EARLY DRUG EFFECTS SIGNAL SUBSEQUENT DRUG EFFECTS: INTRA-ADMINISTRATION ASSOCIATIONS AND THE CONDITIONING ANALYSIS OF TOLERANCE

By

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Abstract

The Pavlovian conditioning analysis of drug tolerance emphasizes that cues present at the time of drug administration become associated with drug-induced disturbances. These disturbances elicit unconditional responses that compensate for the pharmacological perturbation. The drug-compensatory responses eventually come to be elicited by drug-paired cues. These conditional compensatory responses (CCRs) mediate tolerance by counteracting the drug effect when the drug is administered in the presence of cues previously paired with the drug.

Although there are many findings consistent with the conditioning analysis of tolerance, there also are contrary findings. The results of experiments reported in Chapter 2 suggest that some of the apparently contradictory findings result because interoceptive pharmacological cues, as well as exteroceptive environmental cues, are paired with a drug effect. That is, within each administration, early drug-onset cues may become associated with the later, larger drug effect, and these pharmacological cues may overshadow simultaneously-present environmental cues. The results of these experiments demonstrate that such intra-administration associations contribute to tolerance to the analgesic effect of morphine and to the expression of conditional compensatory hyperalgesia.

Although there is substantial evidence that conditioning contributes to tolerance, there has been little research concerning the physiological events that mediate this contribution. The experiments reported in Chapter 3 were designed to evaluate the role of a putative anti-opioid peptide – cholecystokinin-8 (CCK-8) – in the associative

mechanisms of opiate tolerance. The results of these experiments demonstrate that endogenous CCK-8 activity is important to the expression of conditional compensatory responding and the expression of established morphine tolerance.

In addition to the theoretical implications of the work presented in this thesis, the present findings may also be of clinical interest. The clinical report presented in Chapter 4, describes how a conditioning analysis of tolerance that includes intra-administration associations may be relevant to understanding a case report of enigmatic opiate overdose. Patients receiving drugs for pain relief may be at risk for opiate overdose when the route of administration is altered, thus effectively altering the pharmacological drug-onset cues.

The results of these experiments and the clinical report demonstrate that intra-administration associations are important to a compound-conditioning analysis of tolerance.

To my parents

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This thesis is dedicated to my parents who have supported and believed in me through every stage of my life, but worried all the same. See, what was there to be worried about?

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Thank you Shep.

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Chapter 3

Preface

This thesis includes introductory text that is based, in part, on a published manuscript (Siegel, Baptista, Kim, McDonald, and Wiese-Kelly, 2000), experiments based on a published manuscript (Kim, Siegel, & Patenall, 1999) and a manuscript submitted for publication (Kim & Siegel, submitted), and a clinical report based on a published manuscript (Siegel & Kim, 2000). Because the manuscripts have multiple authors, my contribution to each is explained here.

Chapters 1 and 5 are based, in part, on material presented in Siegel, Baptista, Kim, McDonald, and Wiese-Kelly (in press). I was a co-author of this review paper, and some of the relevant information is presented in this thesis.

Chapter 2 includes two experiments. Both experiments are relevant to this thesis.

Chapter 3 includes two experiments. Both experiments are relevant to this thesis.

Chapter 4 describes a case report that illustrates the clinical implications of my research and is relevant to this thesis.

List of Abbreviations

AOP	anti-opioid peptide
CCK	cholecystokinin-8
CCR	conditional compensatory response
CR	conditional response
CS	conditional stimulus
CNS	central nervous system
IAA	intra-administration association
IP	intraperitoneal
IV	intravenous
LMor	long morphine
LSal	long saline
pMor	probe morphine
pSal	probe saline
SC	subcutaneous
SMor	short morphine
SSal	short saline
TFL	tail-flick latency
UCR	unconditional response
UCS	unconditional stimulus

CHAPTER 1: INTRODUCTION

Early chroniclers of drug effects noted that responsivity to drugs often decreased as a function of experience with the drug. For example, in 1612 Jean Mousin, physician to the King of France, wondered why individuals sometimes became progressively more sober while they were continuing to drink alcoholic beverages. Although the term tolerance was not used until some years later, it appears that Mousin observed the phenomenon now termed acute tolerance – decreased responsiveness to a drug within the course of a single administration (Kalant, 1998).

Acute Tolerance

Acute tolerance has been investigated extensively with respect to ethanol (e.g., LeBlanc, Kalant, & Gibbins, 1975), as well as other drugs, such as opiates. For example, over the course of a single, long administration of morphine, accomplished by gradual infusion via an implanted morphine pellet, the analgesic effect of the drug decreases (e.g., Tilson, Rech, & Stolman, 1973; Wei & Way, 1975).

The existence of acute tolerance is evidence that pharmacological stimulation initiates adaptive responses that compensate for the primary drug effect (Haefely, 1986; Ramsay & Woods, 1997; Siegel & Allan, 1998, Siegel, Baptista, Kim, McDonald, & Weise-Kelly, in press). The observed effect of a drug is, therefore, the net result of primary, drug-induced changes and these secondary, compensatory responses. Further evidence for drug-compensatory responses may be seen when the drug effect is abruptly terminated (e.g., by cessation of the delivery of ethanol vapor to the environment, or by removal of a morphine pellet). The compensatory response, now having little to compensate for, may now be seen. Thus, upon termination of an ethanol effect (and the anticonvulsant effect of the drug), a decrease in seizure threshold is noted (e.g., McQuarrie & Fingl, 1958). Similarly, upon termination of a morphine infusion (and the analgesic effect of the drug), an increased sensitivity to painful stimuli is noted (e.g., Tilson et al., 1973; Wei & Way, 1975).

Chronic Tolerance

Typically, drugs are not administered via constant long infusions. Rather, administration is by means of a brief injection, and the effects are measured following the termination of the injection. It has been known for many years that when such measurements are made following each of a series of drug administrations, the drug effect frequently is noted to become progressively smaller over the course of these administrations. This decreasing effect, seen following each successive administration of a drug, is termed chronic tolerance (for historical reviews of chronic tolerance to ethanol and opiates, see Kalant, 1998, and DuMez, 1919, respectively). The term tolerance, as it is generally used, refers to such chronic tolerance.

While acute tolerance results from drug-compensatory processes reflexively elicited by a drug effect (e.g., Haefely, 1986), chronic tolerance (hereafter termed tolerance) results, at least in part, from drug-compensatory processes elicited not only by the drug effect, but also by cues that, in the past, have been associated with the drug effect. That is, learning contributes to tolerance.

Tolerance and Learning

As early as the 1960s, some investigators proposed that a complete analysis of tolerance requires an appreciation of associative principles. For example, in 1965, Cohen, Keats, Krivoy, and Ungar suggested that "the development of tolerance can be considered a form of learning" (p. 383), because actinomycin D, an inhibitor of protein synthesis, retarded the development of tolerance (much as it retards the acquisition of other learned responses). Results of subsequent research demonstrated that many metabolic inhibitors impede the development of morphine tolerance, as do several other manipulations that retard learning (e.g., electroconvulsive shock or frontal cortical stimulation). Moreover, several pituitary peptides that antagonize metabolic inhibitors, and facilitate learning, also facilitate the acquisition of tolerance (see Siegel, 1983, for a historical summary of research concerning the relationship between learning and tolerance).

In addition, some researchers proposed that learning contributed to tolerance because tolerance often was very well retained. That is, if an organism has acquired tolerance to a drug, this tolerance may be manifest even after a prolonged, drug-free period. For example, tolerance to the analgesic effect of morphine in rats persists over a drug-free period of months – indeed, perhaps even a year (Cochin & Kornetsky, 1964; Kornetsky & Bain, 1968). Since learned responses typically display very substantial retention (e.g., Kimble, 1961, p. 281), some investigators suggested that tolerance is "a reaction analogous to memory" (Cochin, 1970, p.19).

The contribution of learning to tolerance, and the importance of drug-associated environmental cues to tolerance, is incorporated in an analysis of tolerance that emphasizes Pavlovian conditioning principles.

Pavlovian Conditioning and Tolerance

Pavlov (1927, pp. 35ff) suggested that the administration of a drug could be viewed as a conditioning trial; the drug effect serves as the unconditional stimulus (US), and the immediately antecedent environmental cues served as the conditional stimulus (CS). Some years ago, we suggested that "conditioned drug responses are commonly opposite in direction to the unconditioned effects of the drug" (Siegel, 1975, p. 499), and these "compensatory" conditional responses (CRs) attenuated the drug effect and mediated tolerance. The pharmacological CR, then, was conceived as being opposite in direction to the pharmacological unconditional response (UR) – at least in instances in which tolerance occurred – a position contrary to Pavlov's view that the CR was similar to the UR.

The conditioning analysis of drug administration subsequently has undergone several important modifications, primarily as a result of critical analyses of pharmacological conditioning by several authors (Dworkin, 1993; Eikelboom & Stewart, 1982; Poulos & Cappell, 1991; Ramsay & Woods, 1997; Wikler, 1973). It is now apparent that the initial application of the Pavlovian conditioning paradigm to drug administration was somewhat superficial. The UR to a pharmacological stimulus, in common with reflex responses to other stimuli, consists of responses generated by the central nervous system (CNS). The drug effect that initiates these CNS-mediated responses is the US (not the UR). For many effects of drugs, the UR consists of responses that compensate for drug-induced perturbations. These unconditionally-elicited compensatory responses are responsible for acute tolerance (Ramsay & Woods, 1997). After some pairings of the pre-drug CS and pharmacological US, drug-compensatory responses can be elicited by pre-drug cues. These conditional compensatory responses (CCRs) mediate the development of tolerance by counteracting the drug effect. As noted by Dworkin (1993), the analysis now closely follows Pavlov's (1927) conceptualization of conditioning – "Conditioned drug responses, when adequately isolated, dissected, and understood, exemplify in an uncomplicated way the phenomenon first described by Pavlov: The conditioned reflex resembles the unconditioned reflex, and as it develops, it augments the effect of the unconditioned reflex" (Dworkin, 1993, p. 38).

Typically, CCRs are observed by presenting the usual pre-drug cues in the absence of the drug. Perhaps the first demonstration of a CCR was provided by Subkov and Zilov over 60 years ago. They injected dogs with adrenaline on a number of occasions (one injection every few days), and noted that the tachycardiac effect (increase in heart rate) of the drug decreased over the course of repeated injections (i.e., tolerance developed). On a final test session, they placed the dog in the injection stand and administered an inert substance (Ringer's solution). On this test, a decrease in heart rate was observed: "It follows that the mere reproduction of the experimental conditions in which the animal is accustomed to receive adrenaline is alone sufficient to set in motion the mechanism, by means of which the animal counteracts the high vascular pressure produced by adrenaline" (Subkov & Zilov, 1937, p. 295).

Subsequently, CCRs have been demonstrated with many drugs (see Siegel, 1991, 1999a), including commonly abused drugs, such as opiates (e.g., Grisel, Wiertelak, Watkins, & Maier, 1994; Krank, Hinson, & Siegel, 1981; Raffa & Porreca, 1986), ethanol (e.g., Larson & Siegel, 1998; Siegel, 1987), and caffeine (Andrews, Blumenthal & Flaten, 1998; Rozin, Reff, Mark, & Schull, 1984).

The original phenomenon implicating CCRs in tolerance has been termed the "situational-specificity of tolerance" (Siegel, 1978, p. 345). After tolerance is established by repeatedly administering the drug in a particular environment, tolerance often is more pronounced in that drug-paired environment than in an alternative environment.

<u>Situational-Specificity of Tolerance</u>

Situational-specificity of tolerance has been demonstrated in experiments that have cues explicitly paired with a drug effect, or that have used <u>opportunistic designs</u> that rely on the subjects' extra-experimental conditioning histories.

<u>Experimental designs</u>. There are several experimental designs that have been used to demonstrate situational-specificity of tolerance (see Siegel, 1983). For example, the <u>paired/unpaired</u> design was used both by Siegel, Hinson, and Krank (1978) to demonstrate the situational-specificity of tolerance, and by Baptista, Siegel, MacQueen, and Young (1998) to evaluate the neurochemical basis of the phenomenon. In these experiments, rats were assigned to paired or unpaired conditions. For paired rats, pretest morphine injections were signaled by an audiovisual cue. Unpaired rats received their

pretest drug injections and cue presentations in an unpaired manner. Following the last pretest injection, analgesia was assessed in the presence of the audiovisual cue. Despite the fact that paired and unpaired rats received the same number of morphine injections, at the same doses, at the same intervals, paired rats were more tolerant to morphine-induced analgesia than were unpaired rats.

Opportunistic designs. An example of an opportunistic design is that used by McCusker and Brown (1990). In their experiment, one group of (human) subjects was given alcohol in a familiar context (beer in a simulated bar, the beer-bar group), and another group was administered the same dose of alcohol in an unusual form and context (alcohol mixed in carbonated water and consumed in an office setting, the alcohol-office group). Subjects in the beer-bar group were less impaired on cognitive and motor tasks than were the subjects in the alcohol-office group. More recently, Remington, Roberts, and Glautier (1997) reported that the same amount of alcohol induced less impairment when college students consumed the alcohol in an alcohol-associated beverage (beer) rather than in a novel liquid (a blue, peppermint-flavored beverage).

Situational-specificity of tolerance to the lethal effects of drugs. The most dramatic demonstrations of the situational-specificity of tolerance concern tolerance to the lethal effects of drugs. Following a series of drug administrations involving escalating doses, each in the context of the same cues, tolerance develops to the potentially lethal effect of that drug as long as it is administered in the usual context. Altering the context of drug administration increases the lethality of several drugs, including heroin (Siegel, Hinson, Krank, & McCully, 1982), pentobarbital (Vila, 1989), and alcohol (Melchior, 1990; Melchior & Tabakoff, 1982, but see Neumann & Ellis, 1986; Tsibulsky & Amit, 1993). There are clinical reports suggesting that an alteration in pre-drug cues may be responsible for some instances of opiate overdoses experienced by drug addicts (Siegel, 1984), and by patients that receive drugs for pain relief [Siegel & Ellsworth, 1986; see Chapter 4 for a further discussion of this possibility (Siegel & Kim, 2000)].

Generality of the situational-specificity of tolerance. Situational-specificity has been demonstrated with respect to tolerance to many effects of a variety of drugs: opiates (reviewed by Siegel, 1991), naloxone (Goodison & Siegel, 1995b), ethanol (e.g., Lê , Poulos, & Cappell, 1979; Seeley, Hawkins, Ramsay, Wilkinson, & Woods, 1996), nicotine (e.g., Cepeda-Benito, Reynosa, & McDaniel, 1998; Epstein, Caggiula, & Stiller, 1989), pentobarbital (e.g., Cappell, Roach, & Poulos, 1981), phencyclidine (Smith, 1991), immunoenhancing drugs (Dyck, Driedger, Nemeth, Osachuk, & Greenberg, 1987), cholecystokinin (Goodison & Siegel, 1995a), carisoprodol (Flaten, Simonsen, Waterloo, & Olsen, 1997), haloperidol (Poulos & Hinson, 1982) and several benzodiazepines (Greeley & Cappell, 1985; King, Bouton, & Musty, 1987; Siegel, 1986b). It has been reported in many species, from snails (Kavaliers & Hirst, 1986) to humans (e.g., Dafters & Anderson, 1982). Situational-specificity typically also is seen with respect to cross-tolerance. Thus, rats tolerant to drug A in a particular context also display cross-tolerance to drug B if drug B is administered in that context, but not if drug B is administered in an alternative context (e.g., El-Ghundi, Kalant, Lê, & Khanna, 1989; Goodison & Siegel, 1995b; but see Carter & Tiffany, 1996).

The fact that tolerance displays situational-specificity is consistent with the conditioning analysis of tolerance. That is, drug-associated cues elicit CCRs that attenuate the drug effect, thus tolerance is greater when assessed in the presence of drug-associated cues than when it is assessed elsewhere.

Parallels Between Pavlovian Conditioning and Tolerance

If conditioning processes contribute to tolerance, it would be expected that nonpharmacological manipulations of putative CSs (cues present at the time of drug administration), known to affect the course of Pavlovian conditioning, should similarly affect the course of CCR acquisition and thus tolerance. The results of many such manipulations have been assessed (see reviews by Goudie, 1990; Ramsay & Woods, 1997; Siegel, 1989, 1991, 1999). For example, like other learned responses, tolerance is vulnerable to extinction procedures.

The magnitude of established CRs is decreased by extinction (i.e., repeated presentations of the CS without the US, or unpaired presentations of both the CS and US). Similarly, tolerance to both the lethal (Siegel, Hinson & Krank, 1979) and analgesic (e.g., Siegel, Sherman & Mitchell, 1980) effects of morphine is attenuated by repeated presentation of the pre-drug cues. Once tolerance to the behaviorally-sedating effect of morphine is established by repeated presentation of the drug in the presence of distinctive cues, this tolerance is attenuated by subsequent unpaired presentation of these cues and the drug (Faneslow & German, 1982). Extinction of morphine tolerance is seen with a variety of routes of administration, [e.g., subcutaneously (Siegel et al., 1980) and directly into the ventricles of the brain (MacRae & Siegel, 1987)]. Furthermore, tolerance to a variety of effects of ethanol, amphetamine, midazolam (a short-acting benzodiazepine), and the synthetic polynucleotide, Poly I:C, also can be extinguished (see reviews by Siegel, 1989, 1991).

In addition to extinction, many other conditioning manipulations have been shown to be relevant to tolerance. Like other conditional responses, drug tolerance displays inhibitory learning (Faneslow & German, 1982; Hinson & Siegel, 1986; Siegel, Hinson, & Krank, 1981), stimulus generalization (e.g., Caggiula et al., 1991), and a flattening of the generalization gradient as a result of extending the interval between acquisition and assessment (Feinberg & Riccio, 1990). Tolerance also displays sensory preconditioning (Dafters, Hetherington, & McCartney, 1983), external inhibition (e.g., Poulos, Hunt, & Cappell, 1988; Larson & Siegel, 1998), and a variety of compound conditioning effects, such as overshadowing (e.g., Dafters & Bach, 1985; Walter & Riccio, 1983) and blocking (Dafters et al., 1983).

In summary, the results of experiments from many laboratories suggest that tolerance may be mediated, in part, by associative mechanisms (see reviews by Dworkin, 1993; Ramsay & Woods, 1997; Siegel & Allan, 1998; Siegel, 1999; Siegel et al., in press). For example, there are many demonstrations that, following a series of drug administrations, drug-paired stimuli elicit CCRs. Furthermore, tolerance often is situationally-specific; that is, tolerance is more pronounced when assessed in the presence of drug-paired cues than when assessed in the presence of alternative cues. In addition, there are many parallels between tolerance and other conditional responses: Nonpharmacological manipulations that attenuate conditioning (e.g., extinction procedures) similarly modulate the acquisition of tolerance.

Many of these pre-drug cue manipulations have focused on external environmental cues that are explicitly paired with the drug effect. However, as elaborated in the following chapter, there is evidence that a variety of cues may come to be associated with the drug effect. For example, an especially effective type of cue that may become associated with the drug effect is the pharmacological drug-onset cue inherent to drug administration. The potential for such intra-administration associations to develop within each drug administration may have profound implications for understanding the contribution of conditioning to drug tolerance.

CHAPTER 2: INTRODUCTION

Experimental studies of the associative basis of drug effects have typically focused on the manipulation of environmental cues (e.g., cues of the room where the drug is administered). However, there is evidence that a variety of stimuli may become associated with a drug and control the display of tolerance. For example, distinctive flavors (McNally & Westbrook, 1998), ambient temperatures (Kavaliers & Hirst, 1986), or magnetic fields (Kavaliers & Ossenkopp, 1985) may, after being paired with morphine administration, influence the display of morphine tolerance. Although such explicitly manipulated exteroceptive stimuli have been shown to contribute to tolerance, there is evidence that naturally occurring interoceptive cues, such as pharmacological cues, may also do so.

Pharmacological Cues for Drugs

There is considerable evidence that, through Pavlovian conditioning, organisms can learn that a stimulus, normally considered to be a US, can signal the delivery of another US (Goddard, 1999); thus, it is not surprising that organisms can associate two drug effects. There have been various types of experiments concerning pharmacological cues for drugs. In some experiments (inter-drug conditioning), a given drug (drug A) is administered before a second drug (drug B). Other experiments have evaluated the ability of a drug to serve as a cue for itself. In such intra-drug conditioning studies, a small dose of drug A is administered prior to a larger dose of drug A. Finally, results of some research suggest that, even in the absence of explicit pairings of a pharmacological CS with a pharmacological US, an association may nevertheless develop within each administration. Because a drug effect may be protracted, the early, small, drug-onset cues may become associated with the later, larger drug effect. That is, there is a possibility for intra-administration associations (Kim, Siegel, & Patenall, 1999).

Inter-drug associations. There is evidence that inter-drug associations may make an important contribution to tolerance (see Krank & Bennett, 1987). For example, Taukulis (1986) described the results of an experiment in which atropine sulfate was routinely injected prior to pentobarbital. Tolerance to the hypothermic effect of the barbiturate was much more pronounced when it was preceded by atropine than when it was presented without the signal provided by the anticholinergic.

As discussed by Siegel (1988b), such pharmacological associations may be manifest as state-dependent learning of tolerance. As elaborated by MacQueen and Siegel (1989), inter-drug associations, and the contribution of such associations to the display of tolerance, may be important considerations in treatment schedules that routinely involve sequential presentations of different drugs (e.g., chemotherapy for cancer).

Intra-drug associations. There is also evidence that intra-drug associations may make an important contribution to tolerance. For example, a small dose of a drug may serve as a cue for a larger dose of the same drug (see Greeley & Ryan, 1995). Greeley, Lê, Poulos, and Cappell (1984) used a paired/unpaired design (see Chapter 1) to provide the first demonstration of such an intra-drug association. In this Greeley et al. (1984) study, rats in the paired group consistently received a low dose of ethanol 60 min prior to a high dose of ethanol. Rats in the unpaired group received the low and high doses on an unpaired basis. When tested for the tolerance to the hypothermic effect of the high dose following the low dose, paired subjects, but not unpaired subjects, displayed tolerance. Moreover, if the high dose of ethanol was not preceded by the low dose, paired rats failed to display their usual tolerance. This tolerance, dependent on an ethanol-ethanol pairing, was apparently mediated by an ethanol-compensatory thermic CR; paired rats, but not unpaired rats, displayed a hyperthermic CR (opposite to the hypothermic effect of the high dose of ethanol) in response to the low dose of ethanol.

Intra-administration associations. Several investigators have proposed that intra-drug conditioning findings have important implications for understanding the contribution of conditioning to tolerance. Within each drug administration, drug-onset cues reliably precede the later and larger drug effect, thus there is the potential for the formation of intra-drug associations whenever a drug is administered (e.g., Greeley et al., 1984; King et al., 1987; Mackintosh, 1987; Tiffany, Petrie, Baker & Dahl, 1983). We have termed such associations, formed within a single administration, intra-administration associations (Kim et al., 1999).

Intra-Administration Associations and Drug Tolerance

The potential for such intra-administration associations to develop within each drug administration may have profound implications for understanding the contribution of conditioning to drug tolerance. For example, Mucha, Kalant, and Birbaumer (1996) have provided evidence that intra-administration associations contribute to tolerance. They evaluated the analgesic effect of morphine, administered either intravenously (IV) or intraperitoneally (IP), on a final test session. Prior to the test, rats had extensive experience with the drug administered by one or the other of the two parenteral routes. Tolerance was maximal when the route on the test corresponded with the route used for pre-test administrations. Mucha et al. (1996) suggested that their findings were "analogous to the specificity of environmental factors of a tolerance treatment situation reported in the literature on classically conditioned tolerance" (p. 371); that is, "interoceptive stimuli produced by morphine acting through a particular route" (p. 371), in common with environmental stimuli, may act as CSs in the control of tolerance.

In summary, many investigations of the associative basis of tolerance have focused on external environmental cues that are explicitly paired with the drug effect. However, a variety of cues may come to be associated with the drug effect. Some cues, such as internal pharmacological cues, are inherent to drug administration. Although many investigators have discussed the importance of cueing effects within a drug administration, the direct investigation of intra-administration associations (IAAs) has been slowed by the unavailability of adequate procedures to measure these hypothesized associations. In the following article, we describe a novel procedure that is suitable for the direct investigation of IAAs. We demonstrate the contribution of such intra-administration associations to tolerance to the analgesic effect of morphine and to the expression of conditional compensatory hyperalgesia. In the discussion that follows, we describe how an appreciation of IAAs and the conditioning analysis of tolerance may reconcile apparently contradictory findings in the psychopharmacological literature.

CHAPTER 2

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Drug-Onset Cues as Signals: Intra-Administration Associations and Tolerance

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Running Head: INTRA-ADMINISTRATIONS AND TOLERANCE

Abstract

On the basis of a conditioning analysis of drug tolerance, drug-associated cues become associated with the drug effect. These cues elicit conditional compensatory responses and modulate the expression of tolerance. Although there are many findings consistent with the conditioning analysis of tolerance, there also are contrary findings. The results of the present experiments suggest that some of the apparently contradictory findings result because interoceptive pharmacological cues, as well as exteroceptive environmental cues, are paired with a drug effect. That is, within each administration, early drug onset cues may become associated with the later, larger drug effect, and these pharmacological cues may overshadow simultaneously-present environmental cues. We demonstrate the contribution of such intra-administration associations to tolerance to the analgesic effect of morphine and to the expression of conditional compensatory hyperalgesia.

The effects of many drugs decrease during the course of a single exposure the first time the drug is administered. For example, over the course of a single, long administration of morphine accomplished by gradual infusion via implanted morphine pellet, the analgesic effect of the drug decreases (e.g., Tilson, Rech, & Stolman, 1973; Wei & Way, 1975). This adaptation to a drug, over the course of the initial administration, is termed "acute tolerance." The existence of acute tolerance is evidence that the pharmacological stimulation initiates adaptive responses that compensate for the primary drug effect (Haefely, 1986; Ramsay & Woods, 1997; Siegel & Allan, 1998). The observed effect of a drug is therefore the net result of primary, drug-induced changes and these secondary, compensatory responses.

Typically, drug effects are not evaluated over the course of a single, protracted infusion. Rather, the administration is rapid (e.g., a brief injection) and the effects are measured following the termination of the administration. When measurements are made following each of a series of such administrations, the drug effect frequently is noted to become progressively smaller. "Chronic tolerance" refers to this decreasing effect seen following each successive administration of a drug. The term "tolerance," as it is generally used, refers to chronic tolerance.

There is considerable evidence that Pavlovian conditioning contributes to chronic tolerance (hereafter termed "tolerance"). Using the usual conditioning terminology, cues accompanying the primary drug effect function as conditional stimuli (CSs). The direct effect of the drug constitutes the unconditional stimulus (UCS). Prior to any learning, this pharmacological stimulation elicits responses that compensate for drug-induced disturbances. These responses that compensate for the drug effect are "unconditional responses" (UCRs). These UCRs are responsible for acute tolerance (see Ramsay & Woods, 1997). After some pairings of the pre-drug CS and pharmacological UCS, drug-compensatory responses are elicited by predrug cues. These conditional compensatory responses (CCRs) mediate the development of tolerance by counteracting the drug effect.

There are many findings that support the conditioning analysis of tolerance (see reviews by Dworkin, 1993; Ramsay & Woods, 1997; Siegel, in press; Siegel & Allan, 1998). There also are contrary findings (e.g., Bardo & Hughes, 1979; Goudie & Griffiths, 1984; Kesner & Cook, 1983; LaHoste, Olson, Olson, & Kastin, 1980; Tiffany & Maude-Griffin, 1988). The purpose of the present experiments was to investigate a basis for the discrepant findings.

Situational-Specificity of Tolerance

The most thoroughly established findings supporting the conditioning analysis of tolerance are demonstrations of the situational-specificity of tolerance. After tolerance is established by repeatedly administering the drug in a particular environment, tolerance often is more pronounced in that drug-paired environment than in an alternative environment. Situational-specificity has been demonstrated with respect to tolerance to many effects of a variety of drugs: opiates (reviewed by Siegel, 1991), naloxone (Goodison & Siegel, 1995b), ethanol (e.g., Lê, Poulos, & Cappell, 1979; Seeley, Hawkins, Ramsay, Wilkinson, & Woods, 1996), nicotine (e.g., Cepeda-Benito, Reynosa, & McDaniel, 1998; Epstein, Caggiula, & Stiller, 1989), benzodiazepines (King, Bouton, & Musty, 1987; Siegel, 1986), pentobarbital (e.g., Cappell, Roach, & Poulos, 1981), phencyclidine (Smith, 1991), immunoenhancing drugs (Dyck, Driedger, Nemeth, Osachuk, & Greenberg, 1987), cholecystokinin (Goodison & Siegel, 1995a), carisoprodol

(Flaten, Simonsen, Waterloo, & Olsen, 1997), and haloperidol (Poulos & Hinson, 1982). It is seen in many species, from snails (Kavaliers & Hirst, 1986) to humans (e.g., Dafters & Anderson, 1982).

The most dramatic demonstrations of situational-specificity concern tolerance to the lethal effects of drugs. Altering the context of drug administration increases the lethality of several drugs, including heroin (e.g., Siegel, Hinson, Krank, & McCully, 1982), morphine (Siegel & Ellsworth, 1986), pentobarbital (Vila, 1989), and ethanol (Melchior, 1990; but see Tsibulsky & Amit, 1993).

Conditional Compensatory Responses

The fact that tolerance displays situational-specificity is consistent with the conditioning analysis of tolerance. That is, drug-associated cues elicit CCRs that attenuate the drug effect, thus tolerance is greater when tolerance is assessed in the presence of drug-associated cues than when it is assessed elsewhere. These CCRs may be seen by presenting the usual predrug cues but not administering the drug. Such CCRs have been demonstrated with many drugs (see Siegel, 1991, in press), including commonly abused drugs, such as opiates (e.g., Grisel, Wiertelak, Watkins, & Maier, 1994; Krank, Hinson, & Siegel, 1981; Raffa & Porreca, 1986), ethanol (e.g., Larson & Siegel, 1998; Siegel, 1987), and caffeine (Andrews, Blumenthal & Flaten, 1998; Rozin, Reff, Mark, & Schull, 1984).

Discrepant Findings

Although the conditioning analysis of tolerance has been assessed with respect to many effects of a variety of drugs in several species, most research has concerned tolerance to the analgesic effect of morphine in the rat. Not all investigators have reported situational-specificity of tolerance and conditional compensatory responding with this preparation. That is, there are reports that such tolerance sometimes is trans-situational, rather than situationally-specific (i.e., the morphine-experienced rat displays analgesic tolerance in the presence of both drug-paired cues and alternative cues), and sometimes a CCR is not apparent (i.e., rats tolerant to the analgesic effect of morphine do not display heightened sensitivity to nociceptive stimuli – "hyperalgesia" – when presented with morphine-associated cues) (e.g., Goudie & Griffiths, 1984; Kesner & Cook, 1983; Sherman, 1979; Tiffany, 1995).

Some investigators have suggested that such failures to find results expected on the basis of the conditioning interpretation of tolerance compromise the generality of the theory (see Goudie & Griffiths, 1984; Kesner & Cook, 1983). Others, however, have indicated that apparently contrary findings may be explicable by recognition that there are several potential signals for a drug (in addition to explicit, experimenter-manipulated environmental cues). Especially relevant to the present research are suggestions that, since each drug administration involves a protracted period of pharmacological stimulation, it is possible that a pharmacological association forms within each drug administration (an "intra-administration association"). That is, the early drug-onset cues may become signals for the later, larger drug effect. If a particular administration procedure promotes such an intra-administration association, it might be expected that the pharmacological cue would be especially effective. Unlike typical exteroceptive CSs (which likely generalize to stimuli encountered outside the conditioning situation), this putative interoceptive CS is both novel and presented in a perfectly positively contingent manner with the subsequent drug effect. This pharmacological cue, then, may overshadow (Kamin, 1969; Pavlov, 1927, pp. 142-143 and 269-270) simultaneously presented environmental cues. In such circumstances, the display of tolerance would not be influenced by environmental cues, and thus appear "nonassociative" (e.g., King et al., 1987; Mackintosh, 1987; Tiffany, Petrie, Baker & Dahl, 1983).

Support for the Contribution of Intra-Administration Associations to Tolerance

If intra-administration pharmacological CSs modulate tolerance because they overshadow simultaneously-present exteroceptive cues, we would expect: (1) relatively weak pharmacological effects should serve as signals for relatively stronger pharmacological effects, (2) drug tolerance should be subject to overshadowing, and (3) manipulations that modulate the likelihood of intra-administration associations should modulate situational-specificity of tolerance and CCRs. There is evidence for all these predictions.

Pharmacological CSs. We hypothesize that an intra-administration association results from the pairing of the small, initial drug effect with the later, larger drug effect, and this association contributes to tolerance. The hypothesis is supported by the results of experiments that have explicitly paired a small dose of a drug (pharmacological CS administration) with a larger dose of the same drug (pharmacological UCS administration), and have demonstrated that the resulting association between the two doses of the same drug contributes to tolerance to the pharmacological UCS (see Greeley & Ryan, 1995). Such associations may be termed "intra-drug associations."

For example, Greeley, Lê, Poulos, and Cappell (1984) injected rats in one group (Paired) with a low dose of ethanol (0.8 g/kg) 60 min prior to injection with a high dose of ethanol (2.5 g/kg). Another group of rats (Unpaired) received the low and high injections on an unpaired basis. When tested for the tolerance to the hypothermic effect of the high dose following the low dose, Paired subjects, but not Unpaired subjects, displayed tolerance. Moreover, if the high dose of ethanol was not preceded by the low dose, Paired rats failed to display their usual tolerance. This tolerance, dependent on an ethanol-ethanol pairing, was apparently mediated by an ethanol-CCR; Paired rats, but not Unpaired rats, evidenced a hyperthermic CCR (opposite to the hypothermic effect of the drug) in response to the low dose of ethanol. More recently, Cepeda-Benito and Short (1997) similarly reported that a small-dose of morphine may serve as a cue for a larger dose of the opiate, and control the display of morphine tolerance. Both Greeley et al. (1984) and Cepeda-Benito and Short (1997) suggested that their reports of intra-drug conditioning suggest that pharmacological associations may play an important role in the effects of repeated drug administrations. That is, a gradual increase in systemic concentration is an inevitable consequence of many drug administration procedures, thus there is the potential for intra-administration associations when a drug is administered.

Results reported by Krank (1987) are consistent with the suggestion that an intra-administration association forms within a drug administration. Krank (1987) assessed pharmacological conditioning in rats that received injections of 5 mg/kg morphine in a distinctive environment. When administered saline in the drug-paired environment, the rats did not display a CCR of hyperalgesia. However, when responsivity was assessed following a small dose of morphine (1 mg/kg), a hyperalgesic CCR was apparent. It is possible that the small dose was effective because the rats had formed an association between the small, early effect of the drug and the later, larger effect, and this pharmacological cue overshadowed the environmental cue.

Overshadowing of tolerance. Overshadowing is seen if two CSs simultaneously signal a UCS, and one CS is more "salient" than the other (other things being equal, a subject trained with a more salient CS will learn more rapidly than a subject trained with a less salient CS). When such a compound CS signals a UCS, subjects learn about the more salient CS at the expense of the less salient CS. Although there are no demonstrations that the pharmacological CS inherent in a drug effect can overshadow an exteroceptive CS, there are several demonstrations that tolerance (in common with other CRs) is subject to overshadowing (Dafters & Bach, 1985; Walter & Riccio, 1983). For example, in the Walter and Riccio (1983) experiment, rats were administered morphine in the presence of a compound (light + noise) CS. In different groups, the noise was either relatively soft and less-salient, or relatively loud and more-salient. Tolerance was assessed in the presence of the light alone. In the group in which the light had been compounded with the less-salient auditory stimulus, rats were tolerant (i.e., there was little overshadowing). In contrast, in the condition in which the light had been compounded with the more salient auditory stimulus, there was little evidence of tolerance; the noise CS overshadowed the light CS.

Although Walter and Riccio's (1983) experiment demonstrated overshadowing of tolerance with exteroceptive cues, the authors speculated about a potential role for "morphine-produced internal stimuli" (p. 661), i.e., intra-administration associations. They suggested that pharmacological cues inherent in a drug effect may compete with simultaneously presented environmental cues in eliciting CRs that mediate drug tolerance.

Modulation of the likelihood of intra-administration associations. Results reported by Grisel et al. (1994) suggest that intra-administration associations do indeed contribute to drug tolerance. Grisel et al. (1994) reported the results of two experiments. In Experiment 1 they found no evidence of environmental-specificity of tolerance; rats were equally tolerant to morphine administered in the drug-paired as in the alternative environment. All injections in this experiment were subcutaneous (sc). Grisel et al. (1994) reasoned that, because sc administration induces a slow and gradual onset of the drug effect, the early drug effect may have served as a CS for the later drug effect. This pharmacological CS would be present in both assessment environments, thus accounting for trans-situational tolerance. Grisel et al. (1994) tested this hypothesis in Experiment 2, using intravenous (iv) injections. With iv administration, the maximum effect of the drug is more immediate than with sc administration, thus there is little opportunity for the drug onset cues to effectively signal the maximal effect. Grisel et al. found complete situational-specificity of tolerance with iv administration, and conditional compensatory responding was apparent.

Grisel et al. (1994) suggested that their results were readily explicable by a conditioning interpretation of tolerance that acknowledged a role for a pharmacological CS:

> "The gradual nature of the increase in morphine action after sc delivery would permit early or weak portions of the drug state to become a cue for the later more potent state or action, thereby competing with the external cues for the acquisition of associative strength. IV injection is likely to minimize the competition from internal cues and maximize the likelihood

of utilizing salient external stimuli associated with the drug effect. Thus, the contribution of iv administration toward the production of stimulus context modulation of tolerance is readily explicable if classical conditioning is, indeed, the mechanism responsible for situationally-specific tolerance" (Grisel et al., 1994, pp. 1033-1034).

Although it is tempting to speculate that the differing pharmacological cue properties inherent in sc and iv administration account for Grisel et al.'s (1994) results, there are alternative interpretations. As discussed by Grisel et al., the kinetics of morphine action after sc and iv administration differ in a number of ways that might complicate interpretation of their findings. The present experiments were designed to assess the role of competition between pharmacological and exteroceptive cues in tolerance to the analgesic effect of morphine using procedures that do not involve the complications inherent in using various routes of drug administration.

Direct Manipulation of Intra-Administration Associations

To evaluate the potential role of intra-administration associations in morphine tolerance, we devised two administration procedures – one more likely to promote such associations than the other. Both procedures involve administration of the same dose of morphine (5 mg/kg) via the same route (iv, through a chronically-implanted venous catheter). The procedures differ in terms of the duration of the infusion. In the long-morphine (LMor) condition, the infusion is gradual (about 25-30 min). This LMor condition, although involving iv administration, is designed to simulate the gradual effects of a drug administered via a slower parenteral route (e.g., sc). We reasoned that if intra-administration associations form with such gradual-onset administration conditions

(Grisel et al., 1994), the LMor condition would promote such associations relatively readily, compared to the typical, more rapid iv administration.

The more rapid iv administration was accomplished in the short-morphine (SMor) condition. In this SMor condition, although the same morphine dose is administered as in the LMor condition, the infusion occurs at over 100 times the rate of the LMor infusion (i.e., the infusion duration is about 14-17 sec). We reasoned that the SMor condition would be less effective than the LMor condition in promoting the development of intra-administration associations.

EXPERIMENT 1

On the basis of a compound-CS analysis of predrug signals, an alteration of the exteroceptive signal for the drug should disrupt tolerance more for SMor than for LMor subjects. That is, SMor subjects, having no effective interoceptive cue, should associate only the exteroceptive cue with the pharmacological UCS. In contrast, altering the infusion parameters should disrupt tolerance more for LMor than for SMor subjects. Since only LMor subjects could associate the early effect of the drug with the maximal effect, administration of morphine to these subjects as a Short infusion on a test involves presentation of the drug without the usual pharmacological signal, and tolerance should be attenuated.

A "discriminative control of tolerance" design (Siegel, 1983) was used in Experiment 1. During the tolerance acquisition phase of the experiment, two groups of rats received 24 intravenous infusions. Six of these infusions consisted of morphine, and were conducted in the presence one distinctive cue (CS+). The remaining 18 infusions consisted of saline, and were conducted in the presence of another cue (CS-). Two

Morphine groups differed with respect to the infusion duration – either long or short (Groups LMor and SMor, respectively). Two additional groups of rats also received 6 infusions in the presence of CS+, and 18 in the presence of CS-, but the infused substance was always physiological saline. These two Saline groups also differed with respect to infusion duration (Groups LSal and SSal).

Following tolerance acquisition, rats were tested to assess the extent to which alteration in exteroceptive cues, and alteration in infusion parameters, disrupted the expression of tolerance.

Method

Subjects and Surgical Preparation

The subjects were 48, experimentally-naive, male, Sprague-Dawley rats (purchased from Charles River Laboratories, St. Constant, Quebec, Canada), weighing 300-350 g at the start of the experiment. They were maintained in individual cages with food and water available ad lib. Rats were handled daily for one week prior to surgery. Thirteen rats were assigned to each of groups LMor and SMor, and 11 rats were assigned to each of Groups LSal and SSal.

Surgery

Intravenous catheters were implanted in the right jugular vein under sodium pentobarbital anesthesia, using a modified version of the technique of Brown and Breckenridge (1975). The tip of the catheter was located approximately 1 cm from the heart. The catheter was constructed from a 9.5 cm length of silastic tubing (Dow Corning, .51-mm inner diameter, .94-mm outer diameter). Each rat's catheter was flushed with a solution of heparin and antibiotic (65 μg sodium heparin and 1.25 mg ampicillin) once daily for a 7-10 day recovery period following surgery

Apparatus, Drugs, Infusion Parameters, and Analgesia Assessment

Infusions took place in one of five, identical, chambers (30.4 cm long X 20.5 wide X 19 cm tall, Lehigh Valley Electronics). Each chamber, which was constructed of clear Plexiglas with a grid floor, was located in a vented, sound-attenuating cubicle, and was fitted with a hydraulically-sealed swivel (see Brown, Amit, & Weeks, 1976), which permitted rats free movement while attached to the infusion apparatus. The rat's cannula was connected to the swivel by a spring-connected cannula connector, and the swivels were connected to a 5-cc syringe by flexible tubing (Tygon, size 13, No. 6409). The tubing between the syringe and the swivel was fitted with an in-line, .025 micrometer micropore filter (Sartorium). The syringe was held in a variable rate syringe pump (Sage Model 341A).

All solutions were infused through the iv cannulae by timed operation of the syringe pump. Morphine was prepared as a 5 mg/ml solution. The rate of infusion was either 1.7 ml/min or .0167 ml/min for the short and long infusions, respectively. The exact duration of the infusion depended on the rat's weight, but was of the appropriate length to administer a dose of 5 mg/kg morphine. Saline infusions were volumetrically equated with morphine infusions, and, infused at the same rate as the morphine infusions (i.e., rats that received LMor in the presence CS+ received LSal in the presence of CS-, and rats that received SMor in the presence of CS+ received SSal in the presence of CS-).

Each cubicle was equipped with a house light and speaker. The house light was provided by a 15-W (nominal at 120 V ac) bulb (luminance was approximately 225 cd/m^2). Flashing the house light (3 flashes per sec) constituted the CS+. A clicking sound (5 clicks per sec) constituted the CS-. The clicks were generated by a Scientific Prototype Model 4041J click generator, set at a nominal scale intensity volume of 2, which corresponded to a volume of approximately 6 dB(SPL) above the ambient background of 73 dB (SPL).

Analgesia was measured with the tail-flick procedure (Fennessy & Lee, 1975). The tail of a lightly-restrained rat was immersed 5 cm into a water bath (located in the same room as the infusion chambers) maintained at 50 deg C. The latency for the rat to lift its tail out of the water (tail flick latency, TFL) was noted.

Procedure

Experiment 1 consisted of four phases: tolerance acquisition, the CS-alteration test, tolerance reacquisition, and the infusion-alteration test. The design of the experiment is summarized in Table 1.

Insert Table 1 about here

Tolerance acquisition. The tolerance acquisition phase of the experiment started 7-10 days after catheter implantation. Rats received two trials on each of 12 days, with about 5 h between trials. On even-numbered days, both trials consisted of presentations of CS-. On odd-numbered days, the first trial consisted of presentation of CS- and the second trial consisted of presentation of CS+. For all rats, presentation of CS- was followed by saline infusion. For rats assigned to Groups LMor and SMor, CS+

presentations were followed by infusion of morphine. For rats assigned to Groups LSal and SSal, CS+ (like CS-) was followed by a saline infusion.

On each tolerance acquisition trial, a rat was placed in the chamber, its cannula was flushed with heparinized saline, and connected to the syringe pump, and the CS was presented for 15 min. Coincidental with CS termination, the infusion started. Following completion of the infusion, the cannula connector was disconnected from the rat, the cannula was filled with a dextrose solution, and the rat remained in the chamber for an additional 90 min before being returned to its home cage.

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

CS-alteration test. Following tolerance acquisition, all rats (including those assigned to Sal groups) were infused with morphine in the presence of the saline-associated cue, CS- (using the same infusion duration that was used during tolerance acquisition). On the basis of the compound-CS analysis of predrug signals, this alteration in the exteroceptive cue should be minimally disruptive of tolerance in the group that received tolerance acquisition sessions under conditions favoring the development of a pharmacological, intra-administration association (LMor). That is, although the exteroceptive cue was altered on the test, the interoceptive, pharmacological association was the same on this test as it was during tolerance acquisition for LMor rats. The responses of rats in groups LSal and SSal indicated the analgesic effect of the opiate in rats receiving the drug for the first time.

Tolerance reacquisition. Following the CS-alteration test, rats received another four days of tolerance acquisition sessions. These sessions were conducted as the first four sessions of the tolerance acquisition phase of the experiment. That is, on the first and third day all rats received one CS+ trial and one CS- trial, and on the second and fourth days they received 2 CS- trials. On each of the 8 reacquisition trials, rats were infused with one of the two substances (5 mg/kg morphine, or physiological saline), at one of the two durations (short or long) in accordance with their treatment during tolerance acquisition.

Infusion-alteration test. Following the tolerance reacquisition phase of the experiment, rats received a second test to assess the contribution of intra-administration associations to tolerance. For this infusion-alteration test, all rats (including those assigned to Saline groups) were infused with morphine following CS+, but the infusion parameters were reversed from those that previously prevailed. That is, LMor and LSal subjects received Short morphine infusions (these groups now designated LMor-SMor Test and LSal-SMor Test, respectively), and SMor and SSal subjects received Long morphine infusions (these groups now designated SMor-LMor Test and SSal-LMor Test, respectively). Although both Morphine-trained groups were infused in the presence of the usual exteroceptive predrug signal, the pharmacological signal was altered for both. For LMor rats, the shortening of the infusion duration on this test would result in the usual dose of morphine being administered without the usual pharmacological cue; thus, LMor-SMor Test rats should display some loss of tolerance. In contrast, SMor-LMor Test rats, having not formed an intra-administration association, should continue to display tolerance to the usual drug dose administered in the context of the usual drug-predictive cue.

Results and Discussion

Tolerance Acquisition

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

Insert Figure 1 about here

The data summarized in Figure 1 were subjected to a mixed design, factorial analysis of variance (ANOVA), followed by post-hoc (LSD) comparisons. The statistical analyses confirmed the trends apparent in the figure: (1) morphine had an analgesic effect, i.e., tail-flick latencies were longer for morphine- than for saline-injected rats, $F(1,44) = 52$, $p < .001$; (2) tolerance developed, i.e., the drug X session interaction was significant, $F(2, 88) = 17$, $p < .001$, and the analgesic effect of morphine (but not saline) decreased from the first to the fifth tolerance-acquisition assessment ($p < .001$); and, (3) similar trends were apparent in short- and long-infused rats [neither infusion duration (short vs. long), nor any interaction involving this variable, was statistically significant, all ps > .19]. Thus, the effects of the 5 mg/kg morphine infusion were similar in LMor and SMor groups.

CS-Alteration Test

On this first test, all rats were infused with morphine in the presence of CS- (using the same infusion duration that was used during tolerance acquisition). The mean TFLs (± 1 SEM) for each group for each post-infusion assessment interval are shown in Figure 2.

Insert Figure 2 about here

The data summarized in Figure 2 were subjected to a mixed design, factorial ANOVA, followed by LSD tests. As can be seen in Figure 2, both groups that received morphine for the first time (SSal and LSal) displayed analgesia, compared to the rats receiving their seventh infusion of morphine on this test (SMor and LMor). This was confirmed by the significant effect of the tolerance-acquisition drug, F (1,44) =31, p < .001. The duration of the morphine infusion minimally affected Sal-trained rats. Analysis of groups LSal and SSal indicated no significant effect of group [F(1, 20) < 1] or group X assessment-interval interaction [F (2, 110) < 1]. In contrast, rats in morphine-trained groups responded differently to the opiate in the presence of CS-: SMor animals displayed slower TFLs (i.e., less tolerance) than LMor animals. Analysis of Mor-trained subjects indicated a significant effect of group (LMor vs. SMor), and a significant group by assessment interval interaction [$F(1, 24) = 5.5$, $p < .03$, and $F(2, 48) = 4.7$, $p < .02$ respectively].

Although rats in both groups were infused with morphine following the saline-associated environmental CS, LMor rats (that would be expected to acquire an intra-administration association) were administered the opiate following the usual pharmacological cue. As we hypothesized on the basis of an intra-administration associative analysis of tolerance, these LMor rats were less affected than SMor rats by the environmental change.

The results obtained on this CS-alteration test are consistent with those previously reported by Grisel et al. (1994). These investigators reported that morphine administration via a gradual-onset route (sc) produced trans-situational tolerance, whereas morphine administration via a rapid-onset route (iv) was environmentally-specific. One possibility is that the functional dose differs with the different administration procedures. That is, the same dose of the drug might be less effective following gradual administration (sc morphine in the Grisel et al. experiment and LMor in the present experiment) than following rapid administration (iv morphine in the Grisel et al. experiment and SMor in the present experiment). Results of both the Grisel et al. (1994) and the present experiments do not support this hypothesis. Grisel et al. (1994) demonstrated that sc morphine led to trans-situational tolerance with several different doses of the opiate (2.5-7.5 mg/kg). The results of the tolerance acquisition phase of the present experiment indicated that the initial analgesic effect of the drug, and the development of tolerance, were similar following LMor and SMor infusions.

As is apparent in Figure 2, there was some tolerance apparent in rats assigned to the SMor group. That is, although SMor rats are less tolerant than LMor rats, they nevertheless respond with shorter latencies than do saline rats (that receive morphine for the first time on this test session). Indeed, the differences in TFLs between SMor rats and rats in groups SSal and LSal are significant (all ps $\leq .01$). There are several possible reasons why SMor infusion in the presence of CS- did not eliminate tolerance in these rats. It is possible that the tolerance displayed by SMor rats is, to at least some extent, "nonassociative." Alternatively, although the SMor infusion was completed in about 15 sec, it is possible that there was nevertheless an intra-administration cue for these rats (although less effective than the intra-administration cue provided in the LMor condition). It is also possible that SMor rats were displaying some environmentally-specific tolerance in the presence of CS- because of generalization between the two CSs.

Infusion Alteration Test

Following tolerance reacquisition, rats received their final test. Four of the original 48 rats (1 originally assigned to the SMor group, and 3 originally assigned to the LMor group) did not participate in this test because of cannulae blockage. On this test, all rats were all infused with morphine, in the presence of CS+, but the duration of the infusion for each rat was altered – rats previously infused at the long duration were now infused at the short duration, and vice versa. The results of this test are summarized in Figure 3.

Insert Figure 3 about here

The two groups that received morphine during tolerance acquisition differed with respect to the response to the opiate in this test. Rats in the group previously infused with the short infusion and tested with the long infusion (SMor-LMor test) were more tolerant than rats in the group previously infused with the long infusion and tested with the short infusion (Group LMor-SMor test). Indeed, the TFLs of rats in group LMor-SMor test were about as long as those of rats in the two saline groups (LSal-SMor test and SSal-LMor test), which had minimal prior experience with the opiate.

These trends apparent in Figure 3 were confirmed by a mixed design, factorial analysis of variance. The effect of tolerance-acquisition drug was significant, $F(1, 40) = 16$, $p < .001$. Group SMor-LMor test rats responded more rapidly than did rats in any other group (all ps $< .02$). There were no significant differences in response latencies among groups LMor-SMor test, LSal-SMor test, and SSal-LMor test (all ps $> .1$).

For this infusion alteration test, both Mor-trained groups were tested with the same dose of the opiate in the presence of the opiate-paired environmental cue (CS+). However, the change in the putative pharmacological cue, effected by the alteration in infusion duration, disrupted the expression of tolerance more in LMor-trained rats than in SMor-trained rats. That is, the group hypothesized to have formed an intra-administration association were more affected by the alteration in infusion duration than were rats that did not form this association.

EXPERIMENT 2

The results of Experiment 1 suggest that intra-administration associations contribute to tolerance, and the pharmacological cues inherent in drug administration may overshadow simultaneously-present environmental cues. On the basis of the conditioning analysis of tolerance, within each administration, small drug-onset cues inherent in the LMor group become associated with the subsequent, larger drug effect and come to elicit CCRs. The purpose of Experiment 2 was to confirm the results of Experiment 1 using a simpler, non-discrimination design. In addition, Experiment 2 was designed to evaluate directly the CCR hypothesized to be elicited by the pharmacological signal provided by drug-onset cues.

We hypothesize that the pharmacological CS for the intra-administration association that forms within each LMor infusion is the pharmacological effect elicited by the onset of this infusion. To evaluate this association, we developed the "probe-morphine" (pMor) procedure. The pMor is designed to duplicate the early effect of the LMor infusion, and consists of approximately the first one-tenth of this infusion. Since the LMor infusion consists of administration of 5 mg/kg morphine in approximately 30 min, the pMor infusion consists of administration of approximately 0.5 mg/kg morphine in 3 min. If intra-administration associations form within a LMor infusion, LMor subjects should respond with conditional compensatory hyperalgesia when presented with the pMor.

Method

Design

The experiment consisted of three phases: tolerance acquisition, tolerance test, and CCR test (with tolerance reacquisition training interpolated between the two tests). During the tolerance acquisition phase, all rats received 6, long infusions (one infusion every other day). Approximately half the rats were infused with morphine (LMor), and half were infused with physiological saline (LSal). Following tolerance acquisition, all rats received two tests: a tolerance test and a CCR test. On the tolerance test, subjects either continued to receive their training drug at the same, long infusion rate (groups LMor-LMor and LSal-LSal), or at the more rapid, short infusion rate (groups LMor-SMor and LSal-SSal). If intra-administration associations contribute to tolerance, tolerance should be attenuated in rats receiving the usual drug dose without the usual pharmacological cue (LMor-SMor).

On the CCR test all rats received a probe infusion, with about half the rats trained with each substance (Mor or Sal) tested with a morphine probe (these groups now designated LMor-pMor and LSal-pMor), and half were tested with the saline probe (these groups now designated LMor-pSal and LSal-pSal). We hypothesized that the presentation of the usual pharmacological signal without the usual drug effect would permit observation of the CCR in LMor-pMor rats.

Subjects, Surgical Preparation, Drugs, and Apparatus

The subjects were 29 experimentally-naive rats of the same age and strain as those used in the previous experiment. They were housed as described previously. All subjects were prepared with chronic iv cannulae, using procedures described previously. Morphine sulfate was prepared as a 5 mg/ml solution. The parameters of long and short infusions were as described previously. The probe infusion consisted of a 3 min infusion at the long-infusion rate. The saline probe infusion consisted of a 3 min infusion of physiological saline. All infusions were conducted in the same apparatus as that used in Experiment 1. In contrast with Experiment 1, exteroceptive CSs were not used in this experiment.

Procedure

Tolerance acquisition. Once every other day, for 12 days, all rats were placed in the infusion chamber and infused either with morphine (n = 15) or saline (n = 14), using the long-infusion procedure described previously. The infusion started 15 min after each rat was placed in the chamber, and the rat remained in the chamber for 90 min following the completion of the infusion. As in Experiment 1, analgesia was assessed following the first, third, and fifth tolerance acquisition sessions; TFL was assessed on three occasions following infusion (0-, 45-, and 90-min post- infusion).

Tolerance test. The tolerance test occurred two days after the last tolerance acquisition session. For rats in groups LMor-LMor (n = 7) and LSal-LSal (n= 7), this tolerance test was identical to the tolerance acquisition sessions. Rats in group LMor-SMor (n = 8) were tested with 5 mg/kg morphine using the short procedure, and rats in group LSal-SSal (n = 7) were infused with saline using the short procedure.

Tolerance reacquisition. Following the tolerance test, all subjects received two, tolerance reacquisition trials. They were conducted in the same manner as the tolerance acquisition sessions.

CCR test. Two days following the final tolerance reacquisition session, 28 rats received a probe infusion (one rat infused with morphine during tolerance acquisition did not participate in this second test, as its cannula became nonfunctional following the first test). Approximately half the rats infused with each substance during tolerance acquisition received a morphine probe (groups LMor-pMor and LSal-pMor, ns = 7 and 9, respectively), and approximately half received the saline probe (groups LMor-pSal and LSal-pSal, ns = 7 and 5, respectively).

Analgesia was assessed at 5, 15, 30, 45, and 75 min following the probe infusion. Hyperalgesia would be manifest as a decreased tail-flick latency, thus the temperature of the water bath was 48-deg C (in contrast with the 50-deg C temperature used to measure analgesia). This decrease in temperature increased the likelihood of observing the increased sensitivity to the thermal stimulation.

Results and Discussion

Tolerance Acquisition

The mean TFLs (± 1 SEM) for each group for each post-infusion assessment are shown in Figure 4.

Insert Figure 4 about here

The data summarized in Figure 4 were subjected to a mixed design ANOVA. Morphine had an analgesic effect, as revealed by the significant groups effect [F (3, 24) =65, $p < .001$]. There were no significant differences between the two morphine-trained groups (LMor-LMor and LMor-SMor), or between the two saline-trained groups (LSal-LSal and LSal-SSal), as would be expected since the pair of groups injected with each substance were similarly treated during this phase of the experiment. Analgesic tolerance developed; that is, the response latencies of morphine- (but not saline-) trained subjects decreased from the first to fifth tolerance-acquisition assessment ($p < .001$).

Tolerance Test

On this first test, rats in group LMor-LMor continued to receive a long morphine infusion, while rats in group LMor-SMor received the same dose of the drug as a short infusion. The infusion rate was similarly either maintained or shortened for saline-trained rats (groups LSal-LSal and LSal-SSal, respectively). The mean TFLs (± 1 SEM) following the tolerance-test infusions are shown in Figure 5.

Insert Figure 5 about here

As can be seen in Figure 5, rats in Group LMor-LMor displayed complete tolerance to the analgesic effect of morphine. The response latencies of these rats following the opiate infusion were similar to those seen in rats trained and tested with saline (groups LSal-LSal and LSal-SSal). In contrast, rats in group LMor-SMor displayed greater analgesia than did rats in the other groups.

A mixed-design analysis of variance of the data summarized in Figure 5 indicated that the groups differed significantly, F (3,25)=190, $p < .001$). Pairwise comparisons (LSD) indicated that the response latencies of group LMor-SMor rats were significantly longer than the response latencies of rats in any other group (all ps < .01). Differences between the other 3 groups did not approach statistical significance.

CCR Test

Following tolerance reacquisition, all rats received a 3-min, 0.5 mg/kg probe infusion of either morphine (LMor-pMor or LSal-pMor) or saline (LMor-pSal or LSal-pSal). Figure 6 displays the mean TFLs (± 1 SEM) at various times following this probe infusion for all groups. As is apparent in Figure 6, rats in group LMor-pMor displayed shorter response latencies than did rats in the other groups (with there being similar response latencies displayed in the other three groups).

Insert Figure 6 about here

A mixed design analysis of variance of the data summarized in Figure 6 indicated that both the effect of groups, and the group X time interaction, were significant, F (3, 24) = 47.8, and F (12, 96) = 3.68, respectively, ps <.001. Pairwise comparisons (LSD) indicated that group LMor-pMor rats displayed significantly shorter response latencies than did rats in any other group (all ps <.001).

In LMor-trained rats, reproduction of the early opiate effect by probe infusion elicited hyperalgesia. Thus, as would be expected if an intra-administration association formed in these LMor rats, administration of the drug-onset cue revealed the CCR hypothesized to mediate tolerance in these rats.

GENERAL DISCUSSION

The results of these experiments suggest that intra-administration associations contribute to tolerance to the analgesic effect of morphine. The early drug-onset cues, having been paired with the later, larger drug effect, become associated with the larger effect and come to elicit CCRs that attenuate the drug effect. Moreover, pharmacological CSs inherent within the drug effect may be more salient than simultaneously-present environmental signals; thus, demonstrations that the display of tolerance is not controlled by environmental signals may be due to overshadowing of exteroceptive predrug cues by interoceptive predrug cues, rather than to the irrelevance of predrug cues in tolerance.

The results of the CS-alteration test of Experiment 1 demonstrated greater environmental-specificity of tolerance to 5 mg/kg morphine when the opiate was administered as a short infusion than as a long infusion. As expected on the basis of an intra-administration analysis of tolerance, the administration condition that promoted the acquisition of an intra-administration association (LMor) also decreased the signaling value of environmental cues.

The results of the infusion-alteration test of Experiment 1 further demonstrated the importance of the intra-administration association acquired by LMor rats. Despite the fact that, on this test, the usual dose of the drug was infused in the presence of the usual predrug environmental cue (CS+), the tolerance displayed by LMor rats was attenuated when morphine was infused without the usual pharmacological cue (i.e., as a SMor infusion).

The results of Experiment 2 confirmed the findings of Experiment 1, and further demonstrated that LMor subjects respond to drug-onset cues (reproduced with the pMor infusion) with a CCR. That is, administration of an analgesic drug, in a manner that mimics the early effect of a larger drug administration, actually elicits hyperalgesia.

Recognition of the importance of intra-administration associations has implications for the use of opiates to control pain, the treatment of addiction, and theories of drug tolerance. Finally, the study of intra-administration associations is relevant to the general issue of the study of associations formed between the early and late components of the same event.

Intra-administration Associations and the Use of Opiates for Pain Control

There is considerable evidence that conditioning contributes to some instances of opiate overdose. This evidence is obtained from laboratory experiments with rats (Siegel et al., 1982), studies of heroin overdose victims (Siegel, 1984), and a report of patient that received morphine for relief of pain (Siegel & Ellsworth, 1986). An appreciation of intra-administration associations may further our understanding of some cases of unexpected failures of tolerance that constitute an overdose.

Although the area is controversial (see Onofrio & Yaksh, 1990; Portenoy & Savage, 1997), there is evidence that some patients that receive opiates for pain control develop tolerance to the analgesic effect of the drug (e.g., Foley, 1991; Johnson & Faull, 1997). In such circumstances, the drug may be administered by a more efficient route in an attempt to reestablish effective analgesia. However, if tolerance is, in part, mediated by an intra-administration association, such an alteration in administration procedure may involve presentation of the drug in the absence of the usual predrug cues (and a failure of tolerance may occur). Indeed, Mucha, Kalant, and Birbaumer (1996) reported that alteration of the route of administration of morphine in rats (from intraperitoneal to iv) attenuated the display of analgesic tolerance, and they suggested that their findings were "analogous to the specificity of environmental factors of a tolerance treatment situation reported in the literature on classically conditioned tolerance" (p. 371); that is, "interoceptive stimuli produced by morphine acting through a particular route" (p. 371), in common with environmental stimuli, may act as CSs in the control of tolerance.

There is at least one report of a patient that suffered an "opioid toxicity" when switched from oral to parenteral opiate. Recently, Johnson and Faull (1997) described the case of a patient that was tolerant to the analgesic effect of orally-administered morphine (a μ opioid receptor-agonist). When narcotic analgesia subsequently was induced with a second μ-agonist administered by a different route – transdermal fentanyl – the expected cross-tolerance was not obtained. Rather, the patient showed evidence of an opioid overdose. It is possible that the CCR that mediated tolerance did not occur on the occasion of this overdose because the alteration in the route of administration altered the drug-onset CS that elicited this CCR (Siegel & Kim, submitted).

Intra-administration Associations and the Treatment of Addiction

There is evidence that the CCRs that mediate tolerance also mediate withdrawal distress and craving, and some treatment strategies are designed to extinguish the association between drug-predictive cues and the systemic effect of the drug (see Childress, McLellan, & O'Brien, 1986; Siegel, 1988, in press). Such treatments consist of presenting predrug cues in the absence of the drug. There are conflicting reports of the efficacy of such "cue exposure" treatments; some clinicians are enthusiastic (e.g., Heather & Bradley, 1990), but others have obtained mixed results (see Drummond, Tiffany, Glautier, & Remington, 1995). As indicated by Cepeda-Benito and Short (1997), if the early effect of a drug is one cue that elicits compensatory CRs, it is possible that mere exposure to pre-drug environmental cues may not effectively extinguish the association between pre-drug cues and the drug effect. Rather, "the inclusion of small drug doses during cue-exposure treatments may better reproduce the CSs responsible for craving" (Cepeda-Benito & Short, 1997, p. 239).

Intra-administration Associations and Theories of Tolerance

The fact that sometimes the situational-specificity of tolerance and CCRs are apparent, and sometimes not, has led some investigators to conclude that there are two types of tolerance: "associative" and "pharmacological" (see Grisel et al, 1994). Expression of the former, but not the latter, is dependent on the availability of the usual pre-drug cues. However, as suggested by Walter and Riccio (1983), trans-situational tolerance, rather than having a fundamentally different mechanism than tolerance attributable to conditioning, may be due to the relatively greater effectiveness of the interoceptive-pharmacological component of the predrug compound CS. Such differential effectiveness may arise because of procedural features of the drug administration procedure that promote overshadowing:

> "Tolerance controlled by internal, morphine-produced stimuli, unlike that mediated by environmental stimuli, would be expected to be relatively trans-situational or 'pharmacological' in nature, even though the same underlying conditioning mechanisms would be involved. The question then becomes one of establishing the extent to which tolerance, in any given case, is controlled by one or the other of these two general classes of stimuli, rather than one of making a distinction between two different 'kinds' of tolerance" (Walter & Riccio, 1983, p. 661).

Walter and Riccio's (1983) speculations about the importance of drug-induced cues were prescient; they antedated demonstrations that such cues may contribute to tolerance (Cepeda-Benito & Short, 1997; Greeley et al., 1984).

Although the question of whether there are, indeed, two types of tolerance, associative and nonassociative, is controversial (see Cepeda-Benito & Short, 1997; Grisel et al., 1994; Tiffany, Drobes, & Cepeda-Benito, 1992), it is appealingly parsimonious to characterize all tolerance that results from repeated drug administration as associative. According to this view, tolerance may appear nonassociative (e.g., it is not modulated by drug-paired stimuli) for one of two reasons: [1] the tolerance results from a single drug administration (or very-closely spaced administrations, see Tiffany et al., 1992), and thus reflects the unconditional compensatory responses elicited by drug-induced disturbances (i.e., the tolerance is "acute," rather than "chronic," see Dworkin, 1993; Ramsay & Woods, 1997; Siegel & Allan, 1998), or [2] the drug administration procedures favor the development of intra-administration associations, and the experimenter is assessing only environment-drug associations.

In summary, in the case of tolerance seen after repeated drug administrations, we agree with Ramsay and Woods' (1997) conclusion that "learning is the primary underlying cause of the development of drug tolerance" (p. 170), but additionally suggest that one important type of learning is the acquisition of an association between drug-onset cues and the later, larger drug effect. Of course, a problem with such an analysis is that it is not readily disconfirmed. A demonstration of chronic tolerance that appears "nonassociative" (e.g., it is not situationally-specific) may be reinterpreted as "associative" by appealing to hypothesized intra-administration pharmacological cues that overshadow simultaneously-present environmental cues. However, recognition that drug-onset cues may be effective CSs does lead to novel predictions. For example, administration procedures that favor the development of intra-administration associations should lead to trans-situational tolerance. This prediction has been confirmed by the results of Experiment 1 of the present report, as well as by results previously reported by Grisel et al. (1994). Administration procedures that minimize the likelihood of intra-administration associations developing should maximize the role of environmental cues in the acquisition of tolerance. For example, tolerance induced by intracerebroventricular administration should display little or no evidence of intra-administration control, and thus be maximally susceptible to environmental manipulations (MacRae & Siegel, 1987;

Melchior, 1988). It would further be expected that if trans-situational tolerance results because pharmacological cues overshadow simultaneously-present environmental cues, procedures that reverse overshadowing (e.g., repeated presentation of the overshadowing, pharmacological CS) should similarly restore environmental control of tolerance (Matzel, Schachtman, & Miller, 1985; Matzel, Shuster, & Miller, 1987). Additional research will be needed to evaluate the utility of an analysis of the contribution of Pavlovian conditioning to tolerance that acknowledges the potential contribution of intra-administration associations.

Associations Between Early and Late Components of a Single Event

In most Pavlovian conditioning research, the CS and UCS are two very different stimuli, presented in different modalities (e.g., light and shock). In the case of intra-administration associations, the CS and UCS are intrinsic parts of the same stimulus. Dworkin (1993) distinguished between these two types of conditioning situations. He applied the term heteroreflexes ("heterotopic conditioned reflexes") to the traditional, two-stimulus conditioning preparation, and distinguished heteroreflexes from homoreflexes ("homotopic conditioned reflexes"). In the case of homoreflexes, the CS and UCS are presented in the same modality, and differ only in intensity. Dworkin (1973, p. 29 and pp. 71-84) suggested that the associations that form when the early components of baroreceptor stimulation serve as signals for later components of baroreceptor stimulation, and the learning that occurs when a small dose of a drug serves as a cue for a subsequent administration of a larger dose of that drug, are examples of homoreflexes. Other examples of homoreflexes are described by Bykov (1959) and Ádám (1967). The type of learning studied in the present experiments, in which (within each administration) the early effect of a drug serves as a cue for a later effect of that drug, is another example of a homoreflex.

As discussed by Dworkin (1993, p. 79), "the heteroreflex makes for a clearer and more dramatic experimental demonstration ... Ultimately, however, homoreflexes may prove to be more basic and more ubiquitous" (Dworkin, 1993, p. 79). There is evidence that associations form especially readily if the CS is similar to the UCS (see review by Mackintosh, 1983, pp. 213-214), and "the stimuli of homoreflexes are about as similar as they can be" (Dworkin, 1993, p. 79). Moreover, many physiological events normally contain the potential for homoreflexes, and such associations may serve an important regulatory function. Dworkin hypothesized that homoreflexes may serve as "in situ mechanisms for adjusting the gain of regulatory reflexes" (Dworkin, 1993, p. 79). Intra-administration associations may be but one example of a homoreflex that modulates the regulatory responses elicited by a pharmacologically-induced perturbation.

Although homoreflexes may be "basic and ubiquitous," the experimental analysis of this type of conditioning presents special methodological challenges. The homoreflex CS is not, like the typical heteroreflex CS, "neutral" – rather, the homoreflex CS is a less-intense version of the UCS. We know little about the optimal way of evaluating conditional responding with this sort of CS. For example, in the case of the intra-administration homoreflex described in these experiments, the response to pMor is actually a combination of a hyperalgesic CCR and analgesic responding unconditionally elicited by this small morphine dose. Furthermore, in the case of homoreflexes (and in contrast with heteroreflexes) the CS is an ineluctable part of the UCS. As discussed by Dworkin (1993, pp. 79-84), the traditional control procedures used by learning researchers, that have been developed in heteroreflex studies, are not readily applicable to the study of homoreflexes (e.g., unpaired CS-UCS control groups). Future research on homoreflexes in general, and intra-administration associations in particular, will have to develop control procedures suitable to this type of learning.

The results of the present experiments suggest that one type of homoreflex (intra-administration associations) contributes to tolerance to repeated presentation of morphine. As discussed by Siegel and Allan (1998) and Dworkin (1993), such associative control of tolerance is but one manifestation of the contribution of learning to homeostasis, or "the wisdom of the body" (Cannon, 1939). Inasmuch as "learning is one of the physiological mechanisms that give the body its wisdom" (Dworkin, 1993, p. 185), the further study of homoreflexes likely will yield new insights into the mechanisms by which Pavlovian conditioning contributes to homeostasis.

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Table 1

Design of Experiment 1

Phases of experiment			
Tolerance Acquisition[a]	CS-alteration test[b]	Tolerance reacquisition	Infusion-alteration test[c]
Morphine groups			
CS+ → LMor and CS– → LSal	CS– → LMor (LMor)	CS+ → LMor and CS– → LSal	CS+ → SMor (LMor-SMor Test)
CS+ → SMor and CS– → SSal	CS– → SMor (SMor)	CS+ → SMor and CS– → SSal	CS+ → LMor (SMor-LMor Test)
Saline groups			
CS+ → LSal and CS– → LSal	CS– → LMor (LSal)	CS+ → LSal and CS– → LSal	CS+ → SMor (LSal-SMor Test)
CS+ → SSal and CS– → SSal	CS– → SMor (SSal)	CS+ → SSal and CS– → SSal	CS+ → LMor (SSal-LMor Test)

[a] Rats in Morphine groups were intravenously infused with morphine following CS+ (a flashing light), and physiological saline following CS- (a clicker). For different morphine groups, the morphine and saline were presented as long infusions (LMor and LSal, respectively), or short infusions (SMor and SSal). Rats in Saline groups were infused with either LSal or SSal following both CS+ and CS-.

[b] Rats were infused with morphine in the presence of CS-, with the same infusion duration as was used during tolerance acquisition. Group designations are indicated in parentheses.

[c] Rats were infused with morphine following CS+, but the infusion parameters were reversed from those used during tolerance acquisition. Group designations are indicated

in parentheses.

Figure Captions

Figure 1. Mean tail-flick latencies (± 1 SEM) observed during the tolerance development phase of Experiment 1. Rats were administered 5 mg/kg morphine as either a long or short iv infusion (groups LMor and SMor, respectively), or were administered physiological saline at one of the two infusion durations (groups LSal and SSal). Tail flick latencies were determined at 0- 45- and 90-min following the first (A), third (B), or fifth (C) tolerance development session.

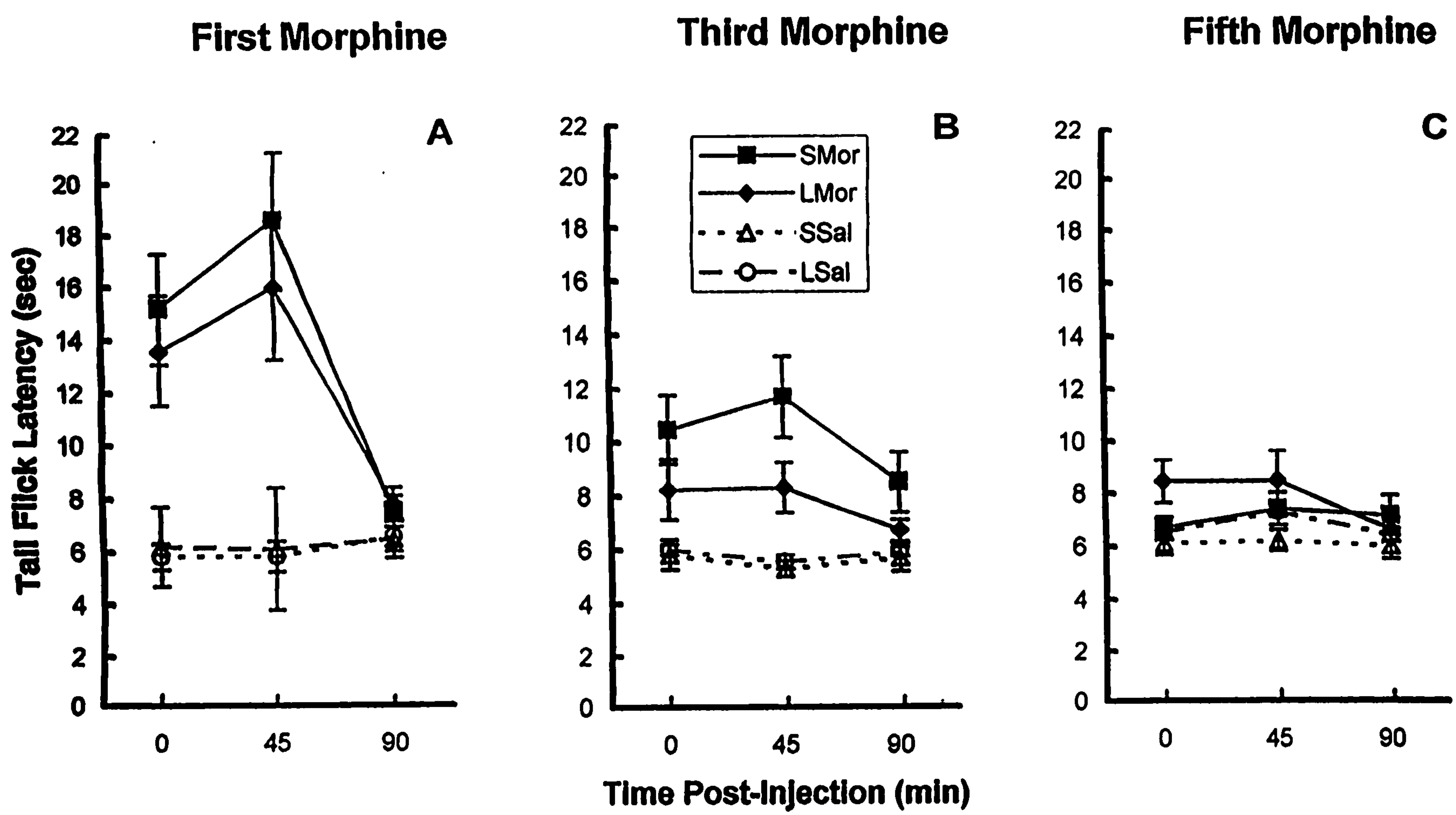
Figure 2. Mean tail-flick latencies (± 1 SEM) observed during the CS alteration test of Experiment 1. All rats were infused with morphine in the presence of CS- (using the infusion durations used during the tolerance development phase).

Figure 3. Mean tail-flick latencies (± 1 SEM) observed during the infusion alteration test of Experiment 1. All rats were infused with morphine in the presence of CS+, but the infusion parameters were reversed from those used during tolerance development: LMor and LSal rats were infused with SMor (groups LMor-SMor test, and LSal-SMor test, respectively), and SMor and SSal rats were infused LMor (groups SMor-LMor test, and SSal-LMor test, respectively).

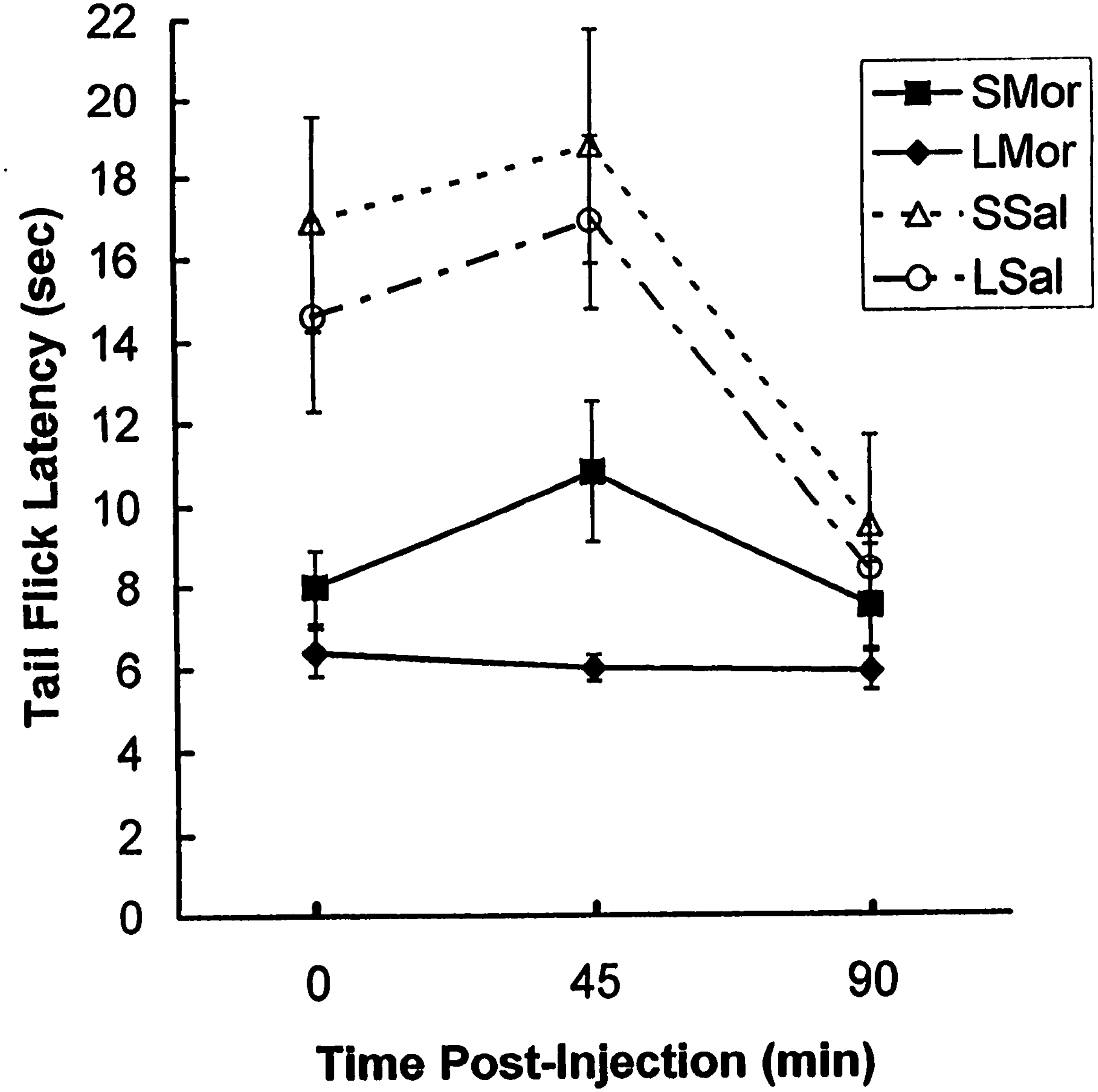
Figure 4. Mean tail-flick latencies (± 1 SEM) observed for each group for each post-infusion assessment during the tolerance development phase of Experiment 2. Tail flick latencies were determined at 0- 45- and 90-min following the first (A), third (B), or fifth (C) tolerance development session.

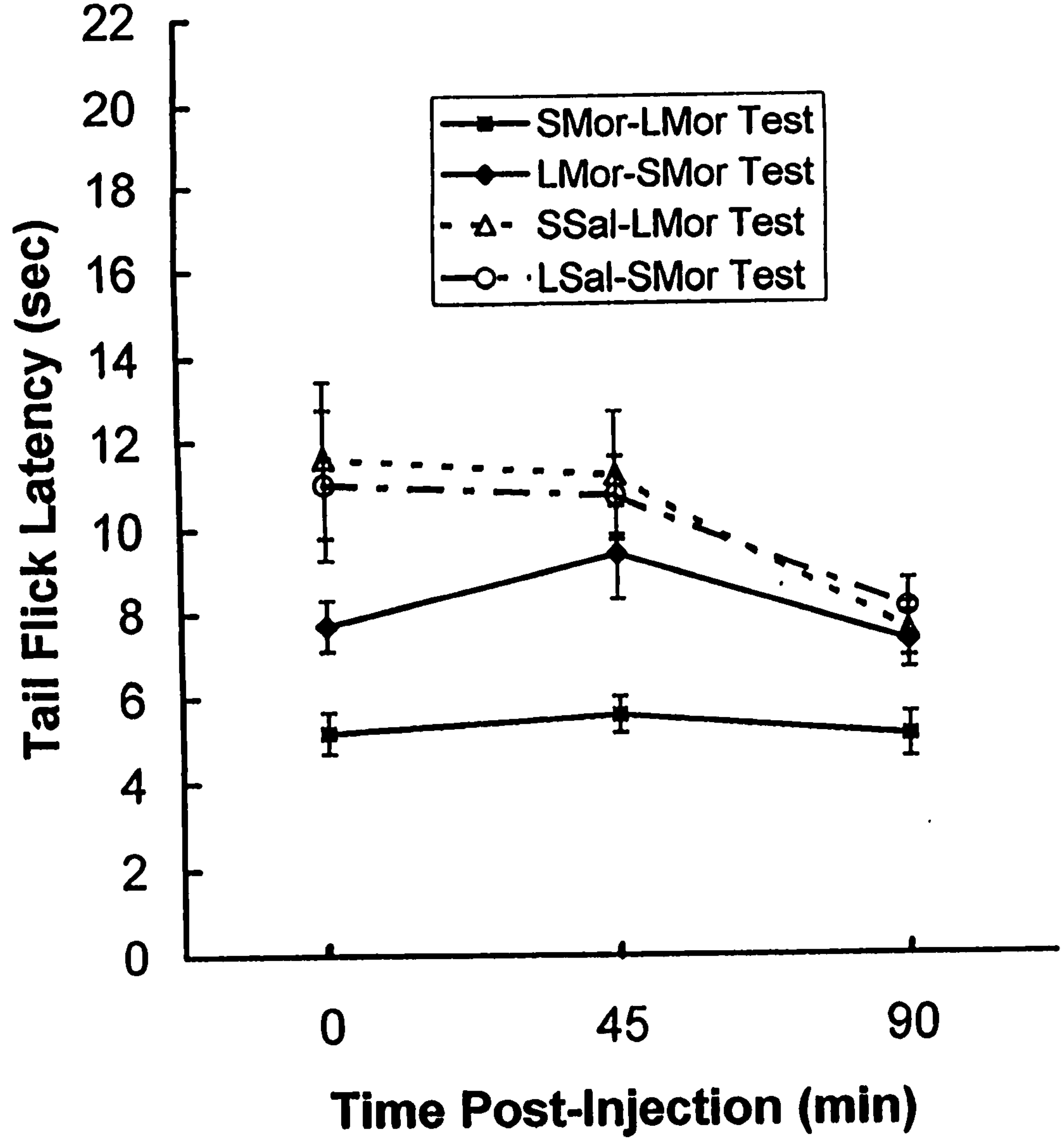
Figure 5. Mean tail-flick latencies (± 1 SEM) observed during the tolerance test of Experiment 2. Rats received iv infusions of the same drug they received during tolerance development, at either the same long infusion duration rate that was used during tolerance development (groups LMor-LMor and LSal-LSal), or at the shorter infusion rate (groups LMor-SMor, and LSal-SSal).

Figure 6. Mean tail-flick latencies (± 1 SEM) observed during the conditional compensatory response (CCR) test of Experiment 2. Rats with a history of long infusions of either morphine or saline (LMor and LSal, respectively) were infused with a probe dose of one of the two substances (pMor and pSal), designed to mimic the early effect of the long infusion.

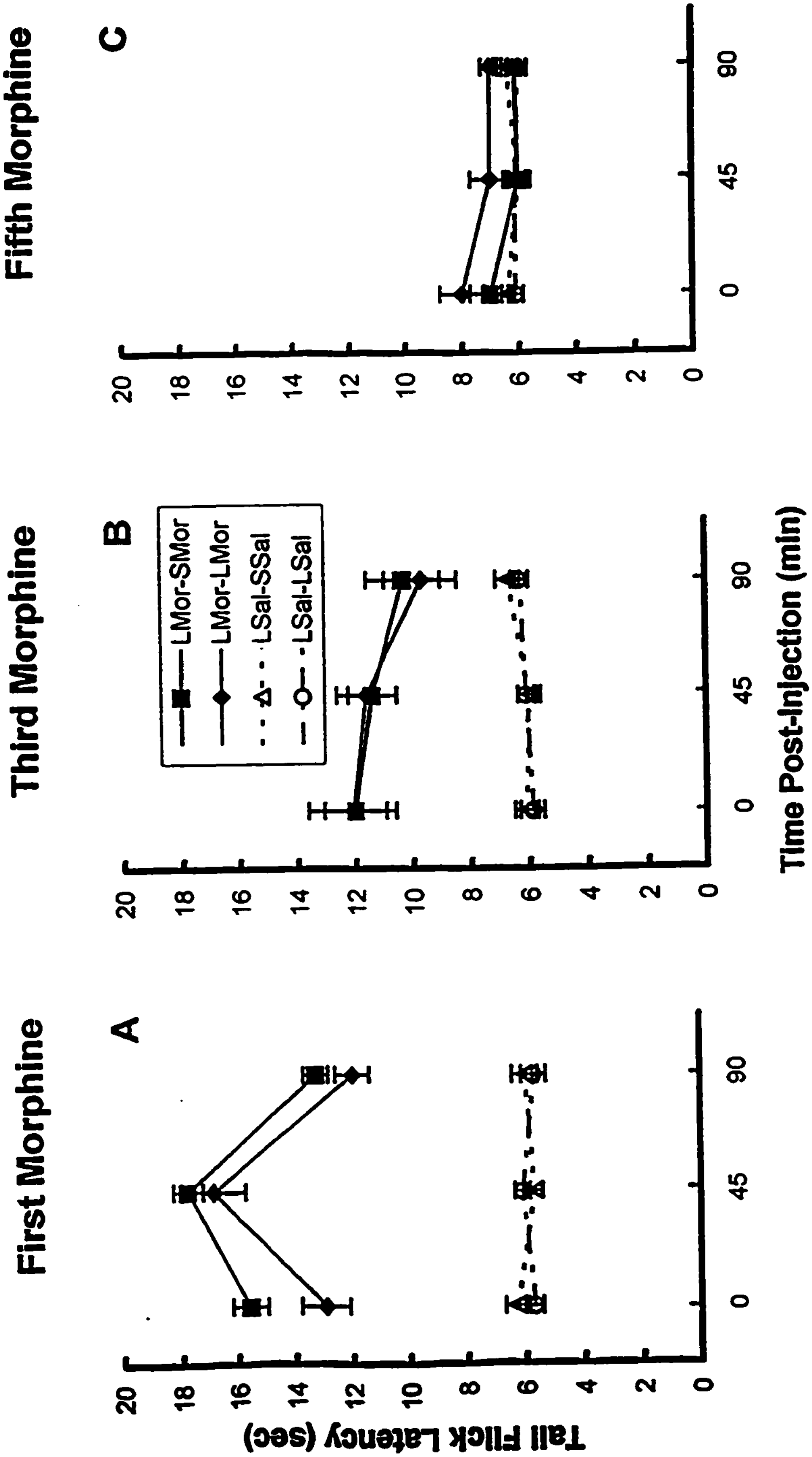


First Morphine
Third Morphine
Fifth Morphine
A
B
C
Tail Flick Latency (sec)
Time Post-Injection (min)
SMor
LMor
SSal
LSal
0
45
90
0
2
4
6
8
10
12
14
16
18
20
22

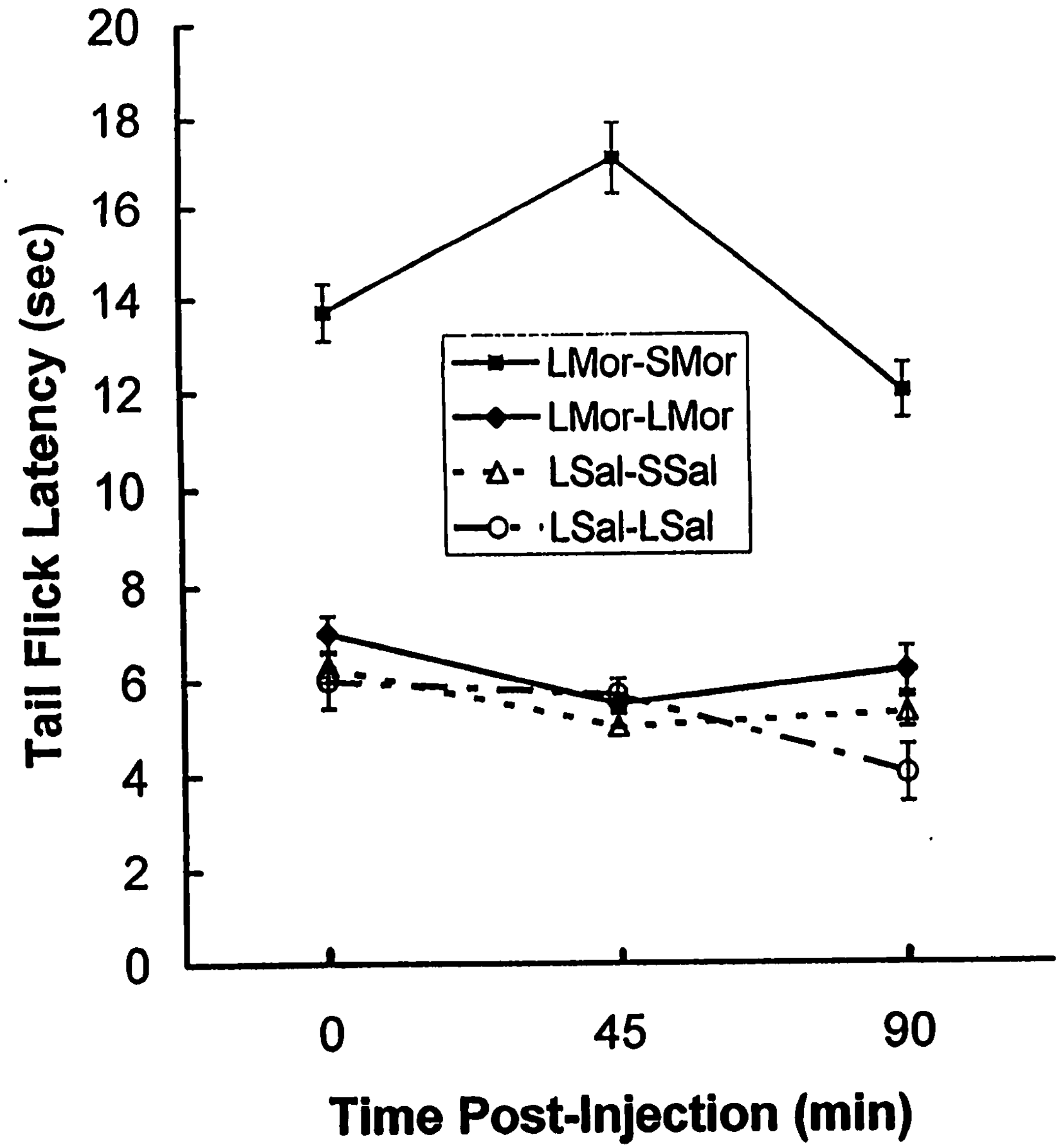

SMor
LMor
SSal
LSal
Tail Flick Latency (sec)
22
20
18
16
14
12
10
8
6
4
2
0
0
45
90
Time Post-Injection (min)

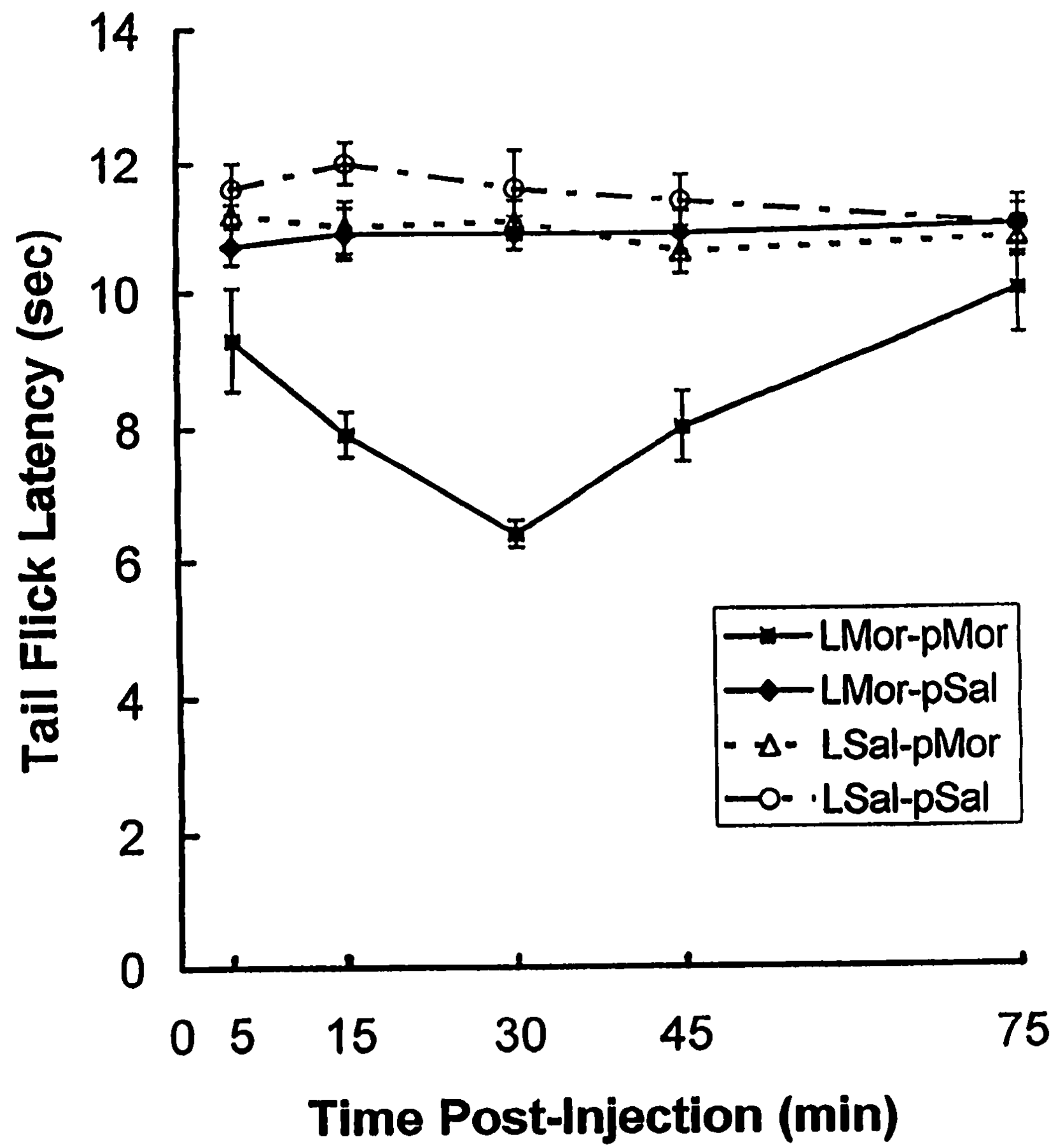


Tail Flick Latency (sec)
0
2
4
6
8
10
12
14
16
18
20
22
SMor-LMor Test
LMor-SMor Test
SSal-LMor Test
LSal-SMor Test
0
45
90
Time Post-Injection (min)



First Morphine
A
Third Morphine
B
Fifth Morphine
C
Tail Flick Latency (sec)
Time Post-Injection (min)
LMor-SMor
LMor-LMor
LSal-SSal
LSal-LSal
0
45
90
20
18
16
14
12
10
8
6
4
2
0


20
18
16
14
12
10
8
6
4
2
0
Tail Flick Latency (sec)
LMor-SMor
LMor-LMor
LSal-SSal
LSal-LSal
0
45
90
Time Post-Injection (min)



Tail Flick Latency (sec)
14
12
10
8
6
4
2
0
0
5
15
30
45
75
Time Post-Injection (min)
LMor-pMor
LMor-pSal
LSal-pMor
LSal-pSal

CHAPTER 3: INTRODUCTION

The results of experiments reported in Chapter 2 demonstrated that in addition to external environmental cues that may be explicitly paired with the drug effect, internal pharmacological cues (inherent to drug administration) may also signal the drug effect. That is, within each administration, early drug-onset cues may become associated with the later, larger drug effect, and these pharmacological cues may overshadow simultaneously-present environmental cues.

These experiments, as well as those reported by many others (see reviews by Dworkin, 1993; Ramsay & Woods, 1997; Siegel & Allan, 1998; Siegel, 1999; Siegel, Baptista, Kim, MacDonald, &Weise-Kelly, in press) provide evidence that conditioning contributes to tolerance; however, there has been little research concerning the physiological events that mediate this contribution. Some researchers have noted conditional metabolic or drug-disposition changes that may function as CCRs (Melchior & Tabakoff, 1985; Roffman & Lal, 1974). More recent research has concerned conditional pharmacodynamic alterations – what happens in the central nervous system in response to pre-drug cues? In Chapter 3, we further our investigation of intra-administration associations: how are drug-onset cues important to the physiological mechanisms of opiate tolerance?

Conditional Neurochemical Alterations

As previously discussed, tolerance results from drug-compensatory processes elicited not only by the drug effect, but also by cues, that in the past, have been associated with the drug effect. In recent years, some investigators have been interested in identifying the neurochemical events that mediate these drug-compensatory processes and the associative contribution to tolerance. For example, in ethanol-tolerant rats, Quertemont and colleagues have suggested that the conditional release of taurine may importantly contribute to ethanol tolerance.

Quertemont, de Neuville, and De Witte, (1998a) used microdialysis to evaluate neurochemical changes in the amygdala during repeated ethanol administrations, and during presentation of a distinctive olfactory cue that had been paired with ethanol. They reported that ethanol elicited an increase in extracellular taurine. Taurine is a neuromodulator believed to attenuate ionic and osmotic changes that occur after ethanol administration. Furthermore, taurine decreases the aversive effects of ethanol, as measured by aversion to the side of a two-choice chamber that contains an ethanol-paired odor (Quertemont, Goffaux, Vlaminck, Wolf, & De Witte, 1998b). In rats with a history of ethanol administration in the presence of a vinegar odor CS, administration of saline in the presence of the olfactory CS elicited an increase in taurine microdialysate content. Quertemont et al. (1998a) suggested that such conditional release of taurine is a CCR in rats presented with a CS for ethanol, and this CCR contributes to tolerance.

Just as there are neurochemical events that may come under conditional control and mediate the associative contribution to ethanol tolerance (e.g., conditional taurine release), there may also exist conditional neurochemical events that mediate the associative contribution to opiate tolerance. For example, drug-onset cues may serve to trigger the release of endogenous anti-opioid peptides (see Rothman, 1992) that compensate for the drug effect. In the experiments reported in Chapter 3, we evaluated the role of a putative anti-opioid peptide – cholecystokinin (CCK-8) – in the associative mechanisms of opiate tolerance. In one experiment, treatment with a CCK2 antagonist was successful in attenuating the expression of the conditional compensatory response elicited by drug-onset cues. In a second experiment, the same CCK2 antagonist treatment also attenuated the expression of established morphine tolerance. A discussion of the theoretical and clinical implications of these findings follows.

CHAPTER 3

Kim, J.A., & Siegel, S. (submitted). The Role of Cholecystokinin in Conditional Compensatory Responding and Morphine Tolerance. (to be submitted to Behavioral Neuroscience, September 2000).

The Role of Cholecystokinin in Conditional Compensatory Responding and Morphine Tolerance

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Running Head: CCK-8 and Morphine

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Abstract

As elaborated in the conditioning analysis of tolerance, cues present at the time of drug administration become associated with the drug effect. One particularly effective type of cue that may become associated with the drug effect is the pharmacological, drug-onset cue inherent to drug administration. Like environmental cues that may be explicitly paired with the drug effect, pharmacological, drug-onset cues contribute to tolerance. The early drug effect, having been paired with the subsequent larger drug effect, elicits a conditional compensatory response that attenuates the drug effect. In the present study, we evaluated the role of a putative anti-opioid peptide – cholecystokinin-8 (CCK-8) – in the associative mechanisms of opiate tolerance. The results of the present experiments suggest that intrathecal pretreatment with the CCK2 receptor antagonist PD-135,158 blocks the expression of cue-elicited conditional compensatory responding (Experiment 1) and the expression of established morphine tolerance (Experiment 2).

The Role of Cholecystokinin in Conditional Compensatory Responding and Morphine Tolerance

The effects of many drugs decrease over the course of repeated administration, the phenomenon being termed tolerance. The results of experiments from many laboratories suggest that tolerance may be mediated, in part, by associative mechanisms (see reviews by Dworkin, 1993; Ramsay & Woods, 1997; Siegel & Allan, 1998; Siegel, 1999; Siegel, Baptista, Kim, MacDonald, &Weise-Kelly, in press). Cues present at the time of drug administration may become associated with the drug effect. After some pairings, the expression of tolerance is modulated by the presence of drug-associated cues. Using the usual conditioning terminology, cues accompanying the primary drug effect function as conditional stimuli (CSs). The direct effect of the drug constitutes the unconditional stimulus (UCS). Prior to any learning, this pharmacological stimulation elicits responses that compensate for drug-induced disturbances. These responses that compensate for the drug effect are unconditional responses (UCRs). After some pairings of the predrug CS and pharmacological UCS, drug-compensatory responses are elicited by predrug cues. These conditional compensatory responses (CCRs) mediate the development of tolerance by counteracting the drug effect.

Situational-Specificity of Tolerance

The most thoroughly established findings supporting the conditioning analysis of tolerance are demonstrations of the situational-specificity of tolerance. After tolerance is established by repeatedly administering the drug in a particular environment, tolerance often is more pronounced in that drug-paired environment than in an alternative environment. Situational-specificity has been demonstrated with respect to tolerance to many effects of a variety of drugs including ethanol (e.g., Lê, Poulos, & Cappell, 1979; Seeley, Hawkins, Ramsay, Wilkinson, & Woods, 1996), pentobarbital (e.g., Cappell, Roach, & Poulos, 1981) and opiates (reviewed by Siegel, 1991). It is seen in many species, from snails (Kavaliers & Hirst, 1986) to humans (e.g., Dafters & Anderson, 1982).

The most dramatic demonstrations of situational-specificity concern tolerance to the lethal effects of drugs. Altering the context of drug administration increases the lethality of several drugs, including ethanol (Melchior, 1990; but see Tsibulsky & Amit, 1993), pentobarbital (Vila, 1989), heroin (e.g., Siegel, Hinson, Krank, & McCully, 1982), and morphine (Siegel & Ellsworth, 1986).

Conditional Compensatory Responses

The fact that tolerance displays situational-specificity is consistent with the conditioning analysis of tolerance. That is, drug-associated cues elicit CCRs that attenuate the drug effect, thus tolerance is greater when assessed in the presence of drug-associated cues than when it is assessed elsewhere. These CCRs may be seen by presenting the usual predrug cues but not administering the drug. Such CCRs have been demonstrated with many drugs (see Siegel, 1991, 1999), including ethanol (e.g., Larson & Siegel, 1998; Siegel, 1987) and opiates (e.g., Grisel, Wiertelak, Watkins, & Maier, 1994; Krank, Hinson, & Siegel, 1981; Raffa & Porreca, 1986).

Drug-associated Cues and CCRs

Although experimental studies of the associative basis of drug effects typically have manipulated environmental cues (e.g., the room where the drug is administered), there is evidence that a variety of stimuli may become associated with a drug and control the display of tolerance. For example, distinctive flavors (McNally & Westbrook, 1998), ambient temperatures (Kavaliers & Hirst, 1986), or magnetic fields (Kavaliers & Ossenkopp, 1985) may, after being paired with morphine administration, influence the display of morphine tolerance. Although such explicitly manipulated exteroceptive stimuli have been shown to evoke CCRs, other naturally occurring interoceptive cues, may also do so.

A particularly salient interoceptive cue that has recently been examined is the pharmacological drug-onset cue inherent to drug administration. Within each drug administration, drug-onset cues reliably precede the later and larger drug effect, thus there is the potential for the formation of an association between early and later drug effects whenever a drug is administered (e.g., Greeley, Lê, Poulos, & Cappell, 1984; King, Bouton, & Musty, 1987; Mackintosh, 1987; Tiffany, Petrie, Baker & Dahl, 1983). We have termed such associations, formed within a single administration, <u>intra-administration associations</u> (Kim, Siegel, & Patenall, 1999). We may expect that pharmacological drug-onset cues, which are naturally "paired" with the drug effect, are particularly salient cues. Unlike typical exteroceptive CSs (which likely generalize to stimuli encountered outside the conditioning situation), this putative interoceptive CS is both novel and presented in a perfectly positively contingent manner with the subsequent drug effect. Indeed, we have recently demonstrated that pharmacological drug-onset cues importantly contribute to the expression of conditional compensatory responding and drug tolerance, and, these pharmacological cues may overshadow simultaneously-presented environmental cues (Kim et al., 1999).

Intra-administration Associations

We have developed a technique to assess directly the contribution of intra-administration associations to tolerance (Kim et al., 1999). With this procedure, rats receive infusions via chronically-implanted jugular cannulae. Morphine is administered as a Long Morphine (LMor) infusion, via a gradual (.0167 ml/min) infusion of a morphine solution (5 mg/ml) for a duration sufficient to administer a dose of 5 mg/kg. The exact duration of the infusion depends on the weight of the rat, but is approximately 30 min. We reasoned that if intra-administration associations form with such gradual-onset administration conditions, the LMor condition would promote such associations relatively readily, compared to a typical, more rapid intravenous administration.

The direct assessment of the intra-administration association involves the presentation of the usual pharmacological signal without the usual drug effect. This is accomplished with the Probe Morphine (pMor) infusion. The pMor consists of the first one-tenth of the LMor infusion; 0.5 mg/kg morphine is administered in about 3-min (at the same infusion rate of .0167 ml/min). We reasoned that, if LMor subjects have acquired an intra-administration association, they would display conditional compensatory responding to this initial drug effect. Thus, in one experiment reported by Kim et al., (1999) rats tolerant to the analgesic effect of morphine induced by LMor infusions were administered the pMor infusion. These rats displayed hyperalgesia (an increase in pain sensitivity) in response to this small, initial drug effect, thus providing direct evidence of the CCR resulting from an intra-administration association.

If drug-onset cues elicit CCRs that contribute to opiate tolerance, what mediates the CCR? There is evidence that the activity of anti-opioid peptides in general, and cholecystokinin in particular, contribute to opiate tolerance.

Anti-opioid Peptides, Cholecystokinin, and Opiate Tolerance

Some evaluations of neurochemical alterations that mediate opiate tolerance have focused on anti-opioid peptides (AOPs). There is evidence that AOPs are released by the central nervous system in response to opiate stimulation, and that they contribute to tolerance by attenuating the effect of the drug (Rothman, 1992). Although several putative AOPs have been proposed, one that has received considerable attention is cholecystokinin-8 (see review by Wiesenfeld-Hallin, Lucas, Alster, Xu, & Hökfelt, 1999). There is evidence that cholecystokinin-8 (CCK-8) attenuates the effect of morphine. For example, if CCK-8 is administered exogenously, it blocks morphine-induced analgesia in a dose-dependent manner (e.g., Han, 1995; Mitchell, Lowe, & Fields, 1998). However, co-treatment with a CCK antagonist prevents CCK-8 from attenuating opioid effects (Suh, Kim, Choi, & Dong, 1995). Blocking CCK receptors potentiates morphine analgesia in rats (e.g., Zhou, Sun, Zhang, & Han, 1992) and humans (e.g., McCleane, 1998). Moreover, morphine administration accelerates the release of CCK-8 from the central nervous system in a dose-dependent manner (Zhou et al., 1992). Treatment with CCK receptor antagonists has been shown to prevent the development of morphine tolerance (e.g., Kellstein & Mayer, 1991) and to attenuate the expression of established morphine tolerance (e.g., Hoffmann & Weisenfeld-Hallin, 1994; Siegel, Kim, & Sokolowska, 1999). Two subtypes of CCK receptors have been identified and termed CCK1 and CCK2 (Wiesenfeld-Hallin et al., 1999). The availability of highly selective receptor antagonists has demonstrated that, at least in rodent models, the anti-opioid function of CCK-8 is active at the CCK2, rather than the CCK1 receptor site (Wiesenfeld-Hallin et al., 1999).

Although many studies have investigated the role of CCK-8 activity (and other AOPs) in opiate tolerance, the mechanism by which CCK-8 release interacts with opioid effects is unclear. Many studies have focused on characterizing the molecular-biological events that may be involved in CCK-opiate interactions (e.g., Zhou et al., 1992; Gustafsson, Afrah, Brodin, & Stiller, 1999). However, with few exceptions (e.g., Wiertelak, Maier, & Watkins, 1992; Grisel, Watkins & Maier, 1996; Mitchell, Basbaum, & Fields, 2000) there has been little research evaluating the associative factors that may also contribute to AOP release and the expression of opiate tolerance.

Inasmuch as opiate tolerance is mediated by associative mechanisms, if CCK-8 may function as an AOP, we would expect that CCK-8 activity would be important to the expression of CCRs and established opiate tolerance. In the present study, we employed conditions that we have previously demonstrated to promote the formation of an intra-administration association and maximize the associative contribution to tolerance (Kim et al., 1999). We evaluated the hypothesis that intrathecal pretreatment with the CCK2 receptor antagonist PD-135, 158 would: (1) block the expression of the drug-onset cue-elicited conditional compensatory response (hyperalgesia) and, (2) block the expression of established morphine tolerance.

EXPERIMENT 1

The purpose of Experiment 1 was to evaluate the role of CCK-8 in the conditional compensatory responding mechanism hypothesized to mediate morphine tolerance (see review by Seigel et al., in press). When expressed, this CCR opposes the analgesic effect of morphine and contributes to tolerance. While the CCR normally is masked by the administered drug, there are techniques that have been developed to directly observe the CCR (Kim et al., 1999).

In the present experiment, rats were infused daily with intravenous morphine (Mor) or saline (Sal) for 8 days. On the subsequent CCR Test, all rats were challenged with a pMor infusion. Fifteen min before this pMor injection, approximately half of the rats in each pretest condition (Mor and Sal) were intrathecally injected with vehicle (Mor-VEH and Sal-VEH), and approximately half were intrathecally injected with the CCK2 receptor antagonist PD 135,158 (Mor-PD and Sal-PD). Suh et al., (1995) have previously demonstrated that this antagonist is effective in preventing CCK-8 activity from attenuating opioid effects. If CCK-8 activity is important to the expression of the CCR, we would expect that pretreatment with the antagonist would block the hyperalgesic response.

Method

Subjects and Surgical Preparation

The subjects were 41 experimentally-naive male Sprague-Dawley rats (Rattus norvegicus, purchased from Charles River Laboratories, St. Constant, Quebec, Canada), weighing between 300-350 g at the start of the experiment. They were maintained in individual cages with ad lib food and water. Rats were handled daily for one week prior to surgery.

Surgery

Intravenous catheters were implanted in the right jugular vein under sodium pentobarbital anesthesia, using a modified version of the technique of Brown and

Breckenridge (1975). The tip of the catheter was located approximately 1 cm from the heart. The catheter was constructed from a 9.5 cm length of silastic tubing (Dow Corning, .51-mm inner diameter, .94-mm outer diameter). Each rat's catheter was flushed with a solution of heparin and antibiotic (65 µg sodium heparin and 1.25 mg ampicillin) once daily for a 7-10 day recovery period following surgery.

Drugs

The dose of morphine (Allen and Hansbury's, Toronto, Ontario, Canada) used was 5 mg/kg and was prepared as a 5 mg/ml solution in physiological saline and delivered intravenously in a volume of 1 ml/kg. The CCK2 receptor antagonist PD-135, 158 [N-methyl-D-glucamine, IC_{50} = 2.8 nM (CCK2), 1233 nM (CCK1), (Hughes et al., 1990)] purchased from RBI (Natick, Massachusetts), was prepared as a 10 ng/µl solution in physiological saline and delivered intrathecally in a volume of 10 µl (100 ng) using a 25-5/8 gauge needle attached to a 50µl Hamilton syringe.

Apparatus, Infusion Parameters, and Analgesia Assessment

Infusions took place in one of five, identical, chambers (30.4 cm long X 20.5 wide X 19 cm tall, Lehigh Valley Electronics). Each chamber, which was constructed of clear Plexiglas with a grid floor, was located in a vented, sound-attenuating cubicle, and was fitted with a hydraulically-sealed swivel (see Brown, Amit, & Weeks, 1976), which permitted rats free movement while attached to the infusion apparatus. The rat's cannula was connected to the swivel by a spring-connected cannula connector, and the swivels were connected to a 5-cc syringe by flexible tubing (Tygon, size 13, No. 6409). The tubing between the syringe and the swivel was fitted with an in-line, .025 micrometer micropore filter (Sartorium). The syringe was held in a variable rate syringe pump (Sage Model 341A).

All solutions were infused through the iv cannulae by timed operation of the syringe pump. During the tolerance acquisition phase, morphine was gradually administered (0.0167 ml/min) as a long infusion (LMor). The exact duration of the infusion depended on the rat's weight, but was of the appropriate length (approximately 30 min) to administer a dose of 5 mg/kg morphine. Saline infusions (LSal) were volumetrically equated with morphine infusions and infused at the same rate as the morphine infusions. The probe-morphine infusion (pMor) consisted of a 3-min infusion at the Long infusion rate (.01667 ml/min) which corresponded to approximately 0.5 mg/kg of morphine. We have previously demonstrated that protracted pharmacological stimulation (i.e., LMor injections) maximizes the association between drug-onset cues and the drug effect; moreover, in LMor infusion-experienced rats, the pMor injection reliably mimics drug-onset cues that elicits a CCR of hyperalgesia (Kim et al., 1999).

Analgesia was measured with the tail-flick procedure (Fennessy & Lee, 1975). The tail of a lightly-restrained rat was immersed 5 cm into a warm water bath maintained at 50 deg C. The latency for the rat to lift its tail out of the water (tail flick latency [TFL]) was noted.

<u>Procedure</u>

<u>Tolerance acquisition.</u> The tolerance acquisition phase of the experiment started 7-10 days after catheter implantation. For eight days, rats were intravenously infused with morphine (n = 22) or saline (n = 19). On each tolerance acquisition trial, a rat was placed in the chamber, its cannula was flushed with heparinized saline, and connected to

the syringe pump. Following a 15 min habituation period, the infusion started. Following completion of the infusion, the cannula connector was disconnected from the rat, the cannula was filled with a dextrose solution, and the rat was returned to its chamber. Thirty min later, the rat was assessed for analgesia using the TFL in a warm water bath maintained at 50 deg C and then returned to its home cage.

CCR test. One day following the last tolerance acquisition block, the rat's lower back was shaved. On the CCR Test, all rats were infused with the pMor injection. 15 min prior to this probe injection, half of the morphine-trained rats were pretreated with PD-135, 158 (Mor-PD, n = 11), and half were treated with vehicle (Mor-VEH, n = 11). Similarly, approximately half of the saline-trained rats were pretreated with PD-135, 158 (Sal-PD, n = 10), and half were pretreated with vehicle (Sal-VEH, n = 9). All pretreatment administrations were direct transcutaneous intrathecal injections into the intervertebral space between L5 and L6, using the method described by Mestre, Pelissier, Fialip, Wilcox, and Eschalier (1994). Following the pMor infusion, rats were assessed for analgesia at 5,15, 30, 45, and 75-min post infusion. Hyperalgesia would be manifest as a decreased tail-flick latency, thus the temperature of the water bath on this test was adjusted to 48-deg C (in contrast with the 50-deg C temperature used to measure analgesia). This decrease in temperature increased the likelihood of observing the increased sensitivity to the thermal stimulation (see Kim et al., 1999).

Results and Discussion

Tolerance acquisition. The TFL scores were collapsed into blocks of two sessions. The mean TFL scores (±1 SEM) for each group for each of the four tolerance acquisition blocks are shown in Figure 1. The analgesic effect of morphine was apparent. Rats administered the opiate 30 min before assessment had longer TFL scores than rats administered saline. Moreover, tolerance developed; the analgesic effect of morphine decreased over blocks.

These observations were confirmed by statistical analysis. A mixed design ANOVA of the data summarized in Figure 1 indicated a significant Group effect, $F(3, 38) = 18.39$, $p < .001$, a significant Block effect, $F(3, 114) = 23.41$, $p < .001$, and a significant Group X Block interaction, $F(9, 114) = 9.65$, $p < .001$. Within-block, between group post-hoc comparisons (LSD) revealed the source of the interaction. Within each block, morphine-treated groups (Mor-PD and Mor-VEH) had significantly higher scores than did saline-treated groups [Sal-PD and Sal-VEH (all ps < .03)]. Rats injected with the same substance behaved similarly. As expected, prior to differential treatments, neither the scores between Mor-PD rats and Mor-VEH rats, nor Sal-PD and Sal-VEH rats were significantly different on the first or final tolerance acquisition blocks (all ps > .85).

The analgesic effect of morphine decreased across blocks. This was confirmed by within-group post-hoc comparisons (LSD) across blocks. For both Mor-PD and Mor-VEH groups, TFL scores significantly decreased from Block 1 to Block 4 (both ps < .001). In contrast, scores for saline-treated groups remained stable. For both Sal-PD and Sal-VEH groups, TFL scores were not significantly different from Block 1 to Block 4.

<u>CCR test.</u> The mean TFLs (±1 SEM) at various times following the pMor infusion for all groups are presented in Figure 2. As is apparent in Figure 2, rats in Group Mor-VEH displayed shorter response latencies than did rats in the other groups (there being similar response latencies displayed in the other three groups).

A mixed design ANOVA of the data summarized in Figure 2 indicated a significant Group effect $F(3, 37) = 4.3$, $p < .01$. Pairwise comparisons (LSD) indicated that group Mor-VEH rats displayed significantly shorter response latencies than did rats in any other group (all ps < .03). Differences among all other groups were not significant (all ps > .31). That is, in morphine-trained rats, the antagonist pretreatment was effective in completely blocking the expression of the CCR (hyperalgesia) such that Mor-PD rats performed no differently than saline-trained rats (Sal-PD and Sal-VEH).

In summary, morphine-naïve rats responded similarly to the pMor infusion, regardless of the pretreatment drug (antagonist or vehicle). As expected, morphine-experienced rats exposed to drug-onset cues displayed a conditional compensatory response of hyperalgesia when pretreated with vehicle; however, pretreatment with the antagonist was effective in blocking this CCR.

EXPERIMENT 2

The purpose of Experiment 2 was to examine whether the treatment that was effective in blocking a conditional compensatory response of hyperalgesia in morphine-experienced rats (Experiment 1), was also effective in blocking the expression of established morphine tolerance. As in Experiment 1, during the 8 day tolerance acquisition phase, one group of rats was intravenously infused daily with 5 mg/kg of morphine presented as a LMor infusion. Controls were infused with an equivalent volume of saline presented as a LSal infusion. On the Tolerance Test, all rats were injected with morphine (5 mg/kg), preceded 15 minutes earlier by intrathecal PD-135,158 (Mor-PD and Sal-PD) or vehicle (Mor-VEH and Sal-VEH).

Method

Subjects, Surgical Preparation, Drugs, and Apparatus

The subjects were 26 experimentally-naive rats of the same age and strain as those used in the previous experiment. They were housed as described previously. All subjects were prepared with chronic iv cannulae, using procedures described previously. Morphine sulfate was prepared and delivered as a LMor infusion, as previously described. PD 135, 158 was prepared and delivered as previously described. All infusions were conducted in the same apparatus as that used in Experiment 1.

Procedure

Tolerance acquisition. The tolerance acquisition phase was conducted in the same manner as described in Experiment 1. For eight days, rats were intravenously infused with LMor (n = 14) or LSal (n = 12) injections, once daily. On each tolerance development trial, a rat was placed in the chamber, its cannula was flushed with heparinized saline, and connected to the syringe pump. Following a 15 min habituation period, the infusion started. Following completion of the infusion (approximately 30 min), the cannula connector was disconnected from the rat, the cannula was filled with a dextrose solution, and the rat was returned to its chamber. Thirty min later, the rat was assessed for analgesia (TFL, using 50-deg C warm water bath) and then returned to its home cage.

Tolerance test. One day following the last tolerance acquisition session, the rat's lower back was shaved. On the Tolerance Test, all rats received a LMor infusion. 15 min prior to this opiate injection, half of the morphine-trained rats were pretreated with PD-135, 158 (Mor-PD, n = 7), and half were pretreated with vehicle (Mor-VEH, n = 7).

Similarly, half of the saline-trained rats were pretreated with PD-135, 158 (Sal-PD, n = 6), and half were pretreated with vehicle (Sal-VEH, n = 6). As in Experiment 1, all pretreatment administrations were direct transcutaneous intrathecal injections into the intervertebral space between L5 and L6, using the method described by Mestre et al., (1994). Following the morphine infusion, rats were assessed for analgesia at 15, 30, 45, and 75-min post-morphine infusion.

Results and Discussion

Tolerance acquisition. As in Experiment 1, TFL scores were collapsed across blocks of two sessions. The mean TFL scores (±1 SEM) for each group for each of the four blocks of tolerance acquisition are shown in Figure 3. The analgesic effect of morphine was apparent. Rats administered the opiate 30 min before assessment had greater TFL scores than rats administered saline. Moreover, tolerance developed; the analgesic effect of morphine decreased over blocks.

These observations were confirmed by statistical analysis. A mixed design ANOVA of the data summarized in Figure 3 indicated a significant Group effect, $F(3, 24) = 25.37$, $p < .001$, a significant Block effect, $F(3, 72) = 48.77$, $p < .001$, and a significant Group X Block interaction, $F(9, 72) = 19.24$, $p < .001$. Within-block, between group, post-hoc comparisons (LSD) revealed the source of the interaction.

As would be expected, groups injected with the same substance (Mor or Sal) behaved similarly. Although Mor-PD rats displayed significantly higher scores than Mor-VEH rats on block 1 ($p < .04$), scores between the two morphine groups were not significantly different on the final block, or any other block (all ps > .07). Saline-treated groups did not differ from each other within any block (all ps > .47).

Within Blocks 1-3, responding between morphine- and saline-trained groups differed, with morphine-treated groups (Mor-PD and Mor-VEH) responding with significantly higher scores than saline-treated groups (all ps $< .004$). However, as is apparent from Figure 3, all groups responded at about the same level by Block 4. On this last block, although Mor-PD rats had TFL scores that were still significantly different from saline-treated rats (all ps$> .02$) and Mor-VEH rats still had significantly higher scores than Sal-PD rats ($p< .03$), Mor-VEH rats were not significantly different from Sal-VEH rats.

The analgesic effect of morphine decreased across blocks. This was confirmed by within-group post-hoc comparisons (LSD) across blocks. For both Mor-PD and Mor-VEH groups, TFL scores significantly decreased from Block 1 to Block 4 (all ps $< .001$). In contrast, scores for saline-treated groups remained stable. For both Sal-PD and Sal-VEH groups, TFL scores were not significantly different from Block 1 to Block 4.

<u>Tolerance test.</u> On this test, all rats were infused with morphine. The mean TFL scores ($\pm$1 SEM) for each group for each post-infusion interval is presented in Figure 4.

The data summarized in Figure 4 was subjected to a mixed design, factorial ANOVA, followed by LSD tests. As is apparent in Figure 4, both groups that received morphine for the first time (Sal-PD and Sal-VEH) displayed higher TFL scores compared to groups with morphine experience (Mor-PD and Mor-VEH). This was confirmed by the significant effect of the Tolerance-Acquisition Drug (Mor or Sal), $F(1, 24) = 30.43$, $p < .001$. The substance intrathecally injected 15 min prior to morphine, minimally affected saline-trained rats. That is, although there was a slight trend for Sal-PD rats to display greater levels of analgesia than Sal-VEH rats, the differences in saline-trained rats was not statistically significant. Analysis of Groups Sal-PD and Sal-VEH indicated no significant effect of Pretreatment Drug (PD or VEH), $F(1, 10) < 1$, or Pretreatment Drug X Time interval interaction, $F(3, 30) < 1$. In contrast, rats in morphine-trained groups responded differently on this test, depending on the intrathecal pretreatment drug: Mor-PD rats displayed less tolerance (i.e., longer TFLs) than Mor-VEH rats. Analysis of morphine-trained rats indicated a significant effect of Pretreatment Drug (Mor-PD vs. Mor-VEH) $F(1, 12) = 16.68$, $p < .002$.

In common with other studies utilizing various CCK2 antagonists (e.g., Hoffmann & Wiesenfeld-Hallin, 1994) the results of Experiment 2 suggest that CCK-8 activity contributes to the expression of established morphine tolerance. However, as is apparent from Figure 4, the loss of tolerance demonstrated by Mor-PD rats was not complete. That is, although Mor-PD rats were less tolerant than Mor-VEH rats, they nevertheless responded with shorter response latencies than did saline-trained rats receiving morphine for the first time on this test. Indeed, the differences in TFLs between Mor-PD rats and rats in Groups Sal-PD and Sal-VEH were significant (all ps < .02, LSD test). Thus, the expression of opiate tolerance, as attenuated by the pretreatment with PD 135,158 (100 ng) is incomplete. Alternatively, the presence of other AOPs (e.g., neuropeptide FF) that are unaffected by PD-135, 158 treatment, may account for some degree of the expressed opiate tolerance.

In summary, just as CCK-8 activity contributes to the expression of the CCR that putatively mediates associative mechanisms of opiate tolerance (Experiment 1), the results of Experiment 2 suggest that CCK-8 activity is also important for the expression of established opiate tolerance.

General Discussion

The results of the present experiments suggest a role for CCK-8 in associative mechanisms of opiate tolerance. In Experiment 1, we demonstrated that CCK2 antagonist pretreatment was effective in attenuating the expression of the CCR hypothesized to mediate morphine tolerance. In Experiment 2, we demonstrated that the same CCK2 antagonist pretreatment was also effective in attenuating the expression of established morphine tolerance. Several investigators have suggested that the spinal cord may be an important site in which CCK-8 plays a modulatory role on opioid effects (e.g., Faris, Komisaruk, Watkins, & Mayer, 1983; Watkins, Kinscheck, & Mayer, 1984; Kellstein & Mayer, 1991; Stanfa, Dickenson, Xu, & Wiesenfeld-Hallin, 1994). Since drug diffusion following a direct transcutaneous intrathecal injection is thought to be limited (Mestre et al., 1994), and the dose employed (100 ng) in the present experiments is likely to be below the minimum effective peripheral dose (see Hoffmann & Weisenfeld-Hallin, 1994), our results suggest that the spinal cord is an important site for the anti-opioid effects of CCK-8.

Our results extend previous research implicating CCK-8 as an AOP. For example, treatment with various CCK2 antagonists attenuates opiate tolerance (e.g., Kellstein & Mayer, 1991; Hoffmann & Weisenfeld-Hallin, 1994; Experiment 2 of the present report). Additionally, the present findings suggest that associative factors may importantly contribute to the expression of morphine tolerance via the conditional release of CCK-8 in response to cues signaling the opiate. Our findings may also have clinical implications for the use of opiates to control pain and the treatment of opiate addiction.

Although the area is controversial (see Onofrio & Yaksh, 1990; Portenoy &

Savage, 1997), there is evidence that some patients who receive opiates for pain control develop tolerance to the analgesic effect of the drug (e.g., Foley, 1991; Johnson & Faull, 1997; Siegel & Kim, 2000). Inasmuch as opiate tolerance is mediated by conditional CCK-8 release, an effective method for reinstating opiate efficacy (in lieu of escalating the opiate dose and risking potential side effects) may be co-treatment with a CCK-receptor antagonist. This prediction has recently been confirmed in tests with humans (McCleane, 1998).

In the present report we have demonstrated that drug-onset cues serve to trigger neurochemical events that compensate for the drug effect (i.e., the conditional release of CCK-8 or other AOPs). Normally, these CCRs attenuate the drug effect. However, when drug-onset cues are presented in the absence of the drug effect, the conditional release of AOPs, now having little to compensate for, manifests as hyperalgesia. This unopposed hyperalgesia may be evidence of opiate withdrawal. Such withdrawal experienced by the addict who is confronted with drug-associated cues may lead to further drug self-administration to self-medicate dysphoria resulting from excess AOP activity (Rothman et al., 1991). In such a case, as suggested by Rothman (1992), a useful adjunct to traditional treatments for these patients may include an AOP antagonist.

Conclusion

The present results are of interest to both learning theorists and psychopharmacologists who have worked to further our understanding of opiate tolerance. Many investigators have demonstrated the role of CCRs and associative processes in opiate tolerance (e.g., Grisel et al., 1994; Kim et al., 1999; Raffa & Porreca, 1986; see review by Siegel et al., in press). Others have worked to characterize the molecular-biological events which allow CCK-8 to function as an AOP (e.g., Zhou et al., 1992; Gustafsson et al., 1999; see review by Wiesenfeld-Hallin et al., 1999). However, little is known with respect to how learning may influence AOP activity; that is, with few exceptions (e.g., Wiertelak et al., 1992; Grisel et al., 1996; Mitchell et al., 2000), little is known about how associative factors may influence AOP release and the expression of opiate tolerance. In the present experiments, treatment with a CCK2 antagonist was effective in attenuating both the expression of the CCR hypothesized to mediate morphine tolerance, and the expression of established morphine tolerance.

In summary, endogenous AOPs such as CCK-8 may be released in response to, but also in anticipation of the opiate. That is, opiate tolerance is mediated both by unconditional and conditional compensatory responses.

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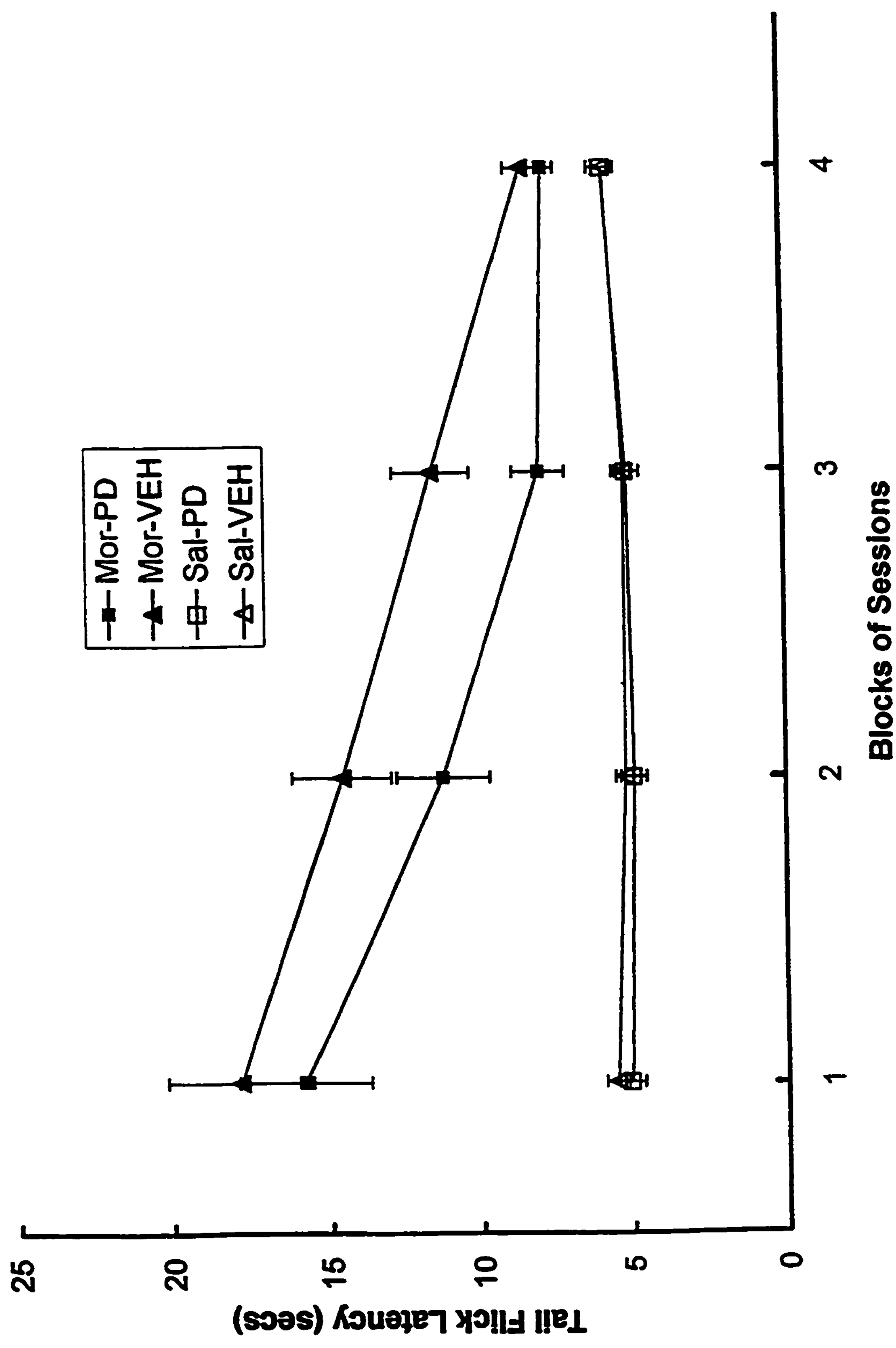
Figure Captions

Figure 1. Mean tail flick latencies (±1 SEM) observed during the tolerance acquisition phase of Experiment 1. Rats were administered 5 mg/kg morphine (groups Mor-PD and Mor-VEH), or physiological saline (groups Sal-PD and Sal-VEH) as a long infusion. Tail flick latencies were determined 30-min following the long infusion.

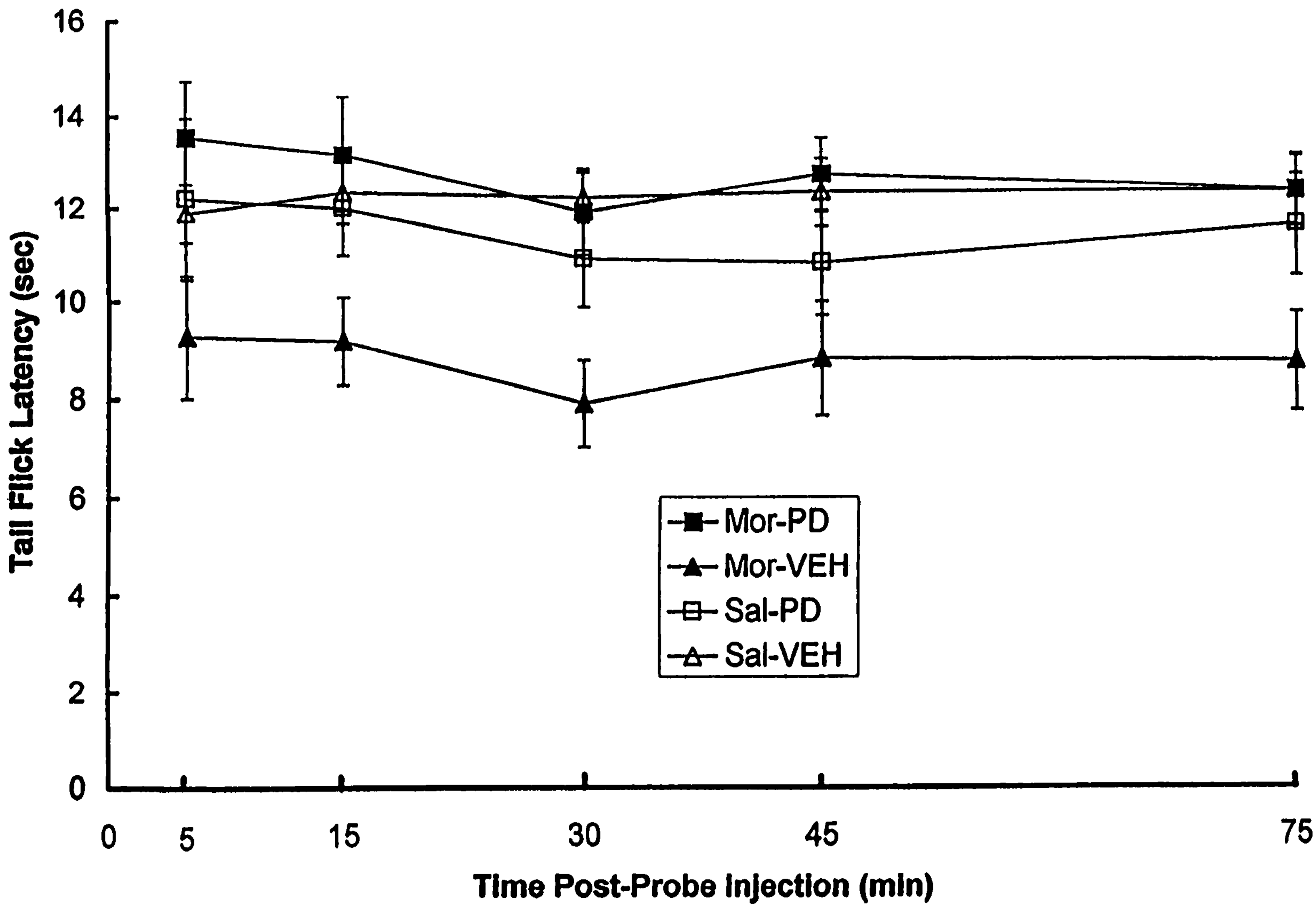
Figure 2. Mean tail-flick latencies (±1 SEM) observed during the CCR Test of Experiment 1. All rats were infused with the pMor infusion. 15 min prior to the pMor infusion, approximately half of the rats were pretreated with PD-135,158 (groups Mor-PD and Sal-PD), and approximately half of the rats were pretreated with vehicle (groups Mor-VEH and Sal-VEH). Tail flick latencies were determined at 5-, 15-, 30-, 45-, and 75-min following the pMor infusion.

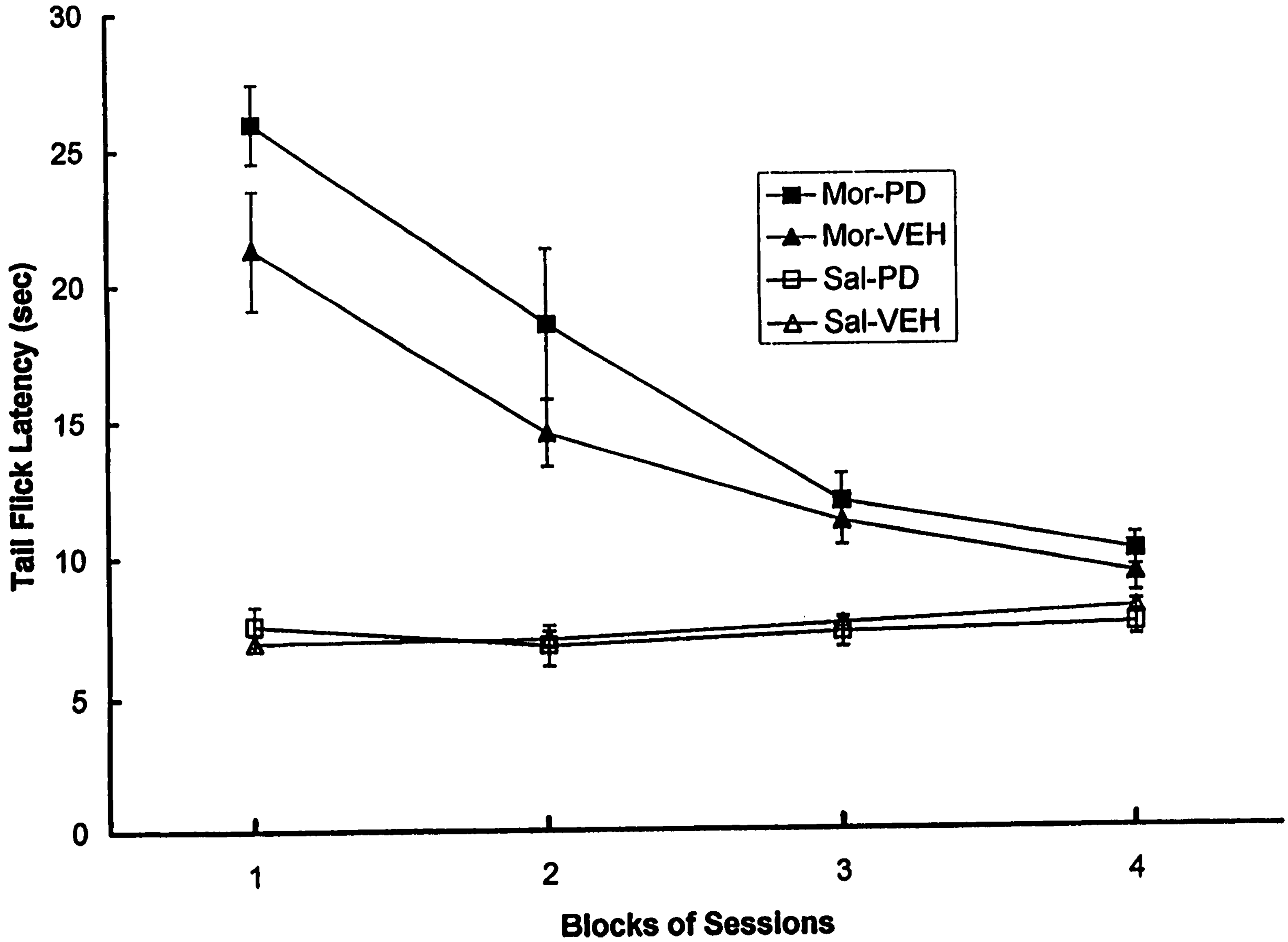
Figure 3. Mean tail flick latencies (±1 SEM) observed during the tolerance acquisition phase of Experiment 2. As in Experiment 1, rats were administered 5 mg/kg morphine (groups Mor-PD and Mor-VEH), or physiological saline (groups Sal-PD and Sal-VEH) as a long infusion. Tail flick latencies were determined 30-min following the long infusion.

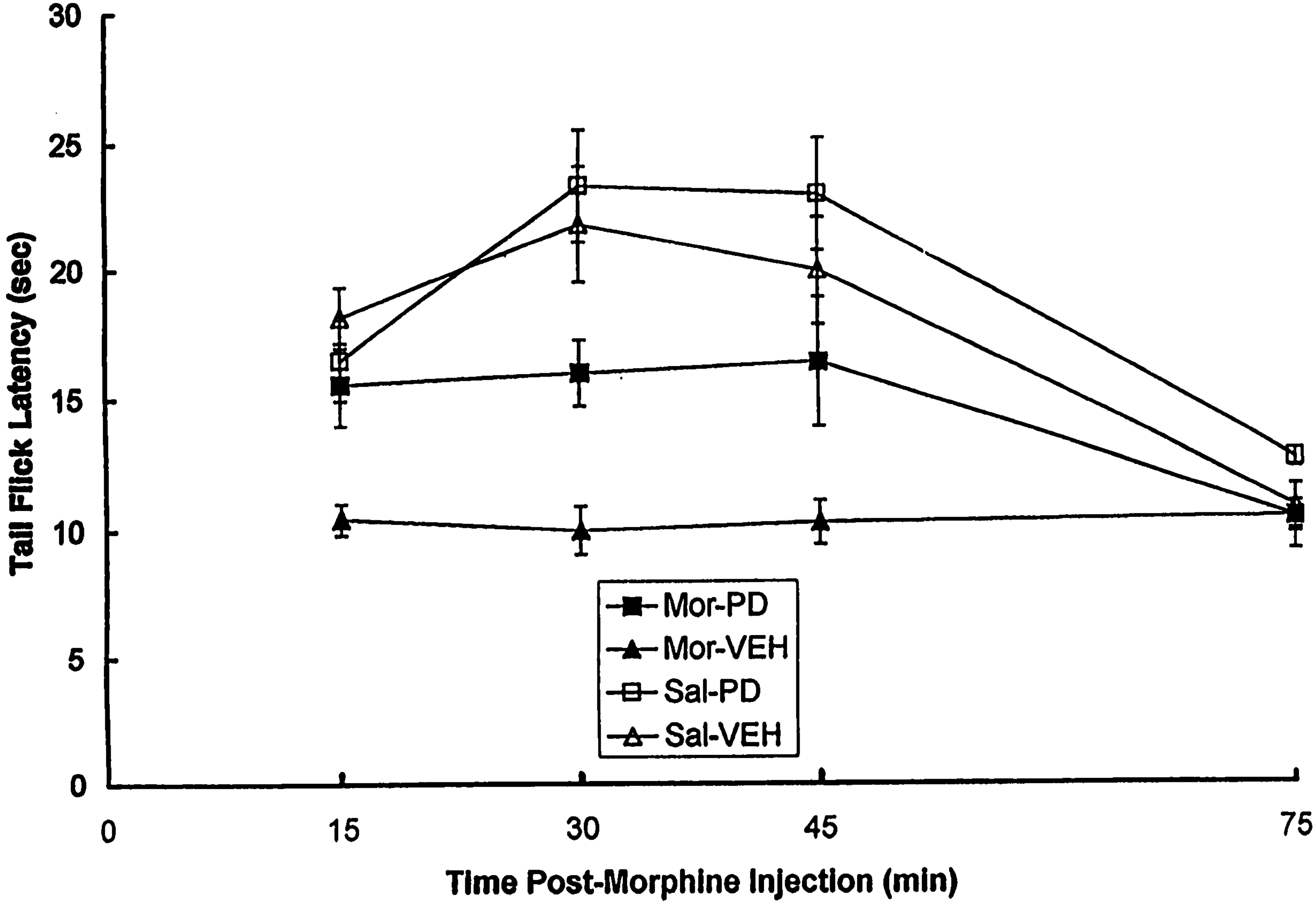
Figure 4. Mean tail-flick latencies (±1 SEM) observed during the Tolerance Test of Experiment 2. All rats were infused with the LMor infusion. 15 min prior to the LMor infusion, half of the rats were pretreated with PD-135,158 (groups Mor-PD and Sal-PD), and half of the rats were pretreated with vehicle (groups Mor-VEH and Sal-VEH). Tail flick latencies were determined at 15-, 30-, 45-, and 75-min following the LMor infusion.



Tail Flick Latency (secs)
0
5
10
15
20
25
Blocks of Sessions
1
2
3
4
Mor-PD
Mor-VEH
Sal-PD
Sal-VEH


16
14
12
10
8
6
4
2
0
Tail Flick Latency (sec)
0
5
15
30
45
75
Time Post-Probe Injection (min)
Mor-PD
Mor-VEH
Sal-PD
Sal-VEH


Tail Flick Latency (sec)
0
5
10
15
20
25
30
Mor-PD
Mor-VEH
Sal-PD
Sal-VEH
1
2
3
4
Blocks of Sessions


30
25
20
15
10
5
0
Tail Flick Latency (sec)
0
15
30
45
75
Time Post-Morphine Injection (min)
Mor-PD
Mor-VEH
Sal-PD
Sal-VEH

CHAPTER 4: INTRODUCTION

The results of experiments presented in Chapter 2 suggest that in addition to external environmental cues that may be explicitly paired with the drug effect, internal pharmacological cues (inherent to drug administration) may also become associated with the drug effect and control the display of tolerance. As elaborated in Chapter 3, such drug-onset cues are important because they serve to trigger neurochemical events that compensate for the drug effect (i.e., the conditional release of cholecystokinin or other anti-opioid peptides). Tolerance results because drug effects are countered by drug-compensatory responses elicited not only by the drug effect, but also by cues, that in the past, have been associated with the drug effect. However, when drug effects are presented in the absence of the usual pre-drug cues, the drug effect (unopposed by a conditional compensatory response) may have unusually greater effects than when presented in the usual context of drug administration. That is, in the absence of pre-drug cues, the drug effect is only countered by unconditional responses to the drug itself and not by conditional compensatory responses. This may leave the subject at risk for drug overdose. In the following case report, we discuss how an enigmatic case of opiate overdose may be understood by appreciating the importance of pharmacological drug-onset cues that are particular to the usual route of administration of a drug. Altering the route of administration of a drug is akin to altering the pharmacological drug-onset cues, and the result is an increased risk of opiate overdose.

CHAPTER 4

Siegel, S., & Kim, J.A. (2000). Absence of Cross-Tolerance and the Situational-Specificity of Tolerance. Palliative Medicine, 14, 75-77.

Absence of Cross-Tolerance and the Situational-Specificity of Tolerance

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key words: analgesic tolerance; cross-tolerance; fentanyl; morphine; overdose

Absence of Cross-Tolerance and the Situational-Specificity of Tolerance

Johnson and Faull[1] described the case of a patient who demonstrated tolerance to morphine but no cross-tolerance to fentanyl. The patient had been treated for pain with a regimen that included oral morphine for about 3 months; however, tolerance to the analgesic effect of the drug developed (he was receiving about 800 mg oral morphine/day) and the patients' condition (advanced carcinoma in the mouth) made swallowing difficult. The patient opted to change to transdermal fentanyl, and cross-tolerance was expected.

Fortuitously, the morphine-fentanyl conversion dose was incorrectly calculated and the patient received one-quarter of the manufacturer's recommended conversion dose. Even with this lesser dose, the patient showed evidence of opioid overdose. The authors concluded that, despite the patient's tolerance to oral morphine, there was apparently no cross-tolerance to fentanyl: "If this man had received the 'correct' dose [of fentanyl] as calculated from the manufacturer's data sheet he would have experienced severe toxicity."

We suggest that this disruption of tolerance seen following a change in route of administration is a further demonstration of the situational-specificity of tolerance. The phenomenon, first characterized in over a dozen experiments reported by Clifford Mitchell and colleagues between 1969 and 1973 (reviewed elsewhere[2]), demonstrated that morphine tolerance is modulated not only by experience with morphine, but also by experience with drug-paired stimuli; following a series of drug administrations, tolerance is more pronounced in the presence of the usual drug-associated cues than in the presence of alternative cues.[3] Situational-specificity of tolerance has been the subject of extensive empirical and theoretical analyses.[4,5,6] It has been seen with respect to tolerance to a variety of effects of opiates and other drugs, and is manifest both by behavioral and neurochemical measures of drug effects.[7] Although most investigations have used rodent models, situational-specificity of tolerance has been demonstrated in many other species (from snails[8] to humans[9,10,11,12,13]). Of special relevance to Johnson and Faull's observation is a report that a patient being treated with injected morphine for pain of pancreatic cancer suffered a lethal overdose when he received the usual morphine dose, but in a novel environment where he had never before been injected with morphine.[14]

Among the stimuli that comprise the "drug-associated cues" are those cues inherent within the administration procedure (such as route of administration and early, weak drug effects experienced immediately after administration). There are several experimental demonstrations of the role of such interoceptive stimuli in the display of tolerance to opiates.[15,16,17] Additionally, Mucha *et al.*[18] recently reported that alteration of the route of administration of morphine in rats (from intraperitoneal to intravenous) attenuated the display of analgesic tolerance, and they suggested that "interoceptive stimuli produced by morphine acting through a particular route" functions similarly to exteroceptive stimuli in modulating the display of tolerance. It is well-established that the display of cross-tolerance, like the display of tolerance, demonstrates situational-specificity.[19,20] Thus, Johnson and Faull's observations concerning a failure of cross-tolerance to occur between two μ-opioid receptor agonists in conjunction with an alteration in administration procedure may represent another demonstration of the situational-specificity of tolerance. The phenomenon has been implicated in unexpected overdose deaths resulting from opiates,[21] alcohol,[22] and pentobarbital,[23] and may also (as

Johnson and Faull's observations suggest) be relevant to understanding and preventing enigmatic overdoses in clinical practice.

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CHAPTER 5: DISCUSSION

An impressive body of research, conducted over a period of about 25 years, across many different laboratories, using a variety of species and drugs, indicates that Pavlovian conditioning plays an important role in the acquisition and expression of drug tolerance. For example, there are many demonstrations that, following a series of drug administrations, drug-paired stimuli elicit CCRs. Furthermore, tolerance often is situationally-specific; that is, tolerance is more pronounced when assessed in the presence of drug-paired cues than when assessed in the presence of alternative cues. In addition, there are many parallels between tolerance and other conditional responses: Non-pharmacological manipulations that attenuate conditioning (e.g., extinction procedures) similarly modulate the acquisition of tolerance.

Since its initial description (Siegel, 1975) the conditioning analysis of tolerance has undergone several important modifications, primarily as a result of critical analyses of pharmacological conditioning by several authors (Dworkin, 1993; Eikelboom & Stewart, 1982; Poulos & Cappell, 1991; Ramsay & Woods, 1997; Wikler, 1973). Additionally, the results of the present experiments, and those by others (e.g., Grisel at al., 1996) require that we add complexity to the model by recognizing that there are a variety of potential pre-drug stimuli. That is, many types of cues present at the time of drug administration may come to elicit the CCRs that contribute to tolerance. Since the 1960s, learning theorists have been concerned with cue interactions in compound conditioning (i.e., conditioning that occurs when a variety of potential CSs signal a US).

It now is apparent that the compound-conditioning effects studied by learning researchers are relevant to understanding drug tolerance. Effective CSs may be public (e.g., exteroceptive cues of the environment of drug administration such as distinctive audio/visual cues) or private (e.g., interoceptive cues incidental to drug administration such as pharmacological drug-onset cues), and several CSs may be simultaneously present at the time the drug effect occurs.

Intra-administration Associations and Conflicting Findings

Although there are many findings that have supported the model, there are apparently conflicting findings. For example, some investigators have reported that sometimes tolerance is not situationally-specific, and sometimes a CCR is not apparent (especially a hyperalgesic CCR following a series of morphine injections) when the usual pre-drug cues are presented without the drug (e.g., Goudie, 1990; Kesner & Cook, 1983; Sherman, 1979; Tiffany, 1995). The exceptions have been characterized as "clearly embarrassing for Siegel's account of tolerance" (Goudie & Griffiths, 1986, p. 193), and indicate "that compensatory responses are not integral components of associational tolerance phenomena" (Baker & Tiffany, 1985, p. 95). Some investigators have concluded that there are two types of tolerance that may result from chronic drug administration: associative and pharmacological (see review by Grisel et al., 1994). The former is dependent on the availability of drug-associated cues (and thus is situationally-specific), but the latter is nonassociative (and thus is trans-situational).

However, as suggested by Walter and Riccio (1983), trans-situational tolerance, rather than having a fundamentally different mechanism than tolerance attributable to conditioning, may be due to the relatively greater effectiveness of the interoceptive-pharmacological component of the predrug compound CS. Such differential effectiveness may arise because of procedural features of the drug administration procedure that promote overshadowing:

> "Tolerance controlled by internal, morphine-produced stimuli, unlike that mediated by environmental stimuli, would be expected to be relatively trans-situational or 'pharmacological' in nature, even though the same underlying conditioning mechanisms would be involved. The question then becomes one of establishing the extent to which tolerance, in any given case, is controlled by one or the other of these two general classes of stimuli, rather than one of making a distinction between two different 'kinds' of tolerance" (Walter & Riccio, 1983, p. 661).

If a particular administration procedure promotes such an intra-administration association, it might be expected that the pharmacological cue would be especially effective. Unlike typical exteroceptive CSs (which likely generalize to stimuli encountered outside the conditioning situation), this putative interoceptive CS is both novel and presented in a perfectly positively contingent manner with the subsequent drug effect. Also, there is evidence that CSs that are physically similar to the USs with which they are paired are especially salient (see review by Mackintosh, 1983, pp. 213-214), and the CS and US that are paired to form an intra-administration association are very similar indeed. This very effective pharmacological cue, then, may overshadow (Kamin, 1969; Pavlov, 1927, pp. 142-143 and 269-270) simultaneously presented environmental cues. In such circumstances, the display of tolerance would not be influenced by environmental cues, and thus the tolerance would appear trans-situational, or nonassociative (e.g., Grisel et al., 1994; Greeley et al., 1984; King et al., 1987; Mackintosh, 1987; Walter & Riccio, 1983).

The results of experiments reported in Chapter 2 (Kim et al., 1999) provided direct evidence supporting the overshadowing interpretation of trans-situational tolerance. We demonstrated that rats that acquired tolerance with Long Morphine infusions (a condition that promotes the development of an intra-administration association) demonstrated trans-situational tolerance; that is, they were equally tolerant in the presence of drug-paired and in the presence of non-drug paired environmental cues. In contrast, rats that acquired similar tolerance but with Short-Morphine infusions (a condition that does not promote the development of an intra-administration association) demonstrated situationally-specific tolerance; they were more tolerant in the presence of drug-paired than non-drug paired environmental cues. Similarly, Grisel et al. (1994) demonstrated that there is greater situational-specificity of tolerance induced with IV morphine administrations than with subcutaneous (SC) administrations. They reasoned that the relatively more gradual onset of the SC opiate effect (compared with the IV opiate effect) resulted in an association between drug-onset cues and the later, larger drug effect, and these pharmacological cues overshadowed simultaneously-present environmental cues.

Intra-Administration Associations and the Physiological Mechanisms of Tolerance

There are relatively few studies that have investigated the physiological mechanisms of the associative contribution to tolerance. Cues that signal drug delivery not only modulate the expression of tolerance, but also modulate the activity of neurotransmitter systems that mediate tolerance. The results of experiments reported in Chapter 3 (Kim & Siegel, to be submitted) suggest that one putative anti-opioid peptide – cholecystokinin – is important to the expression of morphine tolerance. Moreover, the conditional alteration of cholecystokinin neurotransmitter activity may provide a mechanism for the CCRs that mediate the behavioral expression of opiate tolerance.

Clinical Implications

Even at this early stage of research concerning intra-administration CSs, it is clear that such private cues should be of considerable interest to psychopharmacologists and clinicians. As discussed in Chapter 4, patients receiving drugs for pain relief may be at risk of overdose when the route of administration is changed, thus effectively altering the pharmacological drug-onset CS (Kim et al., 1999; Siegel & Kim, 2000). Such intra-administration associations may also contribute to the effect of a priming dose on relapse to drug use. For example, it frequently has been reported that a small dose of alcohol will augment the craving for additional alcohol and enhance subsequent alcohol consumption (see Goddard, 1999; Siegel, 1986a). This loss of control initiated by a priming dose is incorporated in the dogma of Alcoholics Anonymous: "... once he takes any alcohol into his system, something happens, both in the bodily and mental sense, which makes it virtually impossible for him to stop. The experience of any alcoholic will confirm that ... We are without defense against the first drink" (Anonymous, 1939, pp. 34-35). The insalubrious effect of the first drink may be due to the alcoholic's association of that initial effect of alcohol with subsequent larger amounts of the drug: "the signal value of a small drug dose may make a contribution to 'binge' drinking and drug 'priming' effects in humans" (Goddard, 1999, p. 418).

Similarly, a small dose of an opiate may augment additional opiate administration. As discussed in Chapter 3, a small dose of the opiate may elicit the release of endogenous anti-opioid peptides in anticipation of a subsequent larger drug dose. This unopposed anti-opioid peptide activity may contribute to the expression of withdrawal symptoms (e.g., hyperalgesia), leading to further drug self-administration to self-medicate dysphoria resulting from the excess anti-opioid peptide activity (Rothman et al., 1991). In such a case, as suggested by Rothman (1992), a useful adjunct to traditional treatments for these patients may include an anti-opioid peptide antagonist.

Limitations and Future Research

We have demonstrated that compound-conditioning effects and intra-administration associations are relevant to understanding drug tolerance. However, an apparent problem with this analysis of tolerance is that it is not readily disconfirmed. For example, a demonstration of trans-situational tolerance is explicable by appealing to hypothesized private cues, such as intra-administration pharmacological cues, that may overshadow experimenter-manipulated public cues. However, as elaborated in Chapter 2, recognition that private cues may contribute to tolerance does lead to novel predictions, many of which have been confirmed. For example, presentation of the drug effect in the absence of drug-onset cues results in greater drug effects than when presented in the presence of drug-onset cues, both in rats (Kim et al., 1999) and humans (Siegel & Kim, 2000). Further research can evaluate other predictions of a conditioning analysis of tolerance that incorporates the contribution of compound CSs. For example, it would be expected that if trans-situational tolerance is the result of private cues overshadowing simultaneously-present environmental cues, procedures that reverse overshadowing [e.g., repeated presentation of the overshadowing CS (see Matzel, Schachtman, & Miller, 1985; Matzel, Shuster, & Miller, 1987)] should similarly restore environmental control of tolerance.

Although the phenomenon of situational-specific tolerance has been known for many years, researchers are just beginning to elucidate the neurochemical events mediating CCRs. We have provided initial evidence that the activity of one putative anti-opioid peptide, cholecystokinin, is important to the associative mechanisms of morphine tolerance. Further research may be directed to identifying other anti-opioid peptides that may also mediate CCRs and opiate tolerance. Additionally, future research may also uncover the molecular-biological events that interact with learning processes to produce tolerance.

Conclusions

In summary, evidence indicating that a variety of private cues (in addition to public cues) may become associated with a drug effect both complicates and enriches the conditioning analysis of tolerance. Because the more salient private cues may overshadow simultaneously-presented public cues, sometimes tolerance may appear to be trans-situational. Pre-drug cues (whether private or public) contribute to the expression of tolerance by eliciting CCRs, and, cholecystokinin neurotransmitter activity may provide a mechanism for these CCRs to mediate the behavioral expression of opiate tolerance. When opiates are administered in the absence of the usual pre-drug cues, the result is an increased risk for drug overdose. As we learn more about cue interactions in compound conditioning with respect to drug effects, and the physiological mechanisms of the associative contribution to tolerance, new theoretical and clinical insights will undoubtedly emerge to further our understanding of opiate tolerance.

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Chapter 2, Experiment 1
Tolerance Development, Subjects 1-26
Tail Flick Latency Scores

Subject #	GROUP	M1 0'	M1 45'	M1 90'	M3 0'	M3 45'	M3 90'	M5 0'	M5 45'	M5 90'
1	SMor	6	10	5	6	5	4	5	6	5
2	SMor	20	30	9	15	14	15	6	12	5
3	SMor	15	9	7	15	14	18	10	12	15
4	SMor	12	30	6	12	15	9	5	5	8
5	SMor	30	30	5	20	12	8	6	7	4
6	SMor	6	6	6	6	6	7	6	5	5
7	SMor	6	13	8	5	5	4	7	7	8
8	SMor	17	13	8	11	22	9	7	7	8
9	SMor	21	7	11	9	13	8	7	6	8
10	SMor	25	25	8	6	6	5	6	7	6
11	SMor	9	25	6	15	19	9	9	8	8
12	SMor	15	14	7	9	14	7	6	6	6
13	SMor	16	30	12	7	7	7	7	8	7
14	LMor	15	22	7	4	7	4	4	5	5
15	LMor	24	20	6	5	6	5	16	19	7
16	LMor	15	17	10	13	14	7	9	9	6
17	LMor	8	7	7	8	7	6	7	6	8
18	LMor	13	17	6	17	14	7	7	5	5
19	LMor	10	10	7	7	7	6	8	11	7
20	LMor	6	30	6	6	5	7	7	6	4
21	LMor	19	30	5	4	5	7	12	14	6
22	LMor	12	6	8	7	6	7	9	9	9
23	LMor	7	10	8	6	7	7	8	6	6
24	LMor	20	11	8	9	10	10	8	8	10
25	LMor	11	7	8	7	7	6	8	6	6
26	LMor	17	21	15	14	13	8	7	7	7

Chapter 2, Experiment 1
Tolerance Development, Subjects 27-48
Tail Flick Latency Scores

Subject #	GROUP	M1 0'	M1 45'	M1 90'	M3 0'	M3 45'	M3 90'	M5 0'	M5 45'	M5 90'
27	SSal	10	11	11	10	4	10	9	9	9
28	SSal	7	5	6	4	4	5	5	6	4
29	SSal	4	4	5	3	4	3	4	5	4
30	SSal	5	6	7	5	5	6	6	6	7
31	SSal	7	5	5	6	6	5	7	6	5
32	SSal	6	7	7	7	7	7	7	7	7
33	SSal	6	6	5	6	5	5	6	6	5
34	SSal	6	6	8	7	6	5	6	6	8
35	SSal	6	6	5	5	5	5	6	6	5
36	SSal	7	7	7	6	6	6	7	7	7
37	SSal	4	4	5	5	6	6	4	4	5
38	LSal	4	4	6	5	5	5	6	5	6
39	LSal	5	7	8	6	7	7	5	7	8
40	LSal	7	7	7	6	5	6	6	6	7
41	LSal	6	6	6	6	6	6	7	6	7
42	LSal	6	6	6	5	6	6	6	6	5
43	LSal	7	7	8	6	6	6	7	6	6
44	LSal	6	6	6	6	5	5	6	6	6
45	LSal	4	5	6	7	4	6	4	5	6
46	LSal	6	6	6	6	5	6	6	6	6
47	LSal	7	5	6	6	6	6	7	5	6
48	LSal	6	5	7	7	6	6	6	5	7

Chapter 2, Experiment 1
CS-alteration test,
Subjects 1-26
Tail Flick Latency Scores

Subject #	GROUP	0'	45'	90'
1	SMor	4	5	6
2	SMor	8	11	7
3	SMor	15	22	15
4	SMor	5	5	5
5	SMor	9	24	5
6	SMor	8	8	5
7	SMor	4	6	4
8	SMor	8	14	9
9	SMor	7	7	5
10	SMor	9	11	6
11	SMor	9	10	10
12	SMor	5	6	5
13	SMor	13	12	15
14	LMor	4	4	4
15	LMor	4	5	5
16	LMor	5	6	6
17	LMor	5	6	6
18	LMor	8	7	4
19	LMor	10	7	7
20	LMor	5	4	5
21	LMor	8	6	6
22	LMor	5	5	4
23	LMor	6	7	6
24	LMor	7	7	7
25	LMor	6	7	7
26	LMor	10	7	10

Chapter 2, Experiment 1
CS-alteration test,
Subjects 27-48
Tail Flick Latency Scores

Subject #	GROUP	0'	45'	90'
27	SSal	25	30	25
28	SSal	9	10	9
29	SSal	8	20	22
30	SSal	20	15	8
31	SSal	30	30	8
32	SSal	15	30	6
33	SSal	15	18	6
34	SSal	7	7	6
35	SSal	7	7	5
36	SSal	30	10	7
37	SSal	20	30	3
38	LSal	8	12	10
39	LSal	10	17	8
40	LSal	8	20	8
41	LSal	15	13	5
42	LSal	27	15	10
43	LSal	20	25	10
44	LSal	8	7	7
45	LSal	12	12	8
46	LSal	30	30	6
47	LSal	11	24	8
48	LSal	12	11	12

Chapter 2, Experiment 1
Infusion alteration test,
Subjects 1-23
Tail Flick Latency Scores
(Note: Subject 13, 24-26 died)

Subject #	GROUP	0'	45'	90'
1	SMor-LMor Test	2	3	4
2	SMor-LMor Test	8	7	7
3	SMor-LMor Test	6	4	5
4	SMor-LMor Test	3	7	2
5	SMor-LMor Test	7	7	8
6	SMor-LMor Test	4	3	3
7	SMor-LMor Test	6	6	4
8	SMor-LMor Test	4	6	5
9	SMor-LMor Test	6	6	5
10	SMor-LMor Test	6	6	5
11	SMor-LMor Test	5	5	5
12	SMor-LMor Test	5	7	8
14	LMor-SMor Test	7	6	7
15	LMor-SMor Test	6	6	7
16	LMor-SMor Test	8	11	7
17	LMor-SMor Test	7	6	4
18	LMor-SMor Test	12	14	6
19	LMor-SMor Test	9	9	9
20	LMor-SMor Test	8	8	7
21	LMor-SMor Test	7	10	8
22	LMor-SMor Test	5	8	6
23	LMor-SMor Test	8	16	12

Chapter 2, Experiment 1
Infusion alteration test,
Subjects 27-48
Tail Flick Latency Scores

Subject #	GROUP	0'	45'	90'
27	SSal-LMor Test	17	18	10
28	SSal-LMor Test	7	10	7
29	SSal-LMor Test	10	14	8
30	SSal-LMor Test	12	10	8
31	SSal-LMor Test	10	8	5
32	SSal-LMor Test	24	15	9
33	SSal-LMor Test	13	12	11
34	SSal-LMor Test	7	7	6
35	SSal-LMor Test	5	5	5
36	SSal-LMor Test	18	19	9
37	SSal-LMor Test	5	5	5
38	LSal-SMor Test	6	10	4
39	LSal-SMor Test	8	12	8
40	LSal-SMor Test	8	15	6
41	LSal-SMor Test	6	7	7
42	LSal-SMor Test	10	7	8
43	LSal-SMor Test	25	13	10
44	LSal-SMor Test	17	13	10
45	LSal-SMor Test	10	14	12
46	LSal-SMor Test	7	7	7
47	LSal-SMor Test	14	10	7
48	LSal-SMor Test	10	10	10

Chapter 2, Experiment 2
Tolerance Development, Subjects 101-115
Tail Flick Latency Scores

Subject #	GROUP	M1 O'	M1 45'	M1 90'	M3 0'	M3 45'	M3 90'	M5 0'	M5 45'	M5 90'
101	LMor-LMor	15	21	12	16	14	8	7	8	6
102	LMor-LMor	16	22	15	6	7	6	4	4	7
103	LMor-LMor	16	16	13	9	12	8	9	10	8
104	LMor-LMor	11	14	12	9	9	7	9	7	8
105	LMor-LMor	16	18	13	15	12	12	9	7	6
106	LMor-LMor	18	19	16	14	12	12	8	7	7
107	LMor-LMor	17	15	12	16	15	15	10	6	7
108	LMor-SMor	16	19	13	10	12	7	5	7	5
109	LMor-SMor	14	17	11	15	15	12	6	7	6
110	LMor-SMor	14	18	12	13	9	8	8	7	4
111	LMor-SMor	13	16	11	10	8	6	7	5	8
112	LMor-SMor	14	18	13	6	9	7	4	5	6
113	LMor-SMor	17	15	14	14	13	15	10	6	7
114	LMor-SMor	14	15	11	13	13	14	7	4	6
115	LMor-SMor	18	17	11	15	12	13	9	7	7

Chapter 2, Experiment 2
Tolerance Development, Subjects 116-129
Tail Flick Latency Scores

Subject #	GROUP	M1 O'	M1 45'	M1 90'	M3 0'	M3 45'	M3 90'	M5 0'	M5 45'	M5 90'
116	LSal-LSal	5	6	4	6	6	5	7	6	6
117	LSal-LSal	7	7	5	5	6	7	6	5	6
118	LSal-LSal	5	6	6	5	5	6	6	6	5
119	LSal-LSal	6	6	7	6	7	7	7	5	5
120	LSal-LSal	6	5	5	5	6	6	6	7	7
121	LSal-LSal	6	7	7	6	6	7	6	6	7
122	LSal-LSal	5	6	6	8	7	6	5	8	6
123	LSal-SSal	5	5	6	6	5	8	6	6	5
124	LSal-SSal	6	6	6	5	6	6	7	6	7
125	LSal-SSal	7	5	6	6	7	7	6	5	7
126	LSal-SSal	5	7	4	7	7	5	6	6	7
127	LSal-SSal	7	6	6	8	7	6	7	7	6
128	LSal-SSal	6	5	8	6	5	7	6	5	5
129	LSal-SSal	8	6	6	5	5	7	7	7	8

Chapter 2, Experiment 2
Tolerance Test, Subjects 101-115
Tail Flick Latency Scores

Subject #	GROUP	0'	45'	90'
101	LMor-LMor	7	5	5
102	LMor-LMor	7	6	6
103	LMor-LMor	6	5	5
104	LMor-LMor	8	6	7
105	LMor-LMor	8	5	6
106	LMor-LMor	6	6	8
107	LMor-LMor	8	7	6
108	LMor-SMor	16	19	10
109	LMor-SMor	15	15	12
110	LMor-SMor	14	20	15
111	LMor-SMor	13	16	11
112	LMor-SMor	11	15	12
113	LMor-SMor	13	16	12
114	LMor-SMor	14	19	12
115	LMor-SMor	14	15	13

Chapter 2, Experiment 2
Tolerance Test, Subjects 116-129
Tail Flick Latency Scores

Subject #	GROUP	0'	45'	90'
116	LSal-LSal	7	6	3
117	LSal-LSal	6	5	4
118	LSal-LSal	5	6	5
119	LSal-LSal	7	6	5
120	LSal-LSal	6	5	6
121	LSal-LSal	6	6	8
122	LSal-LSal	5	7	6
123	LSal-SSal	6	5	5
124	LSal-SSal	6	5	5
125	LSal-SSal	7	5	6
126	LSal-SSal	7	7	6
127	LSal-SSal	5	5	7
128	LSal-SSal	7	6	7
129	LSal-SSal	7	7	8

Chapter 2, Experiment 2
Tolerance Test, Subjects 151-164
Tail Flick Latency Scores

Subject #	GROUP	5'	15'	30'	45'	75'
151	LMor-pMor	8	7	6	7	9
152	LMor-pMor	7	7	7	10	11
153	LMor-pMor	11	8	7	8	12
154	LMor-pMor	10	9	6	10	12
155	LMor-pMor	7	9	6	7	10
156	LMor-pMor	10	8	7	7	8
157	LMor-pMor	12	7	6	7	8
158	LMor-pSal	10	12	12	10	11
159	LMor-pSal	11	10	11	11	10
160	LMor-pSal	11	11	10	11	12
161	LMor-pSal	12	12	11	12	11
162	LMor-pSal	10	9	11	10	11
163	LMor-pSal	10	11	11	12	10
164	LMor-pSal	11	11	10	10	12

Chapter 2, Experiment 2
Tolerance Test, Subjects 165-178
Tail Flick Latency Scores

Subject #	GROUP	5'	15'	30'	45'	75'
165	LSal-pMor	12	9	10	11	12
166	LSal-pMor	11	10	11	12	11
167	LSal-pMor	11	10	12	11	12
168	LSal-pMor	11	13	12	10	9
169	LSal-pMor	11	12	10	9	10
170	LSal-pMor	12	11	12	12	11
171	LSal-pMor	11	11	10	10	10
172	LSal-pMor	11	12	12	10	11
173	LSal-pMor	11	11	11	10	11
174	LSal-pSal	12	12	11	12	12
175	LSal-pSal	12	12	11	12	10
176	LSal-pSal	12	13	13	11	10
177	LSal-pSal	12	12	13	12	11
178	LSal-pSal	10	11	10	10	12

Chapter 3, Experiment 1
Tolerance Development, Subjects 201-222
Tail Flick Latency Scores

Subject #	Group	Block 1	Block 2	Block 3	Block 4
201	Mor-PD	10	7	5.5	7
202	Mor-PD	10	9.5	6.5	7.5
203	Mor-PD	11	8	6	7
204	Mor-PD	25	15	8	7
205	Mor-PD	15.5	8	5.5	6.5
206	Mor-PD	7	4.5	5	6
207	Mor-PD	19	13	11.5	9.5
208	Mor-PD	27.5	21	12.5	9
209	Mor-PD	18.5	13	9.5	8.5
210	Mor-PD	9	7	6	7
211	Mor-PD	21.5	17.5	12	10.5
212	Mor-VEH	12.5	9	5.5	7
213	Mor-VEH	21	19	10	8.5
214	Mor-VEH	30	18.5	19	10.5
215	Mor-VEH	27.5	14	11.5	9
216	Mor-VEH	6	17	12	8
217	Mor-VEH	14	12	9	7.5
218	Mor-VEH	13.5	18	17.5	12
219	Mor-VEH	18.5	14.5	13	9
220	Mor-VEH	15.5	7	8	5.5
221	Mor-VEH	11	7.5	7	6.5
222	Mor-VEH	27	23.5	14.5	9.5

Chapter 3, Experiment 1
Tolerance Development, Subjects 223-242
Tail Flick Latency Scores

Subject #	Group	Block 1	Block 2	Block 3	Block 4
223	Sal-PD	4	4	4.5	5
224	Sal-PD	2.5	3	2.5	3
225	Sal-PD	5.5	5	4	5
226	Sal-PD	5	4	4	5
227	Sal-PD	5	4.5	5.5	6.5
228	Sal-PD	7.5	7.5	7	7
229	Sal-PD	4	4	4.5	5.5
230	Sal-PD	5.5	6.5	7	8
231	Sal-PD	5	4.5	5.5	6
232	Sal-PD	6.5	6	6.5	7
233	Sal-VEH	6	5.5	4.5	5
234	Sal-VEH	6.5	5.5	5.5	6
235	Sal-VEH	4	4.5	5.5	5.5
236	Sal-VEH	5	5	5	5
237	Sal-VEH	6	4.5	5.5	6
238	Sal-VEH	4	3.5	4	4
239	Sal-VEH	7.5	6.5	6.5	7
240	Sal-VEH	4	4.5	4	6
241	Sal-VEH	6.5	6	6	6
242	Sal-VEH	6	6.5	5.5	7

Chapter 3, Experiment 1
CCR Test, Subjects 201-222
Tail Flick Latency Scores

Subject #	Group	5'	15'	30'	45'	75'
201	Mor-PD	11.5	11.5	11	11.5	13
202	Mor-PD	11	16.5	14.5	15	9
203	Mor-PD	11.5	9.5	8.5	10	12
204	Mor-PD	6	9.5	8	9	11.5
205	Mor-PD	7.5	7	9	9.5	10.5
206	Mor-PD	12	11.5	12.5	13.5	11.5
207	Mor-PD	20.5	21.5	12	18.5	10.5
208	Mor-PD	21	18	9.5	11.5	13
209	Mor-PD	17	17	15	16	16
210	Mor-PD	11	12	12	12	13
211	Mor-PD	18	16	17	16	16
212	Mor-VEH	5.5	7.5	7.5	8.5	8.5
213	Mor-VEH	9	6.5	8	10	10
214	Mor-VEH	5	7	6.5	6.5	7
215	Mor-VEH	15	8.5	5.5	5.5	5.5
216	Mor-VEH	6	6.5	6	6	7
217	Mor-VEH	14.5	13.5	14	11	6.5
218	Mor-VEH	8.5	12.5	11	15.5	9.5
219	Mor-VEH	15	11.5	11	15.5	15
220	Mor-VEH	9.5	10	8	9	10.5
221	Mor-VEH	10	7.5	3.5	5.5	8.5
222	Mor-VEH	12.5	13	10	15	15

Chapter 3, Experiment 1
CCR Test, Subjects 223-242
Tail Flick Latency Scores

Subject #	Group	5'	15'	30'	45'	75'
223	Sal-PD	12.5	12	10	10	12
224	Sal-PD	6	7	4.5	4	4.5
225	Sal-PD	6.5	8.5	8.5	7.5	9.5
226	Sal-PD	10	10	11.5	12	11.5
227	Sal-PD	9	10.5	10.5	10	11
228	Sal-PD	21	18	14.5	16	17
229	Sal-PD	10.5	9.5	12	12.5	9.5
230	Sal-PD	14	15	14.5	13.5	14.5
231	Sal-PD	12	12.5	12.5	14	13.5
232	Sal-PD	13	13.5	13.5	13.5	14
233	Sal-VEH	9.5	10.5	9.5	9.5	10
234	Sal-VEH	13	12	11	12	11
235	Sal-VEH	10.5	12.5	11.5	11.5	11.5
236	Sal-VEH	10.5	13	11	11	10
237	Sal-VEH	10	12.5	11	13.5	12
238	Sal-VEH	12	10	10	10.5	10.5
239	Sal-VEH	13.5	12.5	13	14.5	13.5
240	Sal-VEH	12	14.5	13	12	12.5
241	Sal-VEH	14	14.5	14	14.5	14.5
242	Sal-VEH	12.5	13	13.5	14	15

Chapter 3, Experiment 2
Tolerance Development, Subjects 301-315
Tail Flick Latency Scores

Subject #	GROUP	Block 1	Block 2	Block 3	Block 4
301	Mor-PD	27	22	13	11.5
302	Mor-PD	30	30	12.5	11.5
303	Mor-PD	25	18	9.5	8
304	Mor-PD	30	12	13.5	11.5
305	Mor-PD	28	23.5	16.5	11
306	Mor-PD	20	8.5	9	8.5
307	Mor-PD	22	16	10	9
308	Mor-VEH	30	20.5	11.5	9.5
309	Mor-VEH	25	16.5	14	11.5
310	Mor-VEH	24	17.5	14.5	11.5
311	Mor-VEH	19.5	12	10.5	8
312	Mor-VEH	24	14	13	11.5
313	Mor-VEH	21	14	10	7
314	Mor-VEH	18	9.5	8.5	8
315	Mor-VEH	9	12.5	8	7.5

Chapter 3, Experiment 2
Tolerance Development, Subjects 316-328
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Subject #	GROUP	Block 1	Block 2	Block 3	Block 4
316	Sal-PD	7.5	5.5	8	7.5
317	Sal-PD	8.5	9	8.5	8
318	Sal-PD	11	10	8.5	8.5
319	Sal-PD	8	5.5	8.5	9
320	Sal-PD	6	6	6	5.5
321	Sal-PD	6	5.5	6	7
322	Sal-PD	6	6.5	5.5	7
323	Sal-VEH	8	8	7.5	8
324	Sal-VEH	7	6.5	7.5	8.5
325	Sal-VEH	7	6.5	8	8.5
326	Sal-VEH	6.5	7.5	7.5	8.5
327	Sal-VEH	7.5	7.5	8	8
328	Sal-VEH	6	6.5	7	7

Chapter 3, Experiment 2
Tolerance Test, Subjects 301-315
Tail Flick Latency Scores

Subject #	GROUP	15'	30'	45'	75'
301	Mor-PD	15	12	12	10.5
302	Mor-PD	20	22.5	12	7.5
303	Mor-PD	14	19	12.5	12
304	Mor-PD	15	17.5	15	12
305	Mor-PD	18	17	27	13.5
306	Mor-PD	12	13.5	13	12
307	Mor-PD	16	18.5	21.5	11.5
308	Mor-VEH	10	13	12	11
309	Mor-VEH	11.5	13	12.5	12
310	Mor-VEH	12.5	14	12	10.5
311	Mor-VEH	10.5	9	9.5	11
312	Mor-VEH	12	10.5	11.5	11.5
313	Mor-VEH	9.5	12	11.5	12
314	Mor-VEH	8.5	9	10	9.5
315	Mor-VEH	8.5	8	8.5	8.5

Chapter 3, Experiment 2
Tolerance Test, Subjects 316-328
Tail Flick Latency Scores

Subject #	GROUP	15'	30'	45'	75'
316	Sal-PD	14.5	16	17	13
317	Sal-PD	26.5	29.5	21.5	13
318	Sal-PD	29	30	20	14
319	Sal-PD	19	25.5	24.5	14
320	Sal-PD	15.5	25	22.5	13
321	Sal-PD	17.5	27.5	30	14
322	Sal-PD	18.5	23.5	21.5	14
323	Sal-VEH	17.5	27	18	10.5
324	Sal-VEH	20	27	22.5	11.5
325	Sal-VEH	18	18	17.5	12.5
326	Sal-VEH	15.5	17.5	17.5	12
327	Sal-VEH	18.5	30	29	14.5
328	Sal-VEH	17.5	23.5	20.5	11.5