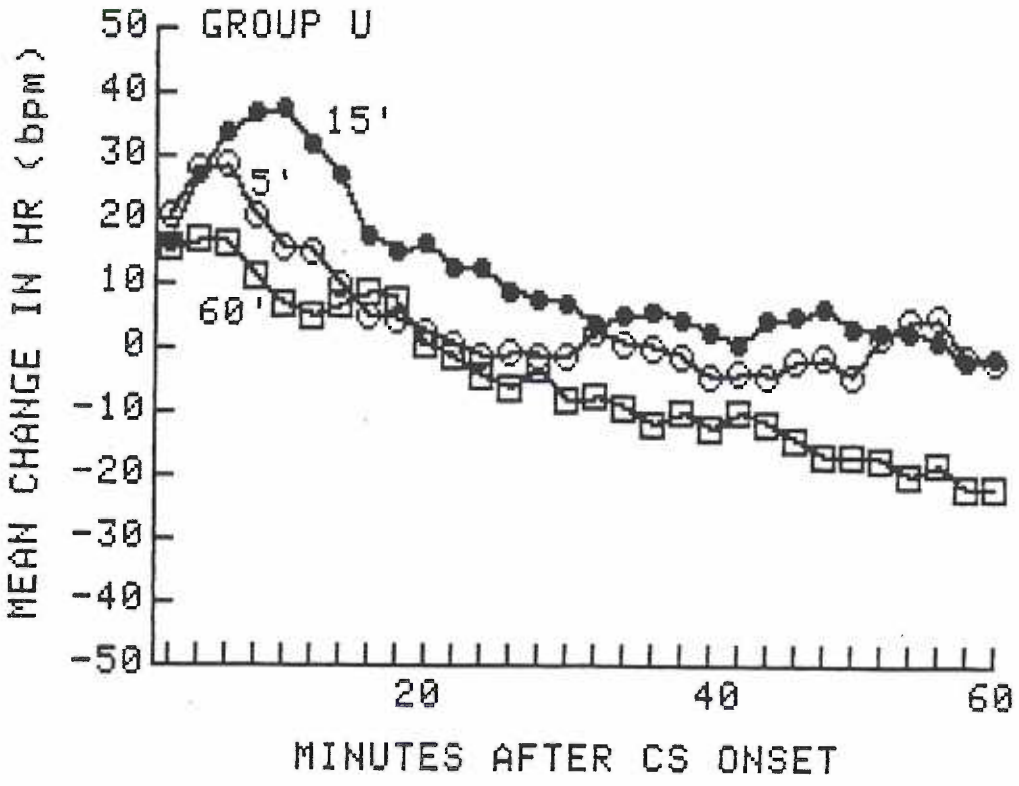

ACQUISITION
MEAN CHANGE IN HR (bpm)
80
70
60
50
40
30
20
10
0
-10
-20
20
40
60
MINUTES AFTER MORPHINE INFUSION
BLOCK 1
BLOCK 4
BLOCK 7
BLOCK 10

x Blocks interaction (data not shown) was due to a greater increase in the mean heart rate response over blocks in Groups P15, P60 and U relative to Group P5. This observation was supported by a significant effect of Blocks in Groups P15, $F(9,45) = 5.83$, P60, $F(9,36) = 2.67$, and U, $F(9,63) = 12.51$, but not in Group P5.

Figure 26 shows the change in heart rate in Group U in response to the three CS durations. The purpose of this figure is to see if differences in Group U's responses to the three CS durations might suggest a non-associative facilitative effect of CS overlap on the Paired groups' responses to morphine. Figure 26 shows that the peak increase in heart rate in response to CS onset is greater in the 15-min CS relative to the 5- and 60-min CSs. Following the increase, heart rate then declines, with the decrease being greater in the 60-min CS relative to the 5- or 15-min CSs.

A two-way ANOVA revealed a significant CS Duration x Sample Periods interaction, $F(58,406) = 1.89$. Significant main effects of CS Duration, $F(2,14) = 5.42$ and Sample Periods, $F(29,203) = 14.92$, were also found. Follow-up analyses of individual sample periods revealed a significant effect of CS duration in Sample Periods 5 and 30, $F(2,14) = 11.59$ and 7.60, respectively, but not in Sample Periods 1 and 15. A contrast analysis of the Duration effect in Sample Period 5 suggested a greater increase in heart after onset of the 15-min CS relative to either the 5- or 60-min CSs, $F(1,14) = 11.35$ and 21.75, respectively. A contrast analysis of Sample Period 30 showed that the decrease in heart rate after CS onset was greater during the 60-min CS relative to both the 5- and 15-min CSs, $F(1,14) = 11.30.$ and 11.50, respectively.

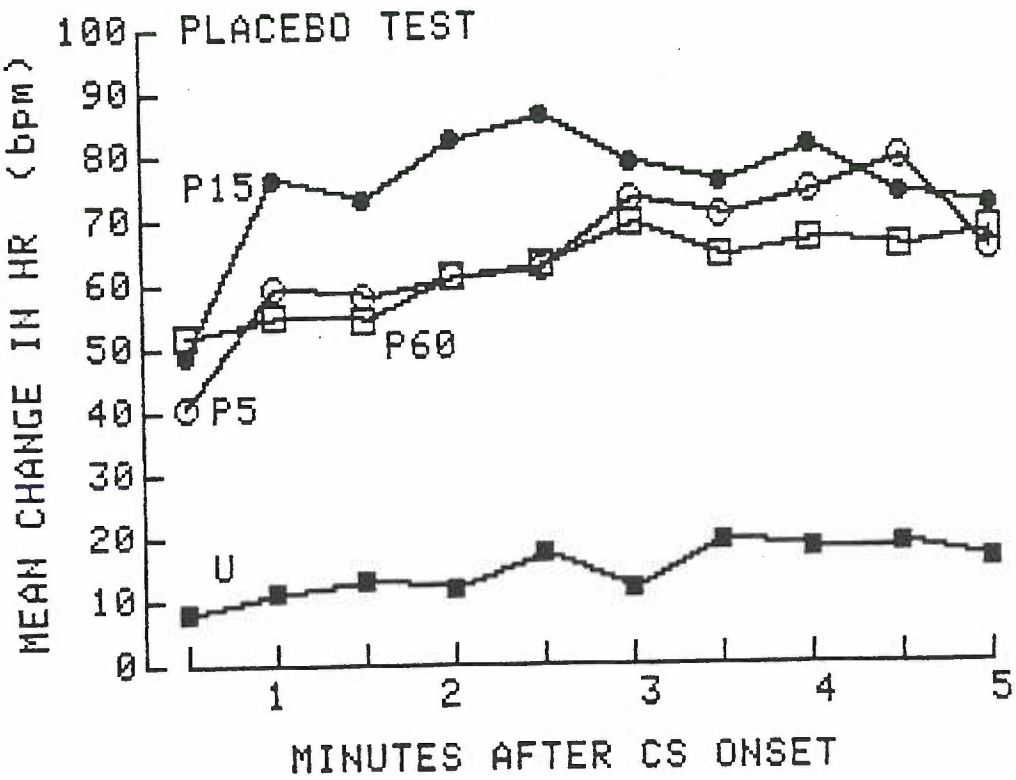
Figure 26. Mean change in heart rate in Group U in response to each CS duration. The data are plotted over the 1-hr period after onset. For the 5-min line, the CS is on only for the first 3 sample periods. For the 15-min line, the CS is on for the first 8 sample periods. The data are collapsed across trials.


GROUP U
MEAN CHANGE IN HR (bpm)
50
40
30
20
10
0
-10
-20
-30
-40
-50
15'
5'
60'
20
40
60
MINUTES AFTER CS ONSET

In summary, during the training phase, rats in the Paired groups showed development of a tachycardic CR during the first 30-sec of the CS, before morphine infusion. In general, all groups developed tolerance to morphine bradycardia. Rats in the Paired groups showed a faster rate of heart-rate increase than Group U. Initially, there was no effect of CS duration on the UR when measured within the first 5 min after infusion; however, when the UR was measured for two hours after infusion, Group P5 showed a smaller magnitude tachycardia relative to other groups. Group U's response to the varous CS durations showed a greater initial increase to the 15-min CS and a greater decrease in heart rate after onset of the 60-min CS.

Placebo Tests. Figure 27 shows the change in heart rate during the first 5 min of the CS in each group collapsed across the two placebo tests. This figure shows that in the absence of morphine, the Paired groups all showed a tachycardic CR. Group U showed little response to the CS. A three-way ANOVA (Groups x Tests x Sample Periods) revealed significant main effects of Groups, $F(3,21) = 5.54$, and Sample Periods, $F(9,189) = 5.10$. In order to evaluate the source of the Groups differences, a contrast analysis comparing the mean response of the Paired and Unpaired groups was performed. This analysis revealed a significant difference between the Paired and Unpaired conditions, $F(1,21) = 17.36$. There were no differences among the Paired groups, suggesting that all Paired groups showed a similar magnitude heart rate CR, regardless of CS-US overlap duration. Also, there was no effect of Tests nor interactions involving Tests, suggesting that the tachycardic CR had reached asymptote by Day 11.

Figure 27. Mean change in heart rate in response to the CS in the Placebo test is plotted over the 5-min period after CS onset. All rats received saline during the second sample period. The data are collapsed across both Placebo tests (Days 11 and 22).


PLACEBO TEST
MEAN CHANGE IN HR (bpm)
100
90
80
70
60
50
40
30
20
10
0
P15
P60
P5
U
1
2
3
4
5
MINUTES AFTER CS ONSET

Drug Tests. Figure 28 shows the change in heart rate over a 5-min period after morphine infusion when given paired with the CS and when given in the absence of the CS. In general, all groups tended to respond to morphine with a greater decrease in heart rate when the CS was absent relative to when it was present. Also, this test difference was much more pronounced in the Paired groups relative to Group U. A three-way ANOVA (Groups x Tests x Sample Periods) supported these observations. A significant Tests x Sample Periods interaction, $F(9,180) = 3.93$ and Groups x Tests interaction, $F(3,20) = 4.92$, were both significant. Main effects of Tests, $F(1,20) = 36.48$, and Sample Periods, $F(9,180) = 17.36$, were also revealed. Analyses of each group showed a significant effect of Tests in Groups P5, $F(1,6) = 26.97$, and P15, $F(1,5) = 19.51$. The effect of Tests in Group P60 approached significance; if the group size were greater, there might have been a significant difference between tests. Follow-up analyses revealed a main effect of Groups in the CS+US test, $F(3,20) = 5.80$, but not in the US-alone test. Separate pairwise comparisons of groups in the CS+US test revealed significant differences between Groups P5 and P15, $F(1,20) = 14.67$, between Groups P5 and P60, $F(1,20) = 4.52$, and between Groups P15 and U, $F(1,20) = 14.61$. Groups P5 and P60 did not differ from Group U.

Figure 29 shows the change in heart rate in response to morphine in each group collapsed across tests. This figure shows the response over the 1 hr period after infusion. The data in the first three sample periods were depicted in Figure 28. Figure 29 allows a look at the response for an extended lenght of time. In general, Group P5 shows a smaller magnitude tachycardia relative to the other groups. The

Figure 28. Mean change in heart rate in response to morphine during the CS+US (left panel) and US-alone (right panel) tests is plotted over the 5-min post-infusion period in each group.

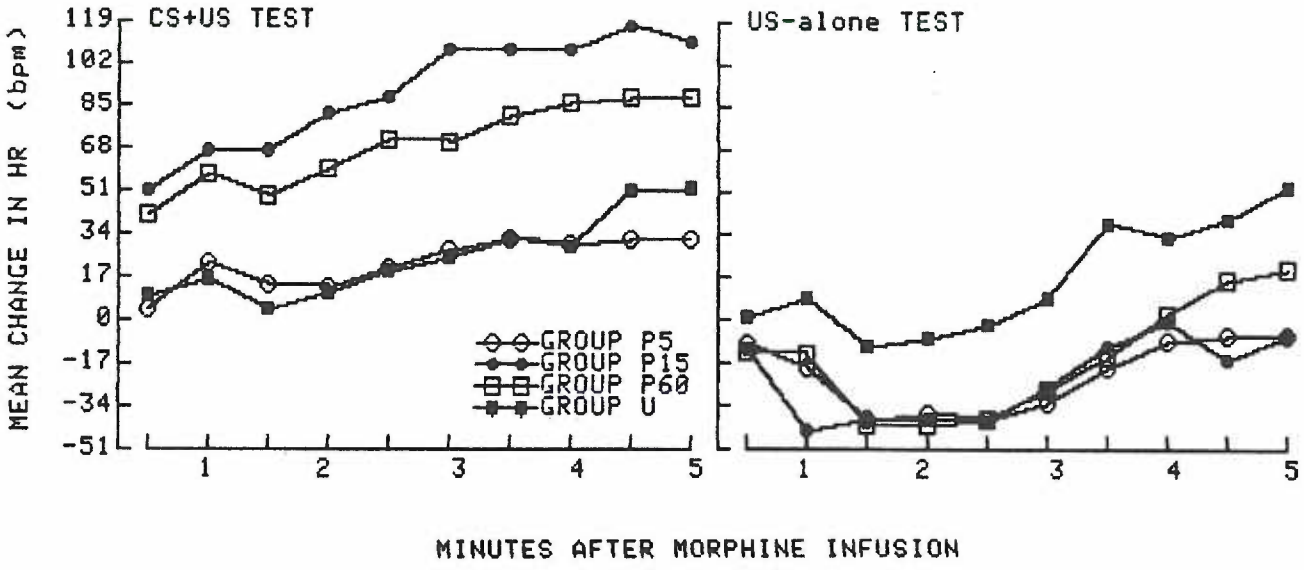
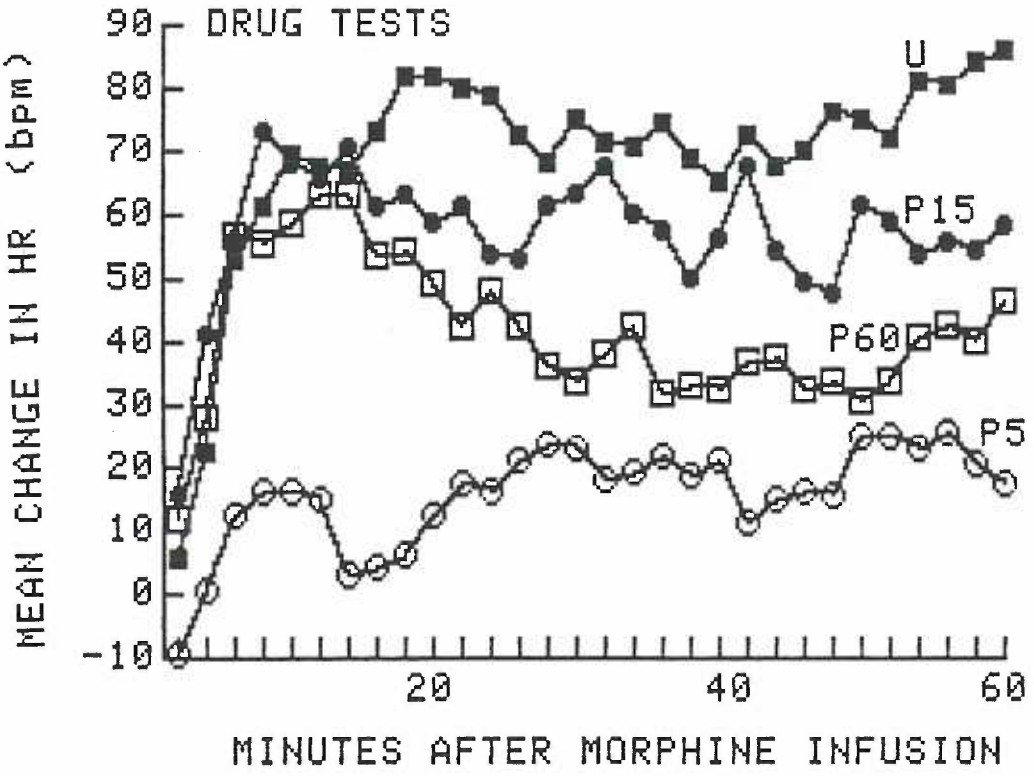

CS+US TEST
US-alone TEST
MEAN CHANGE IN HR (bpm)
119
102
85
68
51
34
17
0
-17
-34
-51
1
2
3
4
5
GROUP P5
GROUP P15
GROUP P60
GROUP U
MINUTES AFTER MORPHINE INFUSION

Figure 29. Mean heart rate in response to morphine is plotted for each group during the 1-hr post-infusion period. The data are collapsed across the CS+US and US-alone Tests.


DRUG TESTS
U
P15
P60
P5
MEAN CHANGE IN HR (bpm)
90
80
70
60
50
40
30
20
10
0
-10
20
40
60
MINUTES AFTER MORPHINE INFUSION

responses of all of the Groups are very similar early after infusion and then diverge at the end of the measurement periods.

Figure 30 shows the heart rate response to morphine in the presence and absence of the CS, collapsed across groups. This figure shows that in the absence of the CS, there is a clear biphasic heart rate response to morphine, but in the presence of the CS, there is a monophasic tachycardic response.

A three-way ANOVA (Groups x Tests x Sample Periods) revealed a significant Groups x Sample Periods interaction, $F(87,580) = 1.33$ and a Tests x Sample Periods interaction, $F(29,580) = 9.14$. Main effects of Tests, $F(1,20) = 5.63$, and Sample Periods, $F(29,580) = 5.78$ were also significant. Follow-up analyses at individual sample periods revealed a main effect of Groups in Sample Period 30, $F(3,20) = 4.22$, but not in Sample Period 1 or 5. To evaluate the Groups effect in Sample Period 30, separate pairwise comparisons were made between Groups. The analyses revealed significant differences between Groups P5 and both P15 and U, $Fs(1,20) = 5.31$ (P5 vs. P15) and 17.61 (P5 vs. U). No other comparisons were significant. A significant effect of Tests was revealed in Sample Periods 1 and 5, $F(1,20) = 24.50$ and 16.99, respectively, but not in Sample Period 30.

Following the CS+US and US-alone tests, two additional tests were given, the CS//US test and NOCS//US tests, in which morphine was administered to all subjects at the time of day normal for Group U. These two tests differed with respect to whether or not the CS had been presented earlier. The purpose of these tests was to see if Group U's heart-rate response to morphine was dependent on prior presentation of the CS. Figure 31 shows the change in heart rate in response to

Figure 30. Mean change in heart rate is plotted during the CS+US (open circles) and US-alone (closed circles) test, collapsed across Groups. The data are depicted in 2-min sample periods after morphine infusion.

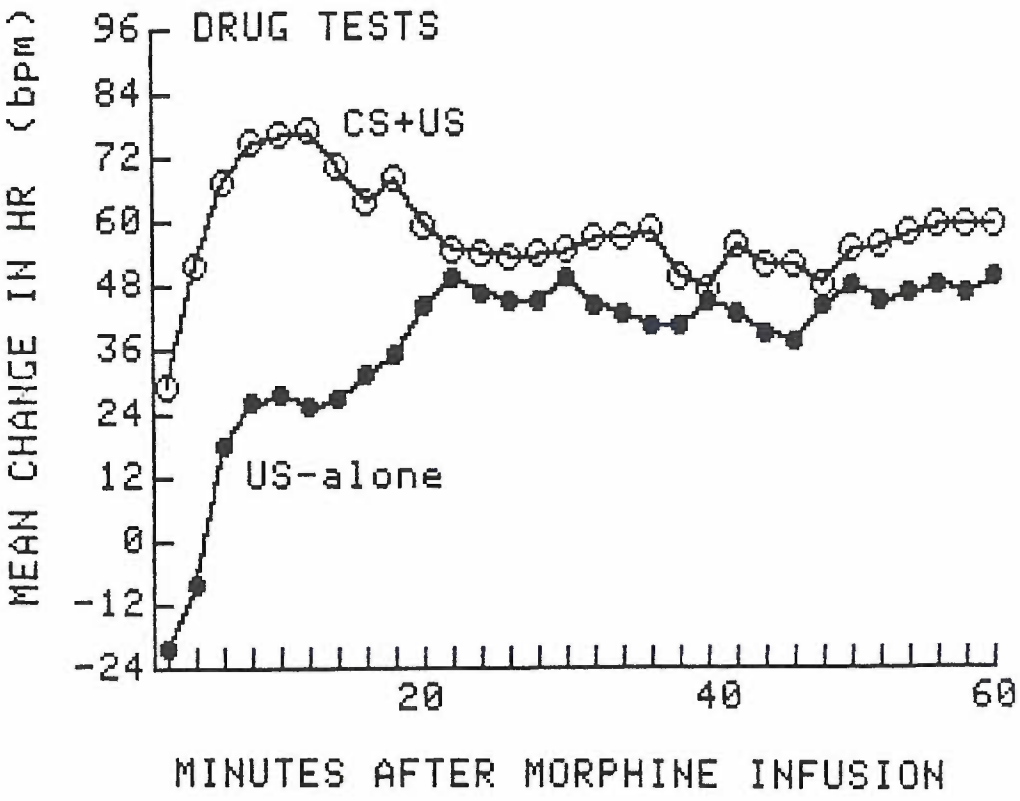
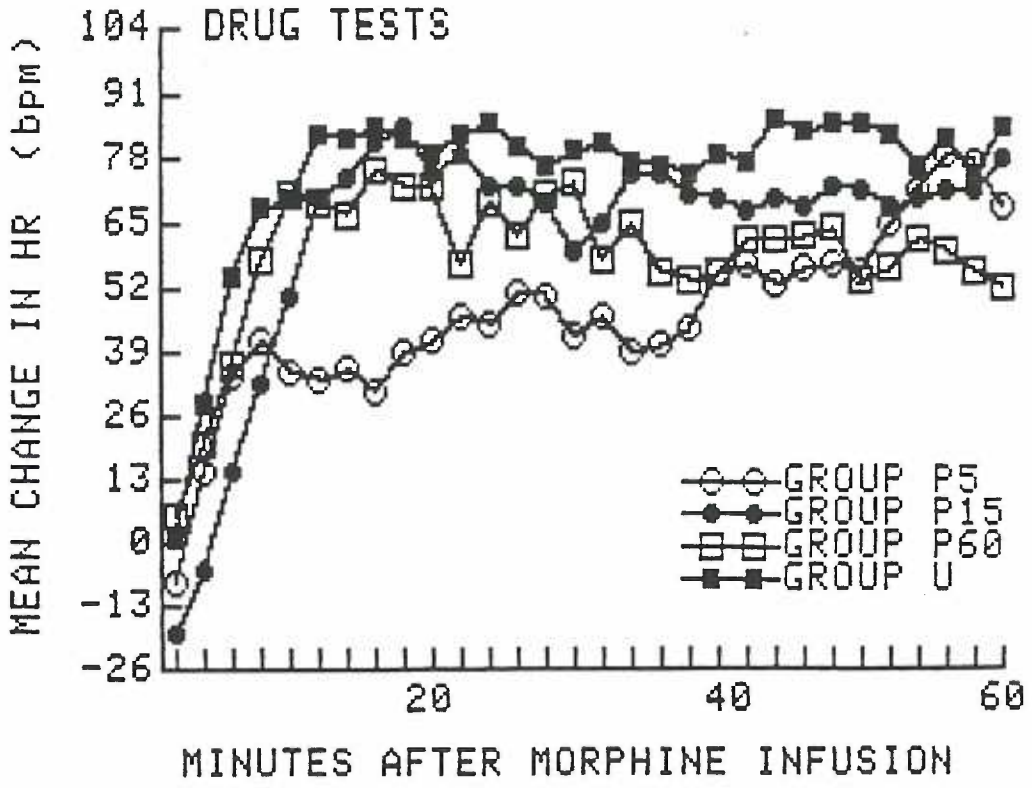

DRUG TESTS
CS+US
US-alone
MEAN CHANGE IN HR (bpm)
96
84
72
60
48
36
24
12
0
-12
-24
20
40
60
MINUTES AFTER MORPHINE INFUSION

Figure 31. Mean change in heart rate is plotted for each group after morphine infusion in the CS//US and NOCS//US tests. The data are collapsed across tests.


DRUG TESTS
MEAN CHANGE IN HR (bpm)
104
91
78
65
52
39
26
13
0
-13
-26
20
40
60
MINUTES AFTER MORPHINE INFUSION
GROUP P5
GROUP P15
GROUP P60
GROUP U

morphine in each group, collapsed across tests. In general, these data are consistent with previous data shown, i.e., Group P5 responded to morphine with a slower increase in heart rate relative to the other groups.

A three-way ANOVA (Groups x Tests x Sample Periods) revealed a significant Groups x Sample Periods interaction, $F(87,551) = 1.39$. A significant effect of Sample Periods was also found, $F(29,551) = 15.63$. No effect of Tests nor interactions involving Tests were significant. Follow-up analyses of the Groups x Sample Periods interaction were performed on individual Sample Periods. No effect of Groups was found at the Sample Periods tested. An interaction contrast analysis comparing the magnitude of tachycardia (Sample Period 8 - Sample Period 1) produced by morphine showed that Group P5 responded with less tachycardia relative to the other three groups, $F(1,551) = 5.92$.

In summary, in the presence of the CS, all of the groups tended to respond to morphine with a monophasic tachycardic response. Differences in responding on the CS+US and US-alone tests were most evident within the first 5 min after morphine administration, and were greater within the Paired groups relative to Group U. In the CS//US and NOCS//US tests, there was no difference in responses in the two tests. As observed in other figures, Group P5 showed less tachycardia after morphine relative to the other groups.

Discussion

Experiment 2 demonstrated development of tolerance to the bradycardic effect of morphine and sensitization to the hyperthermic effect. Furthermore, in the training phase, rats in all three Paired groups showed a faster rate of temperature change (see Figure 14) and no bradycardia (see Figure 24) relative to Group U. Rats in all three Paired groups showed a tachycardic heart rate CR which was apparent within the first 30 sec after CS onset as well as during the 5-min measurement period after CS onset in the Placebo tests. A hyperthermic CR was revealed in Groups P15 and P60; however, Group P5 did not respond differently from Group U in the Placebo tests. In order to assess cue specific tolerance and sensitization, all rats received morphine in the presence and absence of the CS. Both Groups P5 and P15 evidenced cue-specific sensitization to morphine's hyperthermic effect and tolerance to morphine's bradycardic effect. When comparing responses of the Paired groups with the response of Group U in the CS+US test, all Paired groups showed a significantly faster rate of temperature change relative to Group U. Only Group P15 showed more tolerance to morphine's bradycardic effect relative to Group U.

In Experiment 2, the temperature results of Group P15 replicated the results of Group P in Experiment 1. Group P15 showed a hyperthermic CR to the CS in the absence of morphine, and cue-specific sensitization to morphine's hyperthermic effect. Likewise, Group P15 showed a tachycardic CR in the Placebo tests, and cue-specific tolerance to morphine's bradycardic effect. Cue-specific differences in Group P15's temperature and heart rate responses were evident when comparing its

responses on each test as well as when comparing its responses with those of Group U's in the CS+US test.

Both Groups P5 and P60 showed less evidence of conditioning, in that, the results of both groups were less consistent than those of Group P15. Group P5 showed evidence of cue-specific sensitization to morphine's hyperthermic effect when comparing its response to Group U's in the CS+US test. Group P5 demonstrated a tachycardic CR, but Group P5 only evidenced cue-specific tolerance to morphine's bradycardic effect when its response was compared to that in the US-alone test. Group P5 did not differ from Group U in the CS+US test. Group P60 showed both hyperthermic and tachycardic CRs in the Placebo tests. However, Group P60 showed cue-specific sensitization to morphine's hyperthermic effect only when its response was compared to Group U's response in the CS+US test, but not when compared to its response in the US-alone test. Group P60 appeared to show cue-specific tolerance to morphine's bradycardic effect when its response in the CS+US test was compared to that in the US-alone test. It should be noted that this difference approached significance, and might have been significant if the group size had been larger. Group P60's response in the CS+US test did not differ from Group U's.

In terms of Wagner's SOP theory (Donegan & Wagner, 1987; Paletta & Wagner, 1986; Wagner, 1981), associative strength should vary as a nonmonotonic function of CS duration. SOP theory predicts that some intermediate CS duration should provide greater excitatory conditioning relative to very short or long CS durations. An intermediate CS duration allows for a maximum number of CS nodal elements to be in the A1 state at the time of US presentation. In the present experiment, if

it is assumed that less learning occurred in Groups P5 and P60 due to the discrepancies in their results, then it might be concluded that a 5-min CS-US overlap duration was too short for enough CS elements in the A1 state to be activated when the US elements were activated to the A1 state. For Group P60, it might be concluded that the 60-min CS-US overlap duration was so long that overlap of CS nodal elements in the A1 state with US-nodal elements in the A2 state resulted in some inhibitory conditioning. When inhibitory conditioning is summed with excitatory conditioning, one observes less net excitatory conditioning. The 15-min overlap duration, resulted in net excitatory learning which translated into the ability to observe effects of conditioning in all of the tests.

There are problems in interpretation of these results due to the difficulty of distinguishing between associative and nonassociative effects of CS duration on the response to morphine. For example, differences in performance evidenced in Group P5 (i.e., lower peak hyperthermia and tachycardia) may be an effect of CS duration and may not necessarily imply differences in learning. In Block 1, there were no effects of CS duration on the heart rate UR; however, there was an effect on the temperature UR. This lack of difference in the heart-rate response was evident only when the post-infusion measurement period was short. When measurement was extended, a dynamogenic effect of a long CS duration on the UR in Groups P15 and P60 became more apparent. Although there was no difference in rate of temperature or heart-rate increase among the Paired groups, Groups P15 and P60 showed greater peak tachycardia and hyperthermia relative to Group P5. Group U's responses to the different CS durations were analyzed in order to try to clarify the possible dynamogenic effect of the CS on the UR. In the temperature

data, Group U's responses are generally in the direction expected, with the greater increases in temperature over the hour after CS onset occurring to the 15- and 60-min CSs (see Figure 16). A dynamogenic effect of the 15- and 60-min CS on the response to morphine could be thought of as the sum of the nonassociative-based increase to the CS alone with response to morphine. However, in the heart rate data, the increase in heart rate is greater to the 15- and 5-min CSs relative to the 60-min CS (see Figure 26) which does not support the idea of a nonassociative dynamogenic effect of CS duration on the heart-rate response to morphine. The best way to assess the possibility of a nonassociative influence of CS duration would have been to administer morphine paired with each CS duration to all groups. If the depression of peak tachycardia and hyperthermia occurred in all groups consistently when the CS duration was 5 min, this would imply a non-associative effect. If depression occurred in Group P5 consistently with all CS durations, this would indicate a possible associative influence.

Another possible explanation for Group P5's smaller magnitude hyperthermia and tachycardia was its higher baseline heart rate and temperature. In other words, the heart rate and temperature response to drug may have reached a ceiling. The main problem with this possibility is that by Block 10, there was no difference between groups in baseline heart rate, but the smaller magnitude tachycardia to morphine in Group P5 was still evident in the Drug Tests.

As in Experiment 1, during the Drug Tests, Group U showed a shorter latency to maximal hyperthermia (and tachycardia) in response to morphine in the CS+US test relative to the US-alone test. As discussed before, this might have been due to dishabituation to the CS by

morphine. The response observed may have been the sum of the dishabituated response and morphine's UR.

During the CS//US and NOCS//US tests, there was no difference in Group U's temperature or heart rate responses on the two tests. The purpose for these tests originated from Group U's results in Experiment 1. In Experiment 1, Group U showed the development over blocks of a decrease in temperature during the 1-hr period prior to morphine administration (see Figure 4). Also, Group U's response in the US-alone test showed a faster rate of temperature increase relative to that on Day 22 (see Figure 8). These two results taken together, suggested the possibility that the decrease in temperature over the 1-hr pre-infusion period was a learned response to temporal cues (cf., Eikelboom & Stewart, 1979, 1980) and that summation with the UR resulted in slower rate of temperature increase--as long as morphine was given at the same time of day. The CS//US and NOCS//US tests in Experiment 2 were designed to determine whether Group U's response was dependent on temporal cues involving the CS. There was no difference in Group U's temperature or heart rate responses to morphine on these two tests suggesting that previous CS presentation was not a factor in how Group U responded to morphine. However, in the present experiment, Group U did not show the development of a hypothermic response over blocks. Therefore, temporal conditioning in Group U cannot be concluded from these results. One possibility for failure to replicate the development of a decrease in temperature during the pre-infusion hour might be that the presentation of differing CS durations may have interfered with Group U's ability to learn about temporal cues.

The temperature responses of the Paired groups did differ between the CS//US and NOCS//US tests. There was no difference in any of the Groups' heart rate responses on these two tests. The difference in temperature response was most likely due to differences in pre-infusion temperatures, with a higher pre-infusion temperature corresponding to a lower peak hyperthermia implying a ceiling effect. Group U's pre-infusion temperature was about the same in each test. However, an alternative possibility is that in Groups P15 and P60 the prior occurrence of the CS in the CS//US test activated their temperature system so that they responded with a greater magnitude of hyperthermia to morphine relative to when the CS had not occurred previously. Lack of this effect in Group P5 might have been because their CS offset was not close enough in time to morphine administration.

In summary, the results of this experiment demonstrate that changes in both the heart-rate and temperature response to morphine can be achieved when morphine is paired with an explicit CS with an ISI of 30 sec. Effects of CS-US overlap duration on conditioning appeared to support predictions from Wagner's SOP theory, in that, Group P15 showed evidence of conditioning in all tests, while the results of Groups P5 and P60 were less consistent suggesting the possibility of less net excitatory learning relative to Group P15.

GENERAL DISCUSSION

The present experiments demonstrated cue-specific tolerance to the bradycardic effect of morphine and sensitization to the hyperthermic effect after several infusions. The tolerance and sensitization observed in these experiments occurred in conjunction with the development of both tachycardic and hyperthermic CRs. It was thought that summation of the tachycardic CR with the biphasic heart rate UR resulted in tolerance to the bradycardic effect of morphine. In fact, after several pairings of the CS and US, the UR was a monophasic tachycardia. Likewise, summation of the hyperthermic CR with the hyperthermic UR resulted in sensitization to morphine's hyperthermic effect. This additive effect was most apparent in increasing the rate of temperature and heart-rate change, rather than in increasing the magnitude of the response.

The results of the temperature data are in agreement with previous studies of conditioned sensitization to morphine's hyperthermic effect (Eikelboom & Stewart, 1979, 1981; Miksic et al., 1975; Sherman, 1979). In all of these studies a hyperthermic CR was observed when placebo was administered in the presence of morphine-paired cues. The present study demonstrated that a hyperthermic CR can be elicited within 15 min by an explicit CS in a situation where the response was not confounded by handling or the stress of injection and where there was an appropriate control for nonassociative factors.

The heart rate data clearly show conditioning of a tachycardic CR when an explicit CS is paired with a drug US. These results are in agreement with a study by Rush et al. (1970) who reported a tachycardic CR in dogs after pairing morphine administration with a buzzer CS. However, higher doses of morphine (10 and 200 mg/kg) resulted in

inconsistent results (Bykov, 1957; Rush et al., 1970). The results of Experiment 2 also lend support to the notion that the increase in pre-infusion heart rate observed in the study by Schwarz & Cunningham (in press) might have represented a learned response in anticipation of morphine. Stein (1976) failed to observe evidence of heart rate conditioning; however, there were problems in the design of his study which have already been discussed (see pp. 3-4, Introduction).

With respect to the classical conditioning theories of drug tolerance and sensitization, the theories of Kesner and Cook (1983) and Baker and Tiffany (1985), derived from Wagner's (1976) original theory of associatively-primed habituation, can only explain tolerance to the bradycardic effect. Their analysis does this in terms of conditioned diminution of the UR. The habituation theory cannot explain sensitization, or conditioned facilitation, to the hyperthermic effect.

In order to apply Solomon and Corbit's (1974) opponent-process theory, one must assume that positive and negative affective states are reflected by overt physiological responses. In other words, one must assume that different hedonic or emotional states are related to bradycardia and hypothermia relative to those reflected by tachycardia and hyperthermia. As an example, Solomon and Corbit described responses to footshock in dogs. While the shock was on, there was a large increase in heart rate. At shock offset, heart rate decreased below baseline or resting level. These heart rate responses correlated with observations of "terror and panic" during shock followed by "stealth and hesitation" after shock offset. The observations are strictly correlational, and the covert emotional responses assumed in this theory make it difficult to apply. In fact, in the drug conditioning

literature, there is argument about whether the CR reflects aversive withdrawal-like symptoms or positive motivational processes. This issue will be discussed in detail below.

Siegel's (e.g., 1978) and Wagner's (e.g., 1981) theories are both easily applied to these data by arguing that the tachycardic and hyperthermic CRs added to morphine's biphasic heart rate UR and hyperthermic UR, respectively, to produce tolerance to the bradycardia and sensitization to the hyperthermia. Siegel's theory does not suggest any specific mechanisms that underlie the summation of the CR and UR, or why tolerance sometimes results and at other times sensitization results.

Wagner's (1981; Donegan & Wagner, 1987; Paletta & Wagner, 1986) recent SOP theory lays out explicit rules and circumstances about the occurrence of conditioned tolerance or sensitization of the UR. As mentioned previously, the response measured is a function of the ratio of the weighted proportion of elements activated in the A1 state to that of the A2 state. With respect to the biphasic heart rate response, bradycardia must be represented by elements in the A1 state and tachycardia by elements in the A2 state. CS-induced activation (associatively-generated priming) of elements directly to the A2 state leaves fewer elements in the I state at the time of morphine administration resulting in conditioned diminution of the bradycardia. Assuming a monophasic hyperthermic reponse to morphine, the only way sensitization can occur is if the effect of the CS itself is much greater than the priming effect because priming always works toward diminution (Donegan & Wagner, 1987). One could assume a depressant phase preceding the hyperthermia even though it was not apparent in the

thermal response at the dose used. In this case, the underlying unobservable hypothermia would be represented by elements in the A1 state and hyperthermia by elements in the A2 state. As with the heart rate response, conditioned diminution occurs to the hypothermic (or depressant phase) response due to CS-induced activation of elements in the I state directly to the A2 state. This would also be observed as sensitization to the hyperthermic effect to morphine.

This illustrates one problem with Wagner's SOP theory, in that, with monophasic responses, it may not be as useful in predicting the direction of the CR or how the CR and UR interact to obtain either tolerance or sensitization. For example, in the case of ethanol, the UR is generally reported to be a monophasic hypothermia, and the CR is hyperthermia resulting in tolerance (Crowell et al., 1981; Lê et al., 1979; Mansfield & Cunningham, 1980). According to Wagner's theory, tolerance results from either associatively- or self-generated priming. Either no CR should be evident or the CR should be hypothermia. The occurrence of a hyperthermic CR is a problem for Wagner's theory. However, there are two studies suggesting that ethanol's thermic UR is biphasic (Gallaher & Egner, 1987; Sinclair & Taira, 1988). Gallaher and Egner showed that rats responded to ethanol (2 or 4 g/kg, i.p.) with hypothermia. Temperature returned to preinjection baseline levels 4-12 hrs after injection and continued to increase to levels above baseline. Therefore, the hyperthermic CR observed in tolerance studies resembled this secondary hyperthermia, and as predicted by Wagner, tolerance developed to the initial hypothermia.

All of the theories described thus far are classical conditioning theories and assume the results of the present experiments are due to an

association formed between the CS and the US or its effects. However, there is an alternative non-associative explanation for the present results. It is possible that morphine interfered with habituation to the CS in Group P (Izquierdo, 1979; Scoles & Siegel, 1986). Izquierdo measured rearing response to a tone stimulus. Habituation of the rearing response occurred within 20 tone presentations. Immediately following the last tone presentation (within 15 sec) rats received either morphine (1 mg/kg, i.p.) or saline. The rats were tested for their response to the tone on the next day. Those rats that received saline showed no rearing response, but the rats who received morphine exhibited a rearing response suggesting a loss of habituation. The study by Scoles and Siegel assessed the importance of saline trials in place preference conditioning. The results indicated that following a saline injection, or no injection at all, the rat will show preference for the other compartment--whether or not morphine was experienced there. Scoles and Siegel suggested that morphine may attenuate habituation, and therefore, maintain exploratory behavior toward the morphine-paired side and away from the saline-paired side in a no-drug test. In the present experiments, it is possible that morphine interfered with habituation to the hyperthermic and tachycardic orienting responses (ORs) to the CS in Group P. One way to distinguish between interference with habituation and conditioning might be to compare the magnitude of the CR at the end of conditioning with that of the OR on the first trial. By showing that the CR is larger than the OR, this may argue against interference of habituation by morphine. Because previous studies of context-specific drug responses have shown that the development and loss of tolerance and sensitization are subject

to the "rules" of classical conditioning, it will be assumed that the responses measured in the present experiments are associatively-generated CRs.

Speculation about the nature of the CR has always been an important issue in classical conditioning. In particular, of interest was whether the CR mimics the UR or is opposite in direction from the UR. Out of this discussion arose a concept developed by Grings (1960) of perceptual disparity. Badia and Defran (1970) used the concept in describing possible problems in the interpretation of results from various types of test trials used in conditioning experiments. Badia and Defran were concerned with the possibility of confounding ORs and conditioning phenomena. For example, in tests involving the omission of the CS or US, the subject might experience perceptual disparity, that is, there may be an OR to the omission of one of the stimuli. The temperature and heart rate responses to the CS on the Placebo-tests may be one or a combination of three responses: a dishabituated OR to the CS, a response to US omission or an anticipatory CR. As was seen in Figure 11, the CS initially produced an increase in temperature in Group U, which was presumably the OR. Figure 22 illustrates a tachycardic OR to the CS in Block 1. The CRs observed in the Paired groups on the Placebo tests (Figure 9, Experiment 1, and Figures 17 and 27, Experiment 2) were also hyperthermia or tachycardia. Therefore, it may not be possible to differentiate which portion of the hyperthermic response in Group P was OR, CR or dishabituated response to the CS. With respect to the heart rate data, this is not as much of a problem because the ISI is relatively long for heart rate conditioning. A heart rate CR was observed during the 30-sec ISI during the training phase. According to

Badia and Defran, on Placebo test trials, the heart rate CR might be separately identifiable from the OR to US omission, that is, multiple responses during the CS period may occur. However, this was not the case in Experiment 2. Figure 27 shows that the tachycardic CR did not vary with sample periods within 5 min of CS onset.

The heart rate and temperature CRs may share the same physiological or behavioral substrate as the UR. On the other hand, the CR may be less specifically related to the UR, i.e., a positive or negative hedonic anticipatory response, or the CR may be even less specific with respect to the UR, i.e., a nonspecific arousal response in which positive or negative valence does not matter.

One physiological mechanism that may underlie the tachycardic and hyperthermic CRs is an associatively-induced release of norepinephrine and epinephrine from the adrenal medulla. Release of these catecholamines into plasma could account for both the tachycardic and hyperthermic CRs. Morphine has been shown to result in lower adrenal levels of catecholamines, presumably because of their release into the plasma (Anderson & Slotkin, 1975). In another study, adrenalectomized rats failed to show a hyperthermic response to morphine (Wallenstein, 1982), suggesting that plasma catecholamines may be necessary for morphine to effect body temperature. A drug conditioning study by Mansfield et al. (1981) showed a sensitized hyperthermic response in the presence of cues previously paired with morphine (5 mg/kg). Plasma levels of norepinephrine and epinephrine were also significantly greater in morphine-experienced relative to morphine-naive rats. It is possible that a hyperthermic CR was caused by a learned increase in plasma levels

of catecholamines. However, responses to saline in the presence of drug-paired cues were not measured in the study by Mansfield et al.

Another possible physiological mechanism includes learned changes in other heat production or conservation tactics besides the increased secretion of catecholamines. For example, the CR may be piloerection, which serves to conserve heat, or the CR may be shivering or hyperactivity which serve to increase heat production (cf. Guyton, 1986). These possible CRs could be learned to compensate for morphine's initial (but unobservable) hypothermia.

A second possibility for the biological basis of the hyperthermic and tachycardic CRs is not specifically related to morphine's effects on these systems. According to this notion, CRs may represent morphine's rewarding effects. There are two basic viewpoints (cf. Bozarth & Wise, 1984) about the affective quality of the CR. The first viewpoint is that the CR is a manifestation of withdrawal, and the rewarding effect of opioids are a result of their ability to alleviate the withdrawal stress (e.g., Wikler, 1973a). This position cannot explain initial drug-taking. The second viewpoint is that the rewarding effect of opioids is <u>independent</u> of their ability to alleviate withdrawal symptoms. There are data suggesting that the opioid receptors which govern reward and physical dependence are anatomically distinct (Bozarth & Wise, 1984). The rewarding effects of opioids, as determinied by self-administration and conditioned place preference have been shown to be attenuated by pretreatment with dopamine receptor antagonists as well as by opioid receptor antagonists. Based on this evidence, Bozarth and Wise (1987) have developed a psychomotor stimulant theory of addiction that posits that conditioned dopaminergic activity in the ventral

tegmental area of the brain underlies a positive hedonic CR. Because this CR involves dopamine activity, rewarding effects of drugs or CRs should be observable via an increase in locomotor activity. Neisewander and Bardo (1987) performed a conditioning study using morphine as the US which suggested the hyperactive CR can be attenuated by treatment with either naloxone, an opioid antagonist, or pimozide, a dopaminergic antagonist. In the present experiments, it is possible that the hyperthermic and tachycardic CRs were secondary to a hyperactivity CR.

A third possibility is that the CR represents a nonspecific arousal response which may have either positive or negative valence (Cunningham & Schwarz, 1988). This response may not be directly related to the specific physiological or biochemical actions of the drug. For example, ethanol produces a hypothermic UR and after several pairings with environmental cues, a hyperthermic CR develops (e.g., Mansfield & Cunningham, 1980). In contrast, as shown in the present experiments as well as in others (e.g., Sherman, 1979), morphine produces a hyperthermic UR, and after pairings with cues, a hyperthermic CR develops. The similarity in the conditioned thermal responses influenced by ethanol and morphine, drugs that have different effects on the thermoregulatory system, as well as the fact that most drug-induced CRs are excitatory has led to consideration of the possibility that the CR reflects a nonspecific anticipatory arousal response. Although the latter two mechanisms suggested for the CR differ in terms of hedonic specificity, both suggest the possibility that the CR may index the acquired motivational processes that maintain drug-taking and encourage relapse.

In addition to the issue of the physiological, biochemical or behavioral bases for the CR, another issue that has gained interest is whether or not learning influences the type of tolerance (i.e., dispositional or cellular) observed to occur to drug effects (e.g., Melchior & Tabakoff, 1985; Ritzmann, Steece, Lee & DeLeon-Jones, 1985). Tolerance to morphine has been shown to be due to both dispositional and cellular mechanisms. Patrick, Dewey, Huger, Daves and Harris (1978) showed that after six days of a constant i.p. infusion of morphine (increasing dose from 50 to 200 mg/kg/day) rats showed cellular tolerance to morphine's analgesic effect as evidenced by shorter tail flick latencies relative to drug-naive rats with the same brain concentration of morphine. Dispositional tolerance was also observed as evidenced by an increase in concentration of conjugated morphine and an increase in fecal elimination in morphine-experienced relative to morphine-naive rats.

Patrick et al. (1978) were not concerned with whether or not learning played a role in tolerance. In fact, as Siegel (1987) has suggested, nonassociative mechanisms account for the tolerance observed in certain procedures which use continuous drug administration such as constant infusion, liquid diet, etc. Ritzmann et al. (1985) performed a study using mice, in which nonassociative tolerance to the analgesic effect of morphine was produced by pellet implantation. Associative tolerance was induced in a separate group by pairing a daily i.p. injection (40 mg/kg) with an orange scent CS for 12 days. Saline-control groups were also used. After testing for tolerance to the analgesic effect of morphine, brain levels of morphine were determined in all mice. The results indicated that associative

tolerance might have been due to dispositional factors because the morphine-experienced mice showed lower brain levels of morphine relative to morphine-naive mice; however, the differences fell short of statistical significance. The nonassociative type of tolerance was most likely due to cellular mechanisms because brain levels were equal in the morphine-experienced and morphine-naive mice, but the morphine-experienced mice showed significantly less analgesia. There were major problems in this study, including lack of statistical significance among the mice used in the "associative" procedure. Also, there was no control that allowed clear distinction between associative and nonassociative effects of receiving repeated pairings of morphine with orange scent. However, similar (and significant) results have been reported in studies of tolerance to the effects of ethanol (Melchior & Tabakoff, 1985).

Melchior and Tabakoff (1985) have shown that mice given repeated ethanol injections (3.5 g/kg, i.p.) developed tolerance to the hypnotic and hypothermic effects of ethanol only when it is given in the environment previously paired with ethanol. In this study, mice received a 3.5 g/kg dose of ethanol or an injection of saline twice daily for 4 days. On the fifth day, all mice received a 3.5 g/kg i.p. injection of ethanol in the same environment in which they had received previous injections or in a novel environment. Brain levels of ethanol after this injection showed that mice expecting ethanol had lower brain ethanol levels up to 1 hr after injection relative to all other mice. Blood ethanol levels and total amount of ethanol in the body were also lower from 90 to 270 min after injection in mice expecting ethanol relative to other mice suggesting dispositional tolerance to ethanol

only when the drug was administered in the ethanol-paired environment. When ethanol was injected intracerebroventricularly (i.c.v), all ethanol-experienced mice exhibited tolerance to the hypothermic effect of ethanol relative to ethanol-naive mice, regardless of the environment in which the i.c.v. injection was given suggesting some degree of cellular tolerance. In addition, Melchior and Tabakoff showed that mice fed an ethanol diet showed tolerance relative to their pair-fed controls, and it appeared that this tolerance was primarily due to cellular mechanisms. Tolerance to ethanol given in liquid diet did not show cue-specificity.

In summary, tolerance to effects of morphine can be due to either dispositional or cellular mechanisms and can also be influenced by learning. The studies by Ritzmann et al. and Melchior and Tabakoff suggest that cue-specific tolerance is at least partly due to the ability of the subject to change the distribution of a drug, while nonassociative tolerance is due to other factors such as an adaptive change in the target cells. In the present experiment, cue-specific tolerance to the bradycardic effect of morphine in the Paired groups might have occurred as a result of their ability to alter the distribution of morphine, while any tolerance observed in the U groups (and some of the tolerance in the Paired groups) might have been due to cellular mechanisms, e.g., an alteration in the opioid receptors.

Whether similar mechanisms account for cue-specific sensitization to morphine's hyperthermic effect is not clear. Throughout these experiments, sensitization has been defined as a faster increase in heart rate and temperature (i.e., the response occurs sooner after infusion). Perhaps sensitization is not a useful term in that tolerance

to the bradycardia resulted in a monophasic tachycardic response to morphine. The latency of the tachycardic response was reduced because of the absence of the initial bradycardia. Likewise, the decreased latency for hyperthermia might have been due to tolerance to an initial depressant effect. In other words, the decreased latency of the excitatory effects of morphine may not represent sensitization to those effects, but merely tolerance to the initial depressant effect of morphine.

In summary, the present experiments were designed to study the effects of repeatedly pairing an explicit CS with an automatic i.v. infusion of morphine. The purpose of Experiment 1 was to demonstrate successful conditioning of the thermic effect of morphine in the absence of unauthorized cues such as transport from room to room, prick of the i.p. injection or rectal probe to assess temperature. The purpose of Experiment 2 was to determine how CS-US overlap duration affected conditioning of both heart rate and body temperature. Both experiments evidenced context-specific "sensitization" to the hyperthermic UR with a corresponding hyperthermic CR. In addition, context-specific tolerance to the bradycardic effect of morphine was observed with a corresponding tachycardic CR. With respect to CS-US overlap duration, optimal overlap duration for learning occurred in Group P15, whereas Groups P5 and P60 evidenced less net excitatory conditioning in that their results were inconsistent across the various tests done for conditioning.

These experiments have important implications in terms of both the licit and illicit use of drugs. Learning an association between the effects of the drug and the cues surrounding drug-taking results in a response which may include an acquired motivational process, either

pleasant or unpleasant, which may lead to increased need for use or abuse of the drug. Effective treatment of drug addiction must address this issue and include some method for extinction of the learned response in order to prevent relapse.

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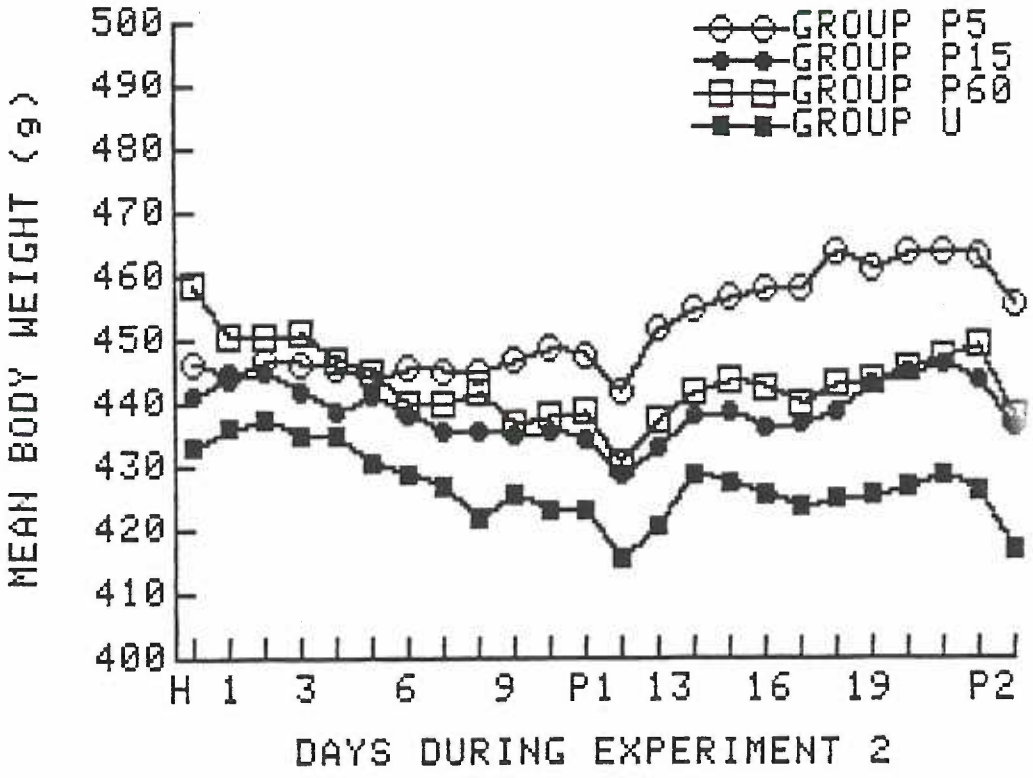
APPENDIX A: Analysis of Body Weight

The mean body weight of all groups from Experiment 2 are plotted in Figure 32. The purpose of presenting this analysis of body weights was the 15.6 % attrition rate observed in this experiment. By the end of the experiment, one rat from Group P5, two rats from Group P15 and two rats from Group P60 had died. A Chi Square test performed on the relative proportion of survivors in the Paired and Unpaired conditions (ignoring subject loss due to procedural problems) showed no relationship between pairing morphine with the CS and attrition ($X^2(1) = 1.25$).

Figure 32 shows body weight plotted over days during the training phase of Experiment 2. This figure shows that initially, all groups had about the same mean body weight, and that the group weights diverge over training. Group P5 actually show an increase in body weight. Group P15 shows little change in weight. Groups P60 and U show a decrease in weight during training. All groups showed a decrease in body weight after the Placebo tests (P1 and P2) suggesting some degree of physical dependence to morphine.

A two-way ANOVA performed on these data revealed a significant Groups x Days interaction, $F(69,506) = 2.41$. A significant main effect of Days was also found, $F(23,506) = 7.57$. Analyses of each group showed a significant effect of Days in Group P5, $F(23,138) = 8.95$, Group P60, $F(23,92) = 2.74$, and Group U, $F(23,161) = 4.00$, but not in Group P15. Separate analyses of individual days revealed no effect of Groups in any of the days tested; therefore, an interaction contrast analysis was performed on the difference between Day 1 and 24. Pairwise comparisons revealed differences between Group P5 and each other group, $F(1,506) =$

Figure 32. Mean body weight (grams) is plotted for each group over days during Experiment 2. P1 refers to the Placebo test on Day 11, and P2 refers to the Placebo test on Day 22.


GROUP P5
GROUP P15
GROUP P60
GROUP U
MEAN BODY WEIGHT (g)
500
490
480
470
460
450
440
430
420
410
400
H 1 3 6 9 P1 13 16 19 P2
DAYS DURING EXPERIMENT 2

5.51 (P5 vs. P15), 21.56 (P5 vs. P60), and 20.18 (P5 vs. U). A significant difference as also found between Group P15 and P60, $F(1,506) = 5.44$. No other comparisons were significant.

In summary, group differences in body weight were not apparent early in Experiment 2. However, as the experiment progressed, differences emerged, most notably Group P5 showed an increase in mean body weight relative to all the other groups.