CONDITIONED MORPHINE WITHDRAWAL

CONDITIONED MORPHINE WITHDRAWAL ELICITED BY ENVIRONMENTAL AND PHARMACOLOGICAL CUES

By

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A Thesis

Submitted to the School of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree

Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY (2001) McMaster University

(Psychology) Hamilton, Ontario

TITLE: Conditioned Morphine Withdrawal Elicited by Environmental and Pharmacological Cues.

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NUMBER OF PAGES: xi, 151

Abstract

Many studies have demonstrated the important contribution of Pavlovian conditioning to the phenomena of drug tolerance and withdrawal. In the Pavlovian analysis, cues that are paired with drug administration come to elicit compensatory responses in anticipation of the subsequent drug-induced physiological disturbance. Furthermore, when such cues are presented in the absence of the drug, the compensatory conditional responses elicited by the drug-paired cues are evident as withdrawal behaviors. The present experiments investigate the validity of both commonly used and novel behavioral indices of morphine withdrawal in the rat model. The results suggest that rearing may not be a valid behavioral index of withdrawal, and that mouth movements may be a sensitive and valid index. The present experiments also investigate the types of stimuli that can serve as effective cues for drug administration. While past studies of conditioned morphine withdrawal have typically employed external environmental stimuli as cues, recent research has suggested that internal pharmacological cues inherent in the drug administration itself may, in some circumstances, come to control the expression of tolerance and withdrawal behaviors. The results of these experiments show that rats conditioned with a high dose of morphine display more withdrawal behaviors when given a small dose of morphine than when given a placebo injection. This result is interpreted as evidence that the early effects of a large dose of a drug, reproduced by the

administration of a small dose of the drug, can serve as conditional stimuli and elicit compensatory conditional responses. The finding that morphine withdrawal can be elicited by administration of morphine has implications for a wide range of issues in drug tolerance, withdrawal, and dependence.

Acknowledgements

It has been a privilege to spend my years at McMaster working with Shepard Siegel. His guidance and support have been essential and invaluable to my academic development. It has been extremely rewarding to work with a supervisor who treats his students as valued colleagues and endeavors to provide opportunities for involvement in all phases of the research process. I am particularly pleased to have been a member of his laboratory at a time when Dr. Siegel's outstanding qualities as a supervisor received official public recognition.

I also wish to express my deep gratitude to a series of teachers, each of whom played a major role in my academic career. Lorraine Allan and John Platt provided me with the benefit of their insights as members of my supervisory committee, and also allowed me to expand my research activities in their respective laboratories. My undergraduate supervisor, Linda Parker, remains a valued source of guidance and support. Thanks are also due to Don Morgenson, who first sparked my interest in psychology, and to Vern Schafer, who first introduced me to the concept of experimental science.

My experience at McMaster was also made more productive and enjoyable by the contributions of my contemporaries in Dr. Siegel's lab. Thanks to Joe Kim for the frequent collaborations, and the willingness to bet money on my inability to differentiate Coke and Pepsi. Thanks to Marco Baptista for many hours of interesting conversation on a variety of topics. Thanks also to Lorraine Weiss-Kelly for her support and friendship, her cat-sitting services, and for listening.

Finally, I wish to thank my parents, Robert and Carolyn McDonald, and the rest of my family for their unfailing love and support. Without them, none of this would have been possible.

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Preface

This thesis contains previously published material. The experiment described in Chapter 2 has been reported as:

McDonald, R. V., & Siegel, S. (1998). Environmental control of morphine withdrawal: Context-specificity or stimulus novelty? Psychobiology, 26, 53-56.

The author of this thesis was primarily responsible for the design and execution of this experiment, the analysis of the results, and the preparation of the manuscript for publication, under the supervision of the second author. Much of the material in the Introduction of the published manuscript has been eliminated here to avoid redundancies. Permission of the copyright holder has been obtained to reproduce this material here.

CHAPTER 1

Introduction

Morphine

Morphine, an alkaloid derivative of the opium poppy (*Papaver somniferum*), is extracted from powdered opium. Along with other opioid alkaloids (papaverine, codeine), and their semi-synthetic (e.g., heroin, hydromorphone) and synthetic (e.g., methadone, meperidine) analogues, morphine has a wide range of effects both peripherally and in the central nervous system (Jaffe & Martin, 1990). While many properties of opiates have long been known, the discovery in the early 1970s of three classes of endogenous opioid peptides, and at least three different receptor types, illuminated the mechanism by which opiates exert their central and peripheral effects (Akil et al., 1984; Goldstein, 1984). Of the three clearly identified receptor types, μ, κ, and δ, morphine produces its most robust effects primarily through agonist properties at μ and κ receptors (Jaffe & Martin, 1990). It has been noted, however, that affinity for the various receptor types varies widely across opioid compounds, and further that a given opioid may act as an agonist, partial agonist, or antagonist simultaneously at different receptor types (Martin, 1983).

Morphine unconditionally produces a variety of effects, including analgesia (e.g., Jaffe & Martin, 1990), respiratory depression (Martin, 1983), decreased gastrointestinal motility (Duthie & Nimmo, 1987), and decreased biliary and pancreatic secretion

(Dooley, Saad, & Valenzuela, 1988). In humans, morphine can also produce drowsiness, mental clouding, and mood changes, with mild to intense euphoria common (Jaffe & Martin, 1990).

Acute Morphine Tolerance and Withdrawal

It has been noted that the effects of morphine may be attenuated over the course of a single administration; that is, acute tolerance develops. When a single administration of morphine is given via an implanted pellet, the analgesic effects are seen to diminish over time (e.g., Tilson, Rech, & Stolman, 1973; Wei & Way, 1975). More importantly, this decrease in the drug's effect is seen across time periods during which the bioavailability of the drug remains relatively constant. The fact that drug effects may be attenuated by compensatory homeostatic processes during the course of the very first administration has led to the observation that the apparent effects of a drug constitute the net effect of both the physiological effects of the drug and the reflexive physiological compensatory response (Ramsay & Woods, 1997; Siegel & Allan, 1998).

Further evidence of these homeostatic compensatory mechanisms is seen when an acute administration of morphine is terminated, either by discontinuation of the administration (e.g., removal of an implanted pellet, Wei & Way, 1975), or by administration of a competitive antagonist, such as naloxone (e.g., McDonald, Parker, & Siegel, 1997). When the unconditional effects of morphine are removed, the unopposed unconditional compensatory responses are evident. These compensatory responses have been "generally characterized by rebound effects in the physiological systems that were

initially modified by the drug" (Jaffe, 1990, pp. 526). For example, Wei and Way (1975) demonstrated increased sensitivity to painful stimuli in rats following removal of an implanted morphine pellet, an effect opposite in direction to the unconditional analgesic effect observed while the pellet was in place.

Chronic Tolerance and Withdrawal

While the acute effects of drug administration are of particular relevance to models of drug tolerance and withdrawal, it is important to note that with many drugs, including opiates, chronic administration is typical of both licit and illicit drug use. It has long been observed that the effects of morphine diminish over a series of administrations (e.g., DuMez, 1919), and that termination of a series of administrations can result in the appearance of so-called withdrawal symptoms opposite in direction to the unconditional effect of the drug (e.g., Wikler & Pescor, 1967). These unopposed compensatory responses seen following termination of series of morphine administrations constitute chronic withdrawal (generally referred to simply as withdrawal).

A number of physiological indices of morphine withdrawal have been developed using the rat model. These include EEG measures (e.g., Stinus, Robert, Karasinski, & Limoge, 1998; Young, Steinfels, & Khazan, 1980), arterial blood pressure and heart rate (Buccafusco, 1982), body weight (e.g., Adams & Holtzman, 1990; Nakamura, Ishii, & Shimizu, 1978), and thermoregulation (e.g., Broadbent & Cunningham, 1996; Jorenby, Keesey, & Baker, 1989; Schwarz & Cunningham, 1990). In addition, a wide range of behavioral indices of withdrawal have also been developed using this model, including

rearing (e.g., Azorlosa, Hartley, & Deffner-Rappold, 1994; Falls & Kelsey, 1989; MacRae & Siegel, 1997), genital licking (Kelsey, Aranow, & Matthews, 1990), ear wiping (MacRae & Siegel, 1997), jumping (Kelsey et al., 1990), startle response (e.g., Mansbach, Gold, & Harris, 1992), ultrasonic vocalization (e.g., Barr & Wang, 1992; Suzuki, Fukagawa, & Misawa, 1990), and disruptions of schedule-controlled patterns of operant responding (e.g., Brady & Holtzman, 1980; Steinfels & Young, 1981). Both the physiological and behavioral measures cited above are theorized to reflect the activity of compensatory responses to the unconditional effects of morphine, now clearly evident in the absence of the opposing drug effect.

The Role of Pavlovian Conditioning in Chronic Tolerance and Withdrawal

There now exists considerable evidence that Pavlovian, or classical, conditioning plays an important role in the development of tolerance to the unconditional effects of morphine; the decreased net effect of a given dose of morphine across a series of administrations is due to the increased efficacy of the compensatory responses, and the increased efficiency of these compensatory responses is seen as the result of associative learning (see Siegel, 1983, for review). The Pavlovian conditioning model of drug tolerance and withdrawal postulates that events occurring during administration of a drug correspond to a Pavlovian conditioning trial (e.g., Dworkin, 1993; Ramsay & Woods, 1997; Subkov & Zilov, 1937). During the course of a series of drug administrations, cues that are paired with the unconditional effects of the drug in a positively contingent manner (i.e., cues that are both positively correlated with the drug effects and negatively

correlated with the absence of drug effects) come to elicit drug-compensatory responses in anticipation of the drug effects. This model proposes that the unconditional effects of the drug constitute the Unconditional Stimulus (US), and that the compensatory corrections constitute the Unconditional Response (UR). Furthermore, cues that are reliably and repeatedly paired with drug delivery, and the consequent US, function as Conditional Stimuli (CSs), and acquire the ability to elicit drug-compensatory responses as Conditional Responses (CRs) (Siegel, Baptista, Kim, McDonald, & Weise-Kelly, 2000). The acquisition of these Conditional Compensatory Responses (CCRs) results in an increased ability to counteract the effects of the drug, and this is overtly manifest as tolerance.

When the CS is subsequently presented in the absence of US (e.g., the subject is placed in an environment where the drug is usually administered, but no drug is given), the CCR is elicited by the CS. This CCR is now unopposed by the absent US, and the CCR is overtly manifest as withdrawal (Siegel et al., 2000). It should be noted that this conception of the Pavlovian conditioning model of drug tolerance and withdrawal represents a fundamental change from the model as originally put forward by Siegel (1975), which proposed that the drug itself was the US and the effects of the drug constituted the UR. This initial version of the model stated that "conditioned drug responses are commonly opposite in direction to the unconditioned effects of the drug" (Siegel, 1975, p. 499). This assertion that the CR was, in fact, the functional opposite of

the UR was at odds with the traditional view that the CR should be similar or identical to the UR (e.g., Pavlov, 1927).

Subsequent critical analyses by many investigators (e.g., Dworkin, 1993; Eikelboom & Stewart, 1982; Poulos & Cappell, 1991; Ramsay & Woods, 1997), based largely on conflicting reports of both drug-similar and drug-opposite CRs in the literature, have resulted in the reformulation of the model as described above. 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).

Control of Tolerance and Withdrawal by Environmental Cues

In past research on the role of Pavlovian conditioning in drug tolerance and withdrawal, many investigators have manipulated environmental cues in conjunction with exposure to the unconditional effects of a drug. Such studies typically involve repeated drug administrations in a particular, distinctive, context (e.g., an experimental room with distinctive lighting or background noise). These environmental cues can come to function as CSs in the development of drug tolerance, as described above. When tolerance develops in the presence of these distinctive environmental cues, that tolerance may be greatly attenuated if the drug is then given in the absence of those cues (i.e., in a different environment, Siegel, 1978).

Subsequent research has demonstrated context-specificity with respect to tolerance to many effects of a variety of drugs, including ethanol (e.g., Seeley, Hawkins, Ramsay, Wilkinson, & Woods, 1996), nicotine (e.g., Epstein, Caggiula, & Stiller, 1989), opiates (reviewed by Siegel, 1991), benzodiazepines (reviewed by Siegel, 1986), pentobarbital (e.g., Cappell, Roach, & Poulos, 1981), phencyclidine (Smith, 1991), immunoenhancing drugs (Dyck, Driedger, Nemeth, Osachuk, & Greenberg, 1987), and haloperidol (Poulos & Hinson, 1982). It is seen in many species, from snails (Kavaliers & Hirst, 1986) to humans (e.g., Dafters & Anderson, 1982).

Similarly, there are demonstrations of context-specificity of withdrawal symptoms. That is, rats display more behavioral withdrawal symptoms in the absence of the drug when assessed in a previously drug-paired environment than an alternative environment (e.g., Azorlosa et al., 1994; Kelsey et al., 1990). Such results are predicted by a Pavlovian analysis of tolerance and withdrawal; in the terms of Pavlovian conditioning, subjects placed in the drug-paired context in the absence of the drug are presented with the CS in the absence of the US, thus rendering the CCR, elicited by drug-paired contextual cues, clearly visible. Furthermore, it has been demonstrated that a wide range of environmental CSs can come to exert control over conditioned drug effects, including distinctive flavors (McNally & Westbrook, 1998), ambient temperatures (Kavaliers & Hirst, 1986), and magnetic fields (Kavaliers & Ossenkopp, 1985).

Control of Tolerance and Withdrawal by Pharmacological Cues

While the majority of investigations into the role of Pavlovian conditioning in tolerance and withdrawal employ manipulations of external contextual cues (e.g., Kelsey et al., 1990), there is also evidence that pharmacological cues can serve as CSs. Although a drug used as a CS may, in fact, have inherent unconditional effects of its own, there are recent demonstrations 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 is evidence that associations between the cues inherent in the separate administration of two different drugs (inter-drug associations) may make an important contribution to tolerance (see Krank & Bennett, 1987). For example, when atropine sulfate is routinely injected prior to pentobarbital, tolerance to pentobarbital's hypothermic effect is much more pronounced when pentobarbital administration is preceded by atropine administration, than when pentobarbital is given in the absence of the signal provided by the atropine (Taukulis, 1986). As discussed by Siegel (1988), such pharmacological associations may be manifest as state-dependent learning of tolerance. That is, in such studies the initial drug may induce a drug state that serves as the context of subsequent drug administration. 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).

It has also been reported that a small dose of a drug may serve as a CS, signaling a subsequent, larger dose of the same drug (intra-drug conditioning, see Greeley & Ryan, 1995). Greeley, Lê, Poulos, and Cappell (1984) used a paired/unpaired design 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 (0.8 g/kg) 60 min prior to a high dose of ethanol (2.5 g/kg). 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.

There is also evidence 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. Although Cepeda-Benito and Tiffany (1993) reported an inability to demonstrate such an intra-drug association with morphine, results of more recent research provide clear evidence of an association (Cepeda-Benito & Short, 1996).

In light of the evidence that a drug can serve as a signal for itself, 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 (intra-administration conditioning, e.g., Greeley et al., 1984; Kim, Siegel & Patenall, 1999; King, Bouton, & Musty, 1987; Mackintosh, 1987; Tiffany, Petrie, Baker & Dahl, 1983).

According to the Pavlovian conditioning analysis of tolerance, cues predictive of the unconditional effects of a drug elicit CCRs. If signaling is inherent within an administration, injection of a smaller dose of the drug to subjects with a history of injections of a larger dose of the drug might be expected to elicit such a CCR; the smaller dose should reproduce the early effect of the larger doses previously administered. Such a finding was reported by Krank (1987). Following 10, daily injections of 5 mg/kg morphine, 1 mg/kg elicited hyperalgesia.

More recently, Mucha, Kalant, and Birbaumer (1996) also 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.

It has further been demonstrated by Kim et al. (1999) that the rate of increase over time in the bioavailability of the drug affects the type of cues that come to control the display of tolerance. Different routes of administration result in peak bioavailability of the drug in the CNS at different latencies following administration. Thus, the temporal relationship among various cues and the peak effects of a drug administration vary depending on the route by which the drug is administered. Kim et al. (1999) demonstrate that external environmental cues acquire control over the display of tolerance when IV drug administration is rapid (i.e., the subject is given a typical IV bolus, where the entire dose of the drug is injected over 15 to 20 s), but internal pharmacological cues acquire control over the display of tolerance when the drug is administered at a slower rate, (i.e., the same dose of the drug is injected slowly over the course of several minutes). This difference is attributed to the latency of onset of the maximal effects of a drug administration; with typical IV administration, the peak effects occur rapidly following administration, whereas with other, less efficient, routes, such as IP or oral administration, it takes longer to reach the maximal effect of the drug (Jaffe & Martin, 1990). The short latency of the peak drug effects with IV administration result in the early and peak effects occurring almost simultaneously, and, therefore, environmental cues that reliably precede the drug effect make better predictors of the upcoming US.

Slower, less efficient, administration provides a larger temporal gap between initial subjective effects of the drug and the later peak effects, in effect allowing early drug cues to function as effective predictors of the later peak effects. Furthermore, when drugs are administered using a less efficient route, the temporally separated early and peak effects are perfectly positively correlated; one never occurs in the absence of the other.

On the basis of the Pavlovian conditioning analysis, withdrawal symptoms – a manifestation of a pharmacological CR – should be elicited not only by drug-associated environmental cues, but also by drug-associated pharmacological cues. Thus, a small dose of the drug might be expected to elicit withdrawal symptoms in subjects experienced with large doses.

Krank's (1987) study demonstrates the ability of a small dose of morphine to elicit hyperalgesia in rats conditioned with a substantially higher dose. While rather counterintuitive, the finding that administration of morphine increased sensitivity to painful stimuli under these circumstances is predicted by a Pavlovian analysis of tolerance and withdrawal. This model also suggests that withdrawal behaviors are unopposed CCRs elicited by drug-paired cues in the absence of the drug. It therefore follows that, to the extent that the early effects of a drug serve as salient cues for the later peak effects, and thereby come to exert control over the display of tolerance, these same early drug cues should elicit withdrawal when presented in the absence of the later peak effect. In other words, it should be possible to elicit so-called morphine withdrawal behaviors by the administration of morphine

There is some experimental evidence that drug withdrawal can be elicited by a small dose of the drug in subjects with a history of exposure to higher doses of the drug. Schachter (1977) reported that some heavy smokers who normally smoked high nicotine cigarettes failed to regulate their nicotine intake when given low nicotine cigarettes (i.e., increase the number of cigarettes smoked). These smokers, who repeatedly self-administered lower than normal doses of nicotine, reported extreme withdrawal distress. Other heavy smokers, who increased consumption when given low-nicotine cigarettes, effectively maintaining their normal nicotine intake, reported no withdrawal distress. The more general prediction of drug-precipitated drug withdrawal, however, remains largely untested.

Conditioned Morphine Withdrawal

This thesis is concerned with the investigation of stimuli capable of eliciting conditioned morphine withdrawal in the rat model. The experiment described in Chapter 2 was designed to assess the potential role of stimulus novelty in studies of context-elicited withdrawal. The results indicate that some behaviors seen as indicative of withdrawal distress may, in fact, be responses to stimulus novelty, and also suggest important methodological considerations for future studies of this phenomenon. The experiment described in Chapter 3 provides evidence of context-elicited withdrawal using an experimental procedure that controls for possible effects of stimulus novelty. Interpretation of the results provides the basis for an assessment of the validity of a range of possible behavioral indices of withdrawal.

Chapter 4 describes an experiment that assesses the potential of pharmacological cues to conditionally elicit withdrawal behaviors. It is shown that withdrawal behaviors are more frequent, following a history of morphine administration, in rats given a small dose of the drug than in rats given placebo injections. The experiment described in Chapter 5 replicates the behavioral data seen in Chapter 4, while also demonstrating a parallel effect in the subject's body temperature. It is further shown that both the behavioral and physiological effects of the small morphine dose reflect conditioned compensatory responses, rather than sensitization; continued administration of the small dose, with the inherent increase in total exposure to the drug, resulted in a decrease in both behavioral and physiological indices of withdrawal. These results indicate that, as expected on the basis of a Pavlovian conditioning analysis of morphine withdrawal, the ability of a small dose of morphine to elicit withdrawal can be extinguished.

CHAPTER 2

Environmental Control Of Morphine Withdrawal: Context-Specificity Or Stimulus Novelty?

As discussed in Chapter 1, a number of investigators hypothesized that, when a drug is administered in the context of the usual drug-administration cues, CCRs contribute to tolerance; that is, they attenuate the drug effect (e.g., Obál, Vicsay, & Madaràsz, 1965; Poulos & Cappell, 1991; Siegel, 1991). Moreover, when a drug is not administered in the context of the usual drug-paired cues, these CRs are expressed as (so-called) withdrawal symptoms. On the basis of the conditioning analysis of tolerance and withdrawal symptoms, then, these phenomena should be more pronounced in the context of the usual drug administration cues than in the context of alternative cues.

There are many demonstrations of context-specificity of tolerance in which the drug-experienced organism is less responsive to the drug if it is administered in the drug-associated environment than if it administered elsewhere. Such context-specificity of tolerance was originally demonstrated with respect to tolerance to the analgesic effect of morphine in a number of experiments by Mitchell and colleagues (e.g., Kayan, Ferguson, & Mitchell, 1973; Kayan, Woods, & Mitchell, 1969). The context-specificity of tolerance has subsequently been widely investigated, as described in Chapter 1, as has the phenomenon of context-elicited withdrawal (e.g., Kelsey, Aranow, & Matthews, 1990). In most demonstrations of the importance of the drug-paired environment to the display

of withdrawal symptoms, "paired" rats are administered morphine in a distinctive environment (E_1, e.g., a room other than the animal colony), and saline in an alternative environment (E_2, e.g., the home cage in the colony room). "Unpaired" rats are administered saline in E_1, and morphine in E_2. Typically, morphine is injected in ascending doses, terminating (in different experiments) at 40-75 mg/kg. Anywhere from 1-10 days following the final morphine administration, rats are administered saline in the E_1, and various signs of morphine withdrawal are tabulated. Context-specificity of withdrawal is inferred if paired rats display more withdrawal behaviors than do unpaired rats.

Recently, Azorlosa and colleagues (Azorlosa et al., 1994; Deffner-Rappold, Azorlosa, & Baker, 1996) have reported that context specificity of withdrawal may be seen after a much smaller amount of morphine exposure than is typical. That is, after as few as seven, 10 mg/kg morphine administrations (at 48h intervals), paired rats display more evidence of withdrawal when administered saline in E_1 than do unpaired rats. Indeed, with some measures, more withdrawal is apparent in E_1 in rats that previously received 10 mg/kg morphine in this environment than in rats that received 75 mg/kg morphine in this environment (Deffner-Rappold et al., 1996). Although such a finding may reflect the context-specificity of morphine withdrawal, there is another interpretation. It is possible that some apparent morphine-withdrawal behaviors are actually exploratory behaviors, and some cases of more withdrawal behaviors in E_1 by paired than by unpaired rats actually represent more exploration by paired-group subjects.

It is well-established that rats explore a novel environment more than they do a familiar environment (e.g., Berlyne, 1955). Although paired and unpaired rats have the same exposure to E_1 and E_2, only paired rats are pretreated with morphine in conjunction with to E_1 placement. There is considerable evidence that if rats are narcotized during exposure to a particular environment their exploration of that environment is attenuated. When subsequently tested while undrugged, they treat this environment as relatively novel (see Scoles & Siegel, 1986).

Although there are various reasons why drugged exposure to an environment may be less effective than undrugged exposure in attenuating exploratory responses, one possibility is that the drug acts as a restraint. Results of conditioned place preference experiments indicate that, if a rat is restrained during exposure to a particular environment, it subsequently displays increased preference for that environment, and that this is due to restraint preserving the "novelty" of the environment (Carr, Fibiger, & Phillips, 1989). It may be that subjects receiving morphine in E_1 are prevented from engaging in exploratory behavior by the locomotor suppression of morphine. That is, morphine may be acting as a "pharmacological restraint" resulting in increased exploratory behaviors when rats are placed in E_1 in the absence of morphine.

Another reason why paired rats may explore E_1 on the test more than do unpaired rats is because of state-dependency. Although rats in both groups had equal exposure to this test-environment prior to actual testing, only paired rats were drugged during pre-test exposures. For paired rats, then, their first exposure to E_1 in the undrugged state occurs

on the test session, and they may treat this environment as they do a novel environment, in that the test trial constitutes the first exposure to E_1 in the drug-free state.

Some of the behaviors seen as indicative of opiate withdrawal, such as wet-dog shakes and piloerection (Emmett-Oglesby, Mathis, Moon, & Lal, 1990), would appear to have little to do with exploratory behavior. However, one commonly reported symptom of opiate withdrawal, rearing (both front paws free of the floor), is also seen in response to novel stimuli. Several investigators have presented rearing as a morphine-withdrawal behavior (e.g., Kelsey et al., 1990; MacRae & Siegel, 1997). Indeed, rearing sometimes is the only measure that provides compelling evidence of context-specificity of such withdrawal -- especially withdrawal seen following minimal morphine exposure (e.g., Azorlosa et al., 1994; Deffner-Rappold et al., 1996). Rearing has also been characterized as an exploratory, or curiosity-related, behavior (Berlyne, 1960), a fear-, or emotionality-related, behavior (Archer, 1973), and the result of nonspecific CNS excitability (Lat & Gollova-Hemon, 1969).

The present experiment was designed to incorporate many features of recent demonstrations of context-specific withdrawal (e.g., Deffner-Rappold et al., 1996). In addition, the design of the experiment also permitted comparison of behaviors seen in a morphine-paired environment and in a novel environment.

Method

Subjects and Drugs

The subjects were 60 male Sprague-Dawley rats, weighing between 325 - 400g at the start of the experiment. All subjects were individually housed in plastic cages containing recycled paper bedding material, with food and water freely available (except during conditioning and test trials), and maintained on a 12:12 h photoperiod (lights on at 0600). All conditioning and testing took place during the light portion of the photoperiod.

Morphine sulfate was prepared as a 10 mg/ml solution. All saline injections were given equivolume to the corresponding morphine injection. Injections were subcutaneous in the dorsal surface of the neck.

Environments, Design, and Procedure

Environments. Morphine and saline were injected in one of three environments (E_1, E_2, or E_3). E_1 was one of 6, identical, clear, acrylic chambers (30 cm X 30 cm X 30 cm) located in a distinctive, brightly illuminated room. These chambers were supported on stands, and a mirror was mounted under the chamber at a 45° angle to allow observation of the rat from below, as well as directly through the walls of the chamber. E_2 was one of 12, identical, clear, plastic cages (like the home cage but with no bedding material) located in a dark, vented, sound-attenuating enclosure (a drawer of a fireproof filing cabinet, see Siegel, Hinson, & Krank, 1978). E_3 was the home cage in the colony room.

Design. Withdrawal behaviors were evaluated in all rats in E_1 on a test session following the conditioning phase of the experiment. Five groups of rats (n/group = 12) differed in their treatments during this pretest conditioning phase. Rats assigned to the Morphine Paired (MP) group were injected with morphine in E_1 and saline in E_2, and rats assigned to the Morphine Unpaired (MU) group were injected with morphine in E_2 and saline in E_1. Rats assigned to the Saline Paired (SP) group were injected with saline in both E_1 and E_2. Thus, the present experiment, like earlier experiments, evaluated contextual contribution to putative drug withdrawal symptoms; more such symptoms should be displayed by MP than by MU rats. In addition, the design of the present experiment included two additional groups to evaluate the extent to which these symptoms seen in E_1 represent responding to a novel environment, rather than a drug-paired environment: Groups Morphine Novel (MN) and Saline Novel (SN). During conditioning, Group MN rats were injected with morphine in E_2 and saline in E_3, and Group SN rats were injected with saline in both E_2 and E_3. These rats had no exposure to test environment, E_1, during conditioning.

Procedure. The procedure was similar to that of Deffner-Rappold et al. (1996), except that the conditioning phase consisted of 12 (rather than 11) days. Rats received two injections on each conditioning day. The first injection consisted of saline. The second injection, 4h later, consisted of either morphine (Groups MP, MU, and MN) or saline (Groups SP and SN). The morphine dose for first morphine injection was 5 mg/kg,

and for the second and subsequent injections it was 10 mg/kg (Deffner-Rappold et al., 1996).

For injections in E_1 and E_2, rats were transported from the colony room to the distinctive environment and placed in the environment for 5 min. They were then removed, injected, and replaced in the environment for an additional 55 min before being returned to their home cages in the colony room. For injections in E_3 (the home cage), rats were simply removed from their cages, injected, and returned to their cages, without leaving the colony room.

Test trials took place 5 days after the final conditioning trial. Subjects were tested in groups of 6. Each test trial consisted of a 5 min pre-injection exposure to the testing environment, an injection of saline (equivolume to injections received on the final conditioning trial), and a 30 min post-injection observation period in the testing environment. The 30 min post-injection interval was divided into 5 blocks of 6 min each. Following injection, each subject was observed for 1 min during each 6-min block in a cycling procedure, resulting in a total observation period of 5 min (1 min from each of 5 blocks) for each subject during the 30 min interval.

Analysis. The behaviors scored during testing were Rearing (both front paws off the floor of the chamber, with body extended upward, with episodes of grooming that resulted in both front paws being off the floor not being scored as rearing, see Azorlosa et al., 1994; Falls & Kelsey, 1989; MacRae & Siegel, 1997), Genital Licking (licking of the external genitalia, presumably reflecting ejaculation, see Kelsey et al., 1990), Ear Wiping

(during grooming, pulling both paws simultaneously over the ears from back to front, see MacRae & Siegel, 1997), and Jumping (all four paws off the ground simultaneously, see Kelsey et al., 1990). The number of feces excreted was also recorded.

Results and Discussion

The mean frequency of Rearing observed during testing for each group is displayed in Figure 1. An one-way analysis of variance of the data summarized in Figure 1 revealed a significant difference among groups, $F(4, 55) = 12.9$, $p < .001$. Subsequent Newman-Keuls tests[1] revealed that Group MP, Group MN, and Group SN all reared more frequently than Group MU and Group SP, and Group MP reared more frequently than Group SN (all $ps < .05$). There were no other significant between-group differences, either in the rearing measure, or in any other measure.

Insert Figure 1 here

That finding that Group MP rats reared more than Group MU rats is similar to other reports that rats conditioned with as little as 10 mg/kg morphine display more rearing in the morphine-paired environment than in a saline-paired environment (Azorlosa et al., 1994; Deffner-Rappold et al., 1996). Although it is tempting to interpret this finding as evidence of environmentally-elicited drug withdrawal, results obtained from the additional groups suggest caution in applying this explanation. Group MN rats

displayed about as much rearing as did Group MP rats, and, like Group MP rats, reared more than did Group MU rats. The difference between Groups MN and MU likely results from the fact that the test environment was novel from Group MN rats, thus they displayed rearing as an exploratory behavior. Indeed, the fact that Group SN rats reared more than did Group SP rats suggests that novelty-induced rearing may be seen in rats with no prior history of morphine.

The primary implication of these results is that previous studies demonstrating context-specific increases in rearing upon termination of relatively low maintenance doses of morphine (e.g., Deffner-Rappold et al., 1996) cannot be taken as unequivocal evidence of context-specific withdrawal. Since paired-group rats were drugged during pretest exposures to the test context, the extensive rearing displayed by these rats may represent heightened exploration of the functionally novel test context.

The phenomenon of context-specific withdrawal is one that merits further study. This phenomenon has been reported in human clinical studies (O'Brien, Ehrman, & Ternes, 1986), and it has been suggested that contextual cues may play a significant role in the relapse to drug use often seen in human drug users following a period of abstinence (Siegel, 1989). While the results of the present study do not rule out the interpretation of rearing as an index of context-specific withdrawal distress, it appears that this interpretation may be problematic where relatively low maintenance doses are used. The fact that there was a significant, although small, difference in frequency of

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[1] Newman-Keuls tests were used in order to replicate the analysis performed by Deffner-Rappold et al. (1996). In subsequent chapters the more conservative Tukey's HSD test was used where parametric data

rearing between Group MP and Group SN may suggest that there is some form of interaction between the effects of stimulus novelty of the environment and the elicitation of context-specific withdrawal in previously drug-paired environments.

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analysis was appropriate.

CHAPTER 3

Context-Specific Morphine Withdrawal Unconfounded By The Effects Of Stimulus Novelty

Introduction

The results described in Chapter 2 suggest that context-elicited morphine withdrawal may be confounded by the effects of testing rats in a functionally novel environment. As demonstrated in the previous chapter, this may be particularly problematic with regard to the use of rearing as a behavioral index of morphine withdrawal. Recently, Azorlosa and Simmons (1999) attempted to assess directly the contribution of exploration to rearing elicited by cues associated with low-dose (5-10 mg/kg) injections of morphine. As in previous experiments, rats were tested in E_1 following a conditioning regime consisting of either morphine in E_1 and saline in E_2 (paired), or morphine in E_2 and saline in E_1 (unpaired). In contrast with previous experiments, rats were confined in tube restraints on each trial, thus neither paired or unpaired rats had the opportunity for exploration prior to the test. As in previous studies, Paired-rats displayed more test-session rearing than unpaired rats: "These results suggest that rearing is an index of conditioned withdrawal" (Azorlosa & Simmons, 1999, p. 557). However, as noted by Azorlosa and Simmons (1999), although restraint does eliminate locomotor exploration, "awareness of other cues (e.g., olfactory, auditory, or visual) may

have been attenuated or altered in the paired rats" (p. 560); thus it is still conceivable that E_1 may be more novel for restrained paired rats than for restrained unpaired rats.

The present experiment adopts a different strategy to evaluate the contribution of E_1 novelty to apparent conditioned withdrawal. As is typical in studies of conditioned withdrawal, rats were unrestrained during pretest sessions. Paired rats received morphine in E_1 and saline in E_2 (with the relationship between injection environment and injected substance reversed for unpaired rats). However, in contrast with earlier experiments, rats in both groups had the same extensive opportunity to explore E_1 (the future test environment) in an undrugged state during the course of conditioning trials. This was accomplished by presenting half the saline injections in E_1 for both paired and unpaired conditions.

Given that the above procedure is expected to control for the possible confounding effects of stimulus novelty at the time of testing, a Pavlovian analysis of tolerance and withdrawal predicts that environmental cues should still elicit more conditioned withdrawal in subjects for whom the test environment was previously paired with morphine administration. While it is true that the additional exposure to the drug-paired environment in the drug-free state reduces the drug-environment contingency for subjects in the paired condition, this contingency remains a positive one. In the unpaired condition, on the other hand, the only pre-test exposure to the test environment occurs in the drug-free state, while morphine is administered in an alternative environment. This results in a negative contingency between the test environment and drug administration

for subjects in this condition. The Pavlovian analysis states that it is these differential contingencies that will result in context-elicited withdrawal in the paired, but not unpaired, conditions.

Given the results described in Chapter 2, the frequency of rearing was recorded and analyzed in the present experiment. The design of this study makes it improbable that any rearing observed at test is the consequence of the functional novelty of the testing environment. Thus, if the testing context elicits rearing differentially in paired and unpaired subjects, this is not attributable to novelty-induced exploratory behavior, and supports the suggestion that rearing remains a valid behavioral index of context-elicited morphine withdrawal (Azorlosa & Simmons, 1999).

It is unclear, however, that rearing is, in fact, a valid index of withdrawal distress in the present preparation. Therefore, other, more established, behavioral indices of morphine withdrawal will also be observed. Furthermore, the paired and unpaired conditions both include subjects receiving either high (50 mg/kg) or low (10 mg/kg) daily doses of morphine. While both these doses are within the range reported to support subsequent context-elicited withdrawal (e.g., Azorlosa & Simmons, 1999; Deffner-Rappold et al., 1996), it is of interest to examine any apparent dose-related differential in the frequencies of the recorded behavioral measures.

Method

Subjects and Drugs

The subjects were 42, experimentally-naïve, male Sprague-Dawley rats, weighing between 250 - 280g at the start of the experiment. All subjects were individually housed in plastic cages containing recycled paper bedding material, with food and water freely available (except during conditioning and test trials), and maintained on a 12:12 h photoperiod (lights on at 0600). All conditioning and testing took place during the light portion of the photoperiod.

Morphine sulfate was prepared as 10 mg/ml and 50 mg/ml solutions, and injected IP at a volume of 1 ml/kg. All saline injections were given equivolume to the corresponding morphine injection.

Environments, Design, and Procedure

Environments. Morphine and saline were injected in one of two environments (E_1 or E_2). E_1 was one of 6 identical, clear, acrylic chambers (30 cm X 30 cm X 30 cm) located in a distinctive, brightly illuminated room. These chambers were supported on stands, and a mirror was mounted under the chamber at a 45° angle to allow observation of the rat from below, as well as directly through the walls of the chamber. The E_2 environment was the rat's cage in the colony room.

Design. Withdrawal behaviors were evaluated in all rats on a test session following the conditioning phase of the experiment. During the conditioning phase, all rats were injected 20 times – twice per day for 10 days. The first injection (am) consisted

of physiological saline, and the second (pm) consisted of morphine. For different rats, the morphine dose was either 10 mg/kg or 50 mg/kg. For paired rats, all 10 morphine injections (either 10 mg/kg or 50 mg/kg, groups P10 and P50 respectively), and 5 of the saline injections, occurred in E_1. The remaining 5 saline injections occurred in E_2. For unpaired rats, all 10 morphine injections (either 10 mg/kg or 50 mg/kg, groups U10 and U50 respectively), and 5 of the saline injections, occurred in E_2. The remaining 5 saline injections occurred in E_1. The design of the experiment is summarized in Table 1.

Insert Table 1 here

In summary, although paired and unpaired rats differed in the environment in which they were injected with morphine (E_1 and E_2, respectively), rats in both groups also received additional exposure to the drug-paired environment in the undrugged state. Thus, paired rats had a total of 15 exposures to E_1 (10 while drugged and 5 while undrugged), and unpaired rats had a total of 5 exposures to E_1 (all while undrugged). The test environment, E_1, should be equally novel for paired and unpaired rats (if novelty depends on undrugged exposure to E_1), or less novel for paired than for unpaired rats (if novelty depends on total exposure to E_1). In neither case is E_1 more novel for paired than for unpaired rats, thus differential withdrawal behaviors among the groups cannot be attributed to the relatively greater novelty of the test environment for paired rats.

Procedure. Rats received two injections on each conditioning day. The first injection consisted of saline. The second injection, 5-6 hours later, consisted of either 10 mg/kg morphine (groups P10 and U10) or 50 mg/kg morphine (groups P50 and U50). The morphine injections were always given after saline on conditioning days so as to maximize the interval between morphine administration and the subsequent conditioning trial, thereby minimizing the possibility that the effects of morphine administration would still be present during a saline injection. For injections in E_1, rats were transported from the colony room to the distinctive environment, injected, and placed in the one of the chambers for 1 h. For injections in E_2, rats were removed from their home cages, injected, and returned to their cages.

On the test session that followed the last conditioning day, each rat was individually transported to E_1, injected with saline, and placed in the observation chamber. Behavior was videotaped for a 10 min period, and videotapes were later analyzed using behavioral data analysis software (The Observer: Noldus, NL).

A number of withdrawal behaviors were scored (see Azorlosa et al., 1994; Falls & Kelsey, 1989; MacRae & Siegel, 1997; McDonald & Siegel, 1998): Rearing (both front paws off the floor of the chamber, with body extended upward, with episodes of grooming that resulted in both front paws being off the floor not being scored as rearing), mouth movements (chewing and swallowing motions, which may also be accompanied by tongue protrusions), wet dog shakes (rapid shaking of the head and/or body), and ear wipes (front paws drawn over the ears from back to front).

Results

Although rats in all groups displayed substantial rearing on the test session, as displayed in Figure 2, there were no significant differences in the display of this behavior among the groups. Figure 3 summarizes the mean frequency of occurrence (± 1 SEM) of mouth movements. A 2 x 2 ANOVA performed on the mouth movement data revealed a significant interaction between dose (10 mg/kg or 50 mg/kg) and condition (paired or unpaired) (F [1, 38] = 6.52, p = .015). Subsequent pairwise comparisons using Tukey's HSD tests revealed that rats in Group P50 displayed a higher frequency of this behavior than did any other group (all ps < .001), and that rats in Group P10 displayed a higher frequency of this behavior than did rats in Group U10 (p = .04).

Insert Figures 2 and 3 here

Figures 4 and 5 show the mean frequencies of occurrence (± 1 SEM) for the measures of wet dog shakes and ear wipes, respectively. The large number of zero cell in the data matrices for both these measures required the use of non-parametric statistical analyses. As shown in Figure 4, wet dog shakes were infrequent in all groups, although a Kruskal-Wallis ANOVA by ranks revealed that the difference among groups was significant (H [3, N=42] = 8.03, p = .045). Subsequent pairwise comparisons with Mann-Whitney U tests, however, show that the only significant pairwise difference is that between Groups P10 and U10 (p = .005). Figure 5 shows that rats in paired groups

displayed more ear wipes than did rats in unpaired groups, and also displayed more variability. A Kruskal-Wallis ANOVA by ranks reveals that the difference between the groups on this measure is marginally significant (H [3, N=42] = 7.68, p = .05). Subsequent pairwise comparisons (Mann-Whitney U tests) revealed that the only significant between-group difference occurred with respect to Groups P50 and U10 (p = .014).

Insert Figures 4 and 5 here

Discussion

The results of the present experiment support the suggestion that morphine withdrawal can be elicited conditionally by contextual cues that have previously been paired with morphine administration (e.g., Azorlosa et al., 1994; Deffner-Rappold et al., 1996; Kelsey et al., 1990). However, they also support the suggestion raised in the previous chapter that rearing may not provide a valid, reliable, or particularly sensitive, behavioral index of conditioned morphine withdrawal. As can be seen in Figure 2, there were no differences in the mean frequency of rearing across either dose or condition. This may be due to the failure of the present manipulations to elicit conditioned withdrawal, or it may be due to relative familiarity of the test environment to all subjects. The present experiment controls for the possibility that the test environment may be functionally novel for Paired subjects. When testing occurred in a relatively familiar

environment for all subjects, no differences were observed in the mean frequency of rearing, despite the use of experimental procedures similar to those that have previously been demonstrated to produce context-elicited morphine withdrawal (e.g., Azorlosa et al., 1994; Deffner-Rappold et al., 1996; Kelsey et al., 1990). This supports the suggestion that rearing may not provide a sensitive behavioral index of withdrawal distress.

The above suggestion is further supported by the data summarized in Figure 3. The differential display of mouth movements across groups suggest that conditional morphine withdrawal was elicited by the drug-paired context in both Paired groups. Further, this effect was dose-dependent, with subjects conditioned with the higher dose displaying a greater frequency of mouth movements at test. Mouth movements have previously been observed to occur more frequently in rats experiencing morphine withdrawal (e.g., MacRae & Siegel, 1997), and may constitute a relatively sensitive behavioral index of withdrawal; the relatively high frequency of this behavior in almost all subjects allows for the use of more powerful parametric, rather than non-parametric, statistical analyses.

The data summarized in Figures 4 and 5, wet dog shakes and ear wipes, respectively, do not suggest the same dose by condition interaction seen in Figure 3. With regard to the behavior of wet dog shakes (Figure 4), the difference between Groups P10 and U10 was significant, although a similar difference was not observed between Groups P50 and U50. It is possible, however, that a conditioning history of repeated administration of the 50 mg/kg dose results in the unconditional display of wet dog

shakes at test in this preparation. Thus, wet dog shakes may be a sensitive index of context-elicited morphine withdrawal when relatively low conditioning doses are employed, but not when higher conditioning doses are used. The mean frequency of ear wipes across groups, as displayed in Figure 5, suggests a main effect of condition, with rats in Paired groups displaying a higher mean frequency than rats in Unpaired groups, but does not suggest that this effect is dose dependent. Paired subjects also displayed high within-group variability on this measure. Both wet dog shakes and ear wipes have served as behavioral indices of conditioned morphine withdrawal distress in previous studies (e.g., MacRae & Siegel, 1997), and the failure to observe significant dose by condition interactions in the present experiment may be due in part to the relatively short duration of the observation at test, and the fact that the observation period began immediately upon placement in the test environment. It may be that a longer test period, allowing for the collection of more behavioral data, would reveal the hypothesized interaction among groups for these measures; in particular, extending the test interval might serve to eliminate many of the zero cells in the data matrices, and reduce within-group variability.

Azorlosa and Simmons (1999) report the use of a procedure involving the physical restraint of subject animals when in the drug-paired environment during conditioning trials, and suggest that, when exploration of the drug-paired environment is controlled for in this manner, greater frequency of rearing is seen in subjects for whom the test environment was previously paired with drug administration. The study

described in the present chapter takes a different methodological approach to the issue of the functional novelty of the test environment; where Azorlosa and Simmons (1999) equated subjects by restricting all pre-test exploration of the environment, the present study equates subjects by allowing all subjects extensive opportunity to explore the test environment in the drug-free state prior to testing. As mentioned in the introduction to this chapter, physical restraint may prevent overt exploratory behaviors, but may still expose the subjects to visual, auditory, or olfactory cues associated with the test environment. Paired subjects will receive all their pre-test exposure to these cues in the drug state, and therefore these sensory properties of the test environment may be functionally novel for these subjects when tested in that environment in the drug-free state. This suggests that the differential frequencies of rearing observed by Azorlosa and Simmons (1999) may still be the result of the stimulus novelty of the test environment.

The substantial pre-test exposure of subjects to the test environment in the present study was intended to accomplish the same purpose as the restraint measures employed by Azorlosa and Simmons (1999), which was to equate various subjects in terms of pre-test exposure to the test environment. When the test environment is made familiar to all subjects prior to test, no differences in frequency of rearing are observed between Paired and Unpaired groups. There is, however, evidence of context-elicited withdrawal, as indicated by other behavioral measures. Both the present study and that conducted by Azorlosa and Simmons (1999) were designed to address the criticism of past studies of context-elicited withdrawal raised in the previous chapter. Given the nature of this

criticism, specifically that the differential expression of some behaviors previously seen to be evidence of context-elicited morphine withdrawal may, in fact, be the result of stimulus novelty, it would seem preferable to adopt control procedures that attempt to eliminate stimulus novelty for all subjects, rather than procedures that attempt to preserve stimulus novelty for all subjects. It would seem that these two approaches produce different results, as evidenced by the contrasting findings of Azorlosa and Simmons (1999) and the present study, with regard to the behavior of rearing. While the results described in the previous chapter make clear the need to control for the potential effects of stimulus novelty in studies of context-elicited morphine withdrawal, future investigators will have to choose between the two methodological approaches outlined above. It is suggested here that attempting to reduce or eliminate potential stimulus novelty will increase the validity of obtained behavioral measures, while attempting to preserve stimulus novelty through physical restraint may still result in differential exposure to environmental cues.

In summary, the results of the experiment described in the present chapter demonstrate context-elicited morphine withdrawal in an experimental preparation that eliminates the potentially confounding effects of stimulus novelty at test. While any between-group differences in frequency of behavioral indices of withdrawal observed in this preparation would therefore be attributable to manipulations of drug dose and drug-context pairing, no such differences were observed in frequency of rearing. This finding has implications for the work of other researchers who have reported instances of

context-elicited morphine withdrawal in preparations where the potentially confounding effects of stimulus novelty were not taken into account, particularly those studies where frequency of rearing provides the most compelling evidence of withdrawal distress (e.g., Azorlosa et al., 1994; Deffner-Rappold, et al., 1996). Mouth movements, on the other hand, would appear to provide a sensitive behavioral index of morphine withdrawal. The frequency of this behavior differed significantly across groups, with subjects in the Paired groups displaying more mouth movements than subjects in Unpaired groups, and subjects conditioned with the 50 mg/kg dose displaying more mouth movements than subjects conditioned with the 10 mg/kg dose. The behavior of ear wipes also provided evidence of context-elicited withdrawal, although this measure did not appear to be sensitive to conditioning dose.

Studies of context-elicited withdrawal (e.g., Azorlosa et al. 1994; Deffner-Rappold et al., 1996; Kelsey et al., 1990) can be, and have been, interpreted as confirming predictions generated by a Pavlovian conditioning analysis of drug tolerance and withdrawal. While the data described in both the previous and present chapters call into question the interpretation of the results of some of these previous putative demonstrations of context-elicited morphine withdrawal, the results of the present experiment show that evidence of context-elicited morphine withdrawal can still be seen in a preparation where the test environment is familiar to all subjects. Furthermore, the behavioral indices of withdrawal in the present study are not readily attributable to exploratory behavior.

CHAPTER 4

Withdrawal Responses Elicited By Pharmacological Cues:

Morphine-Precipitated Morphine Withdrawal

Introduction

As discussed in Chapter 1, most experiments designed to assess the role of pre-drug cues in drug effects have assessed diffuse environmental cues (e.g., Azorlosa et al., 1994; Azorlosa & Simmons, 1999; Kelsey et al., 1990). In such studies it is generally demonstrated that rats are more tolerant to a drug when it is administered in the usual drug-paired environment (e.g., the room where the drug had been administered in the past) than when it is administered elsewhere (e.g., in a different room, Kayan, Woods, & Mitchell, 1969). Similarly, rats display more withdrawal symptoms when, in the absence of the drug, they are placed in the drug paired environment than if they are placed in an alternative environment (e.g., Kelsey et al., 1990).

Although studies of the associative basis of tolerance have typically paired such environmental cues with drug administration, there is evidence, as discussed in Chapter 1, that a small dose of a drug may serve as a CS, signaling a subsequent, larger dose of the drug (Greeley & Ryan, 1995). Small doses of both ethanol (Greeley, et al., 1984), and morphine (Cepeda-Benito & Short, 1997) have been shown to function as drug-predictive cues in subjects with a history of exposure to larger doses of those respective drugs.

The fact that a small dose of a drug may serve as a cue for a larger dose of that drug may have important implications for an associative analysis of drug effects. A gradual increase in systemic concentration is an inevitable consequence of many drug administration procedures. That is, typically small, drug-onset cues reliably precede subsequent larger drug effects, thus there is the potential for intra-administration associations whenever a drug is administered. These drug-onset cues may constitute an important component of the CS that elicits the CCRs that mediate tolerance (see Grisel, Wiertelak, Watkins, & Maier, 1994; Kim et al., 1999; Krank, 1987; Poulos & Cappell, 1991; Tiffany, Petrie, Baker & Dahl, 1983; Walter & Riccio, 1983).

On the basis of the conditioning analysis, withdrawal symptoms – a manifestation of a pharmacological CR – should be elicited not only by drug-associated environmental cues, but also by drug-associated pharmacological cues. Thus, if an intra-administration association was formed during a series of morphine administrations, presenting a small dose of the opiate might be expected to reproduce the early effect of the drug – an effect that had become associated with the subsequent, larger effect. The CR elicited by this pharmacological CS, in common with the CR elicited by an environmental CS, should be manifest as withdrawal symptoms.

Typically, for the organism with a history of morphine administration, administration of the opiate prevents withdrawal symptoms from occurring. We are suggesting that a small dose of the drug should actually elicit the symptoms. The purpose of this experiment was to evaluate this counterintuitive prediction.

Method

Subjects

The subjects were 81, male, Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada), weighing between 250-450g on the first day of the experiment. All subjects were individually housed in plastic cages, with food and water available ad lib. except during conditioning and test trials. Water, but not food, was available ad lib. during habituation sessions. Subjects were maintained on a 12:12 hr photoperiod, (lights on at 0600). All conditioning and testing took place during the light portion of the cycle.

Drugs and Apparatus

Morphine sulfate (British Drug House) was dissolved in physiological saline such that the IP injection volume for both large (50 mg/kg) and small (5 mg/kg) doses of the opiate was 1 ml/kg. Saline was also injected IP at this volume.

Testing was conducted in one of six identical, clear, acrylic observation chambers (30 cm X 30 cm X 30 cm) located in a distinctive, brightly illuminated room. These chambers were supported on stands, and a mirror was mounted under the chamber at a 45° angle to allow observation of the rat from below, as well as directly through the walls of the chamber.

Procedure

Prior to conditioning, all subjects were habituated to the testing chambers during a single, 6 h session. Following habituation, subjects were randomly assigned to one of six groups as follows: Group Mm (n = 17), Group Ms (n = 17), Group sm (n = 16),

Group ss (n = 16), Group mm (n = 8), and Group ms (n = 7). For each group the first letter of the group designation indicates the treatment during conditioning; 50 mg/kg morphine (M), 5 mg/kg morphine (m), or saline (S). The second letter indicates the treatment at test; 5 mg/kg morphine (m) or saline (s). Conditioning began the day following habituation, and consisted of IP injection of morphine or saline once daily for 10 consecutive days. All injections took place in the colony room. Subjects were removed from their home cage, injected, and immediately returned to the home cage.

On the day following the 10th conditioning day, subjects were transported to the testing room in groups of six, selected randomly across experimental groups. Subjects were placed individually in testing chambers for 5 min, removed, injected with morphine (5 mg/kg) or saline, and returned to the testing chamber. Observation of behavior began 10 min following injection, and lasted for 30 min. The 30 min observation period was divided into 5 blocks of 6 min each. During this phase each subject was observed for 1 min during each 6-min block in a cycling procedure, resulting in a total observation period of 5 min (1 min from each of 5 blocks) for each subject during the 30 min interval. Behaviors were scored by a rater blind to testing conditions (m or s).

Analysis

The behaviors scored during testing were rearing (both front paws off the floor of the chamber, with body extended upward, with episodes of grooming that resulted in both front paws being off the floor not being scored as rearing), wet dog shakes, and mouth movements (consisting of chewing or swallowing motions, as well as tongue protrusions). Rearing was included as a behavioral index of withdrawal, as the 6h pre-

exposure to the testing environment for all subjects eliminates the possibility that this behavior is a response to the functional novelty of the test environment.

Results and Discussion

Figures 6 through 8 show the mean frequency of withdrawal behaviors for each group. As can be seen in these figures, the small dose of morphine suppressed all behaviors in subjects in receiving the drug for the first time on this test session (Group sm). However, this same small dose elicited considerable withdrawal behaviors in subjects that had pretest experience with the large dose of morphine (Group Mm). In fact, Group Mm subjects displayed a greater mean frequency of both rearing and mouth movements than did subjects in any other group. Wet dog shakes (Figure 8) were only observed in subjects conditioned with the 50 mg/kg dose, and then only at low frequencies; most subjects displayed no wet-dog shakes, and no group had a mean frequency of this behavior greater than 1.

Insert Figures 6, 7, and 8 here

Because of heterogeneity of variance, the results summarized in Figures 6 through 8 were analyzed using Kruskal-Wallace nonparametric analyses of variance. As seen in Figure 6, there was a significant difference among groups in mean frequency of rearing (H [5, N = 81] = 37.37, $p < .0001$). Subsequent Mann-Whitney U tests indicated that subjects in Group Mm reared more frequently than did subjects in any other group (all ps $< .005$). The data summarized in Figure 7 shows that there was also a significant

difference among groups in mean frequency of mouth movements (H [5, N = 81] = 55.85, $p < .0001$). Additional Mann-Whitney U tests revealed that Group Mm subjects displayed more mouth movements (all $ps < .01$) than did subjects in any other group. Wet dog shakes (Figure 8) occurred very infrequently, and only rats in Group Ms displayed a mean frequency significantly greater than zero for this measure.

The results of this study indicate that animals pretreated with 50 mg/kg morphine display greater frequency of withdrawal behaviors when tested following administration of 5 mg/kg morphine than when tested following administration of saline (Group Mm vs. Group Ms). These increases in behavior were seen following a dose of morphine (5 mg/kg) that produced a complete suppression of behavior in morphine-naive animals (Group sm). Furthermore, animals that received 5 mg/kg of morphine during both conditioning and testing (Group mm) displayed significantly less withdrawal behavior than did Group Mm, thus demonstrating that the 5 mg/kg dose was only effective in eliciting withdrawal behaviors when it followed conditioning with the 50 mg/kg dose. These results support the initial hypothesis that the early effects of a drug administration serve as CSs signaling the subsequent full effects of the drug.

Interestingly, similar evidence of withdrawal following a low dose of nicotine has been observed in human smokers who regularly consume high-nicotine cigarettes (Schachter, 1977); as discussed in Chapter 1, more withdrawal distress was reported by subjects who were self-administering lower than normal doses of nicotine than was reported by subjects who maintained nicotine intake. While the main focus of Schachter's (1977) study was self-regulation of nicotine intake, rather than nicotine

withdrawal, the results may reflect an instance of nicotine-precipitated nicotine withdrawal.

Past studies of the contribution of pharmacological cueing in drug effects (Cepeda-Benito & Short, 1997; Greeley et al., 1984) have used discrete drug administrations as CSs (i.e., injection of a small amount of the drug, followed later by injection of larger amounts of the drug). The results of the present study indicate that pharmacological associations are formed within the course of a single drug administration (a possibility entertained by several investigators, e.g., Grisel, Wiertelak, Watkins, & Maier, 1994; Kim & Siegel, in press; Kim et al., 1999; Krank, 1987; Poulos & Cappell, 1991; Tiffany, Petrie, Baker & Dahl, 1983; Walter & Riccio, 1983).

The results of the present study, indicating that pharmacological intra-administration signals elicit drug withdrawal symptoms, are consistent with the results of other studies apparently indicating that environmental signals can elicit drug withdrawal symptoms (e.g., Azorlosa, Hartley, & Deffner-Rappold, 1994; Deffner-Rappold, Azorlosa, & Baker, 1996; Kelsey, Aranow, & Matthews, 1990). However, as noted in Chapter 2, there is some ambiguity in interpreting the results of prior demonstrations of the role of drug-paired environmental cues in withdrawal-like behaviors – the withdrawal behaviors might actually represent exploratory behaviors. That is, when a rat with a history of morphine administration is tested in the drug-paired environment while drug-free, this environment may be treated as novel (the rat having been narcotized on prior occasions when it was placed in this environment). Some behaviors typically scored as

withdrawal symptoms (e.g., rearing) may actually be exploratory responses elicited by this functionally-novel test environment. Novelty-elicited exploration, however, is not applicable to the present experiment. All rats received the same, 6-hr, undrugged habituation to the observation chamber prior to the start of the experiment. Following pretest injections (M, m, or S) in their home cage environment, on the test session all rats were again placed in the same test chamber to assess withdrawal-behaviors. Thus, in contrast with some studies that have evaluated the effect of environmental cues on withdrawal behaviors, the rats in the present study were all equated with respect to pretest, undrugged exposure to the test environment.

Another alternative interpretation of the present results would suggest that the behaviors observed at test in Group Mm subjects were stereotypical locomotor behaviors resulting from sensitization to the locomotor effects of morphine. Sensitization to the effects of morphine administration would result in an increased response to a given dose over repeated administration, and thus the magnitude of the drug effect would increase with increased exposure to the drug. If this were the case, it would be expected that rats in Group Mm would display the highest frequency of such behaviors, as they had received far larger amounts of the drug during conditioning, and would, thus, display the greatest sensitization. Reports of increases in stereotypical behaviors following repeated exposure to morphine have been common in the literature for some time (e.g., Martin, Wikler, Eades, & Pescor, 1963). In the present study, the behavior of mouth movements was observed to be more frequent at test in Group Mm than in any other group. Sensitization of oral stereotypies following repeated exposure to morphine has also been

reported (Kraus, Piper, & Kornetsky, 1997; Pollock & Kornetsky, 1996), leading to the suggestion that the behaviors measured in the present study reflect sensitization, rather than a conditioned compensatory response. While this interpretation offers another explanation for the frequency of mouth movements in subjects with a history of extensive exposure to morphine, the behaviors observed and recorded as mouth movements in the present study differ markedly from the oral stereotypies described by Kornetsky and colleagues (Kraus et al., 1997; Pollock & Kornetsky, 1996). These oral stereotypies typically consist of gnawing or chewing on some physical substrate (e.g., on the floor or walls of an observation chamber). Observations of this sort are also frequent in taste reactivity studies employing relatively high doses of morphine (e.g., McDonald, Parker, & Siegel, unpublished data). The mouth movements analyzed in the present study would appear to be a fundamentally different type of behavior. The chewing/swallowing motions and tongue protrusions recorded here were not directed at any physical substrate, but usually occurred while the rat was moving around the observation chamber. Furthermore, if these behaviors were directed at some physical substrate, they were not recorded as mouth movements for the purpose of analysis. The behaviors scored as mouth movements in the present experiment would appear to be reflective of excessive salivation, an expected compensatory response to the unconditional drying effects of morphine on secretory membranes (Jaffe & Martin, 1990). As such, they would be indicative of pharmacologically-elicited withdrawal rather than sensitization. Nevertheless, the issue of sensitization of certain locomotor behaviors in rats with a history of morphine exposure warrants further investigation, particularly with regard to

the potentially confounding effects such sensitized behaviors may have in studies of conditionally-elicited morphine withdrawal. Two possible methods of resolving this issue are explored in the experiment described in Chapter 5: Inclusion of a physiological index of morphine withdrawal and the extension of the testing phase over several days. The latter modification should be particularly effective in distinguishing between sensitization- and conditioning-based explanations of the results obtained in the present experiment; the sensitization-based explanation would suggest that the sensitized behaviors would only increase with additional exposure to morphine, whereas a Pavlovian conditioning analysis would suggest that, for subjects in Group Mm, additional test trials would, in effect, constitute extinction trials. That is, repeated exposure to the small drug effect cue in the absence of the larger drug effect associated with the larger dose would be expected to attenuate the ability of the small dose to serve as an effective CS, and this should result in decreased, not increased, withdrawal behaviors over time.

In summary, these results suggest that intra-administration associations are a feature of drug administration. That is, within each administration an early small drug effect may become associated with the later larger drug effect. Associative analyses of drug tolerance and dependence should incorporate such pharmacological associations that may develop even if there is no explicit, environmental signal for the drug.

CHAPTER 5

Morphine-Precipitated Morphine Withdrawal:

Behavioral And Physiological Measures

Introduction

As demonstrated in the experiment described in Chapter 4 and elsewhere (e.g., Kim et al., 1999), the early, small effects of a single administration may come to signal the later peak effects, thus producing intra-administration associations. In subjects with a history of exposure to large doses of morphine, a small dose of morphine may act as a signal for the expected later effects of the usual, much larger, dose. In such a case, the small dose can elicit CCRs in anticipation of the peak effects of the larger dose. If this larger dose fails to materialize, and the CCRs are unopposed by the unconditional effects of the larger dose, they are evident as withdrawal behaviors (see Chapter 4). While counterintuitive, the phenomenon of morphine-elicited morphine withdrawal is predicted under such circumstances by a Pavlovian analysis of drug tolerance and withdrawal (see Siegel et al., 2000).

While the data described in Chapter 4 support the prediction that morphine withdrawal may, under such circumstances, actually be elicited by administration of morphine, the literature suggests a possible alternative explanation for this data; that is, those rats displaying the greatest frequency of withdrawal behaviors also have the greatest total exposure to morphine, and behaviors interpreted as reflective of CCRs may

in fact be the product of sensitization to the locomotor effects of the drug (e.g., Kraus, Piper, & Kornetsky, 1997; Pollock & Kornetsky, 1996). In light of this criticism, the present experiment attempts to demonstrate morphine-elicited morphine withdrawal under circumstances that render a sensitization-based explanation problematic.

The present study was designed to monitor the thermoregulatory effects of morphine, as well as the thermoregulatory effects of morphine-precipitated withdrawal. Administration of morphine over the range of doses employed in the present study has been observed to elicit hyperthermia (e.g., Broadbent & Cunningham, 1996; Clark, 1979; Eikelboom & Stewart, 1979; Geller, Hawk, Keinath, Tallarida, & Adler, 1983; Mucha, Kalant, & Kim, 1987; Numan & Lal, 1981; Paolino & Bernard, 1968). In contrast with the hyperthermic unconditional effects of morphine, hypothermia has been shown to be "one of the most consistent and reliable parameters of [morphine] withdrawal in the rat" (Gianuttsos, Drawbaugh, Hynes, & Lal, 1975, p. 302). Temperature was monitored using biotelemetry in order to avoid the potentially confounding effects of stress due to handling, which are inherent in temperature measurement by rectal probe (Broadbent & Cunningham, 1996). A second potential confound, inherent in the handling and IP injection of morphine (Broadbent & Cunningham, 1996), was minimized by recording temperature for a period of over 5 h following injection; the temperature changes associated with handling and injection are expected to be relatively transient with respect to the more robust and longer-lasting thermoregulatory effects of morphine itself.

A second measure was also taken in the present study to address the potential confound of sensitization of morphine-induced oral stereotypies. Sensitization, by definition, involves an increase in the frequency or magnitude, or decrease in latency, of a response to a stimulus as a function of cumulative exposure to that stimulus. A decrease, rather than an increase, in responding across repeated presentation of the drug-predictive CSs would argue against a sensitization-based interpretation of the results.

It has been demonstrated that repeated presentation of drug-predictive CSs in the absence of subsequent drug administration attenuates tolerance to both the lethal (Siegel, Hinson, & Krank, 1979) and analgesic (Siegel, Sherman, & Mitchell, 1980) effects of morphine. That is, CSs that were previously paired with drug administration, and subsequently came to elicit CCRs, will lose the power to elicit those CCRs if they are repeatedly presented without the US. Furthermore, even when drug administration is continued, but now in an explicitly unpaired manner with the CSs, the ability of those CSs to elicit CCRs is attenuated (Faneslow & German, 1982). If the behavioral and/or thermoregulatory responses to a small dose of morphine in rats conditioned with a large dose of morphine are seen to be attenuated over repeated administrations of the small dose, this result would argue strongly against a sensitization interpretation.

The present experiment was designed to assess both the behavioral and thermoregulatory effects of a small dose of morphine (5 mg/kg) in rats previously conditioned with a large dose of morphine (50 mg/kg). In addition, the effects of the small dose were measured over repeated administrations following conditioning with the

large dose. A conditioning-based analysis of morphine tolerance and withdrawal suggests that the small dose will produce both behavioral and thermoregulatory indices of withdrawal, and further that these effects will be attenuated, not potentiated, by repeated administrations of the small dose. This attenuation, which is predicted to occur despite the steadily increasing history of exposure to the drug, would provide support for the counterintuitive prediction of morphine-precipitated morphine withdrawal and argue against interpretations based on sensitization to the effects of morphine.

Method

Subjects

The subjects were 35, male, Sprague-Dawley rats (Charles River, St. Constant, Quebec, Canada), weighing between 230-285g on the first day of the experiment. All subjects were individually housed in clear plastic cages, with food and water available ad lib. except during a 10 min behavioral assessment period on test days. Water, but not food, was available ad lib. during habituation sessions. Subjects were maintained on a 12:12 hr photoperiod, (lights on at 0600). All conditioning and testing took place during the light portion of the cycle. During the 11 to 15 days of conditioning and testing the subjects were housed in their home cages in the experimental environment.

Surgery

One week prior to the start of conditioning all subjects were implanted with radiotelemetric transmitters (Mini-Mitter, Bend, WA, model PDT4000). These transmitters were enclosed in a 22 x 8 mm capsule, and weighed 1.6 g. Subjects were

anaesthetized with a 2 : 1 : 1 mixture of ketamine, xylazine, and saline injected IP at a volume of 2 ml/kg. A small (approx. 1.5 cm) incision was made through the abdominal wall and the transmitter capsule was inserted into the abdominal cavity. Subjects were then administered an oral antibiotic (Novo-Trimmel) in their drinking water during the one-week recovery period following surgery. No sign of post-surgical infection was observed in any of the subjects.

Drugs and Apparatus

Morphine sulfate (British Drug House) was dissolved in physiological saline such that the IP injection volume for both large (50 mg/kg) and small (5 mg/kg) doses of the opiate was 1 ml/kg. Saline was also injected IP at this volume.

Behavioral testing was conducted in one of six identical, clear, acrylic observation chambers (30 cm X 30 cm X 30 cm) located in the experimental room. These chambers rested on the telemetric receiving units (Mini-Mitter, Bend, WA, model ER4000), which also provided the power to operate the telemetric system. Temperature data were automatically collected by the controlling software (VitalView, Mini-Mitter, Bend, WA) at 20 min intervals during all conditioning and test trials.

Procedure

Prior to conditioning, all subjects were habituated to the testing chambers during a single, 6 h session. Following habituation, subjects were randomly assigned to one of six groups as follows: Group Mm (n = 10), Group Ms (n = 5), Group sm (n = 5), Group ss (n = 5), Group mm (n = 5), and Group ms (n = 5). For each group the first letter of the

group designation indicates the treatment during conditioning; 50 mg/kg morphine (M), 5 mg/kg morphine (m), or saline (S). The second letter indicates the treatment at test; 5 mg/kg morphine (m) or saline (s). Conditioning began the day following habituation, and consisted of IP injection of morphine or saline once daily for 10 consecutive days. On the first conditioning day the subjects were transported to the testing room in their home cages, and these cages were placed on the receiving pads . Temperature data were recorded for a minimum of 30 min prior to injection, following which subjects were removed from their home cage, injected, and immediately returned to the home cage. Temperature data were recorded for 330 min following injection. Subjects remained in the testing room for the duration of the experiment, and the time of the daily injections was varied from 1000 to 1600 on various conditioning days in order to minimize the possibility that cues inherent in the subjects circadian patterns would come to acquire control over conditional thermoregulatory responding (Eikelboom & Stewart, 1981).

On the day following the 10^{th} conditioning day, subjects were individually removed from their home cages and placed in the observation chambers, which replaced the home cages on the receiving pads. After a minimum of 30 min of temperature data collection, subjects were injected with 5 mg/kg morphine or saline, depending on group assignment, and videotaped for a period of 10 min following injection (temperature data collection continued throughout behavioral testing). Videotaped behaviors were later analyzed using behavioral data collection software (The Observer, Noldus, NL). At the end of the 10 min behavioral data collection period, subjects were transferred back to the home cage and temperature data collection continued for the rest of the 330 min interval.

All subjects in Groups Mm were given additional test trials for a further 4 days to assess the effects of repeated administration of the small dose of morphine. These trials were identical in all respects to the first test trial on Day 11.

Analysis

The behaviors scored during testing were rearing, wet dog shakes, mouth movements, ear wipes, and genital licks. All the behaviors recorded on Day 11, with the exception of rearing, displayed a marked heterogeneity of variance among groups, and were therefore analyzed using a non-parametric procedure (Kruskal-Wallace ANOVA). Rearing data was analyzed using a 3 x 2 ANOVA. The behavioral data collected from subjects in Group Mm on Days 11 through 15 were analyzed using a Friedman ANOVA X^2 across testing days.

Temperature data from Day 1 (first conditioning day), Day 10 (final conditioning day), and Day 11 (first test day) are presented for all subjects. In addition, temperature data gathered from subjects in Group Mm on Day 13 (third test day) and Day 15 (fifth test day) are reported. All temperature data are presented as Plot plus Error.

Results

Behavioral Data

Day 11

Behavioral data collected on the first test day following 10 days of conditioning (Day 11) are summarized in Figures 9 through 13. As can be seen in Figure 9, there were no significant differences among groups in the mean frequency of rearing. There were,

however, significant differences among groups in mean frequency of mouth movements (Figure 10, $\underline{H}$ [5, N = 35] = 26.41, $\underline{p}$ = .0001), wet dog shakes (Figure 11, $\underline{H}$ [5, N = 35] = 15.95, $\underline{p}$ = .007), ear wipes (Figure 12, $\underline{H}$ [5, N = 35] = 16.81, $\underline{p}$ = .0049), and genital licks (Figure 13, $\underline{H}$ [5, N = 35] = 21.31, $\underline{p}$ = .0007). Subsequent pairwise analyses using Mann-Whitney U-tests revealed that subjects in Group Mm, who were tested with 5 mg/kg of morphine, displayed a greater mean frequency of mouth movements than all other groups ($\underline{p}$s < .05). Subjects in Group Mm also displayed a greater mean frequency of wet dog shakes than did subjects in Groups mm, sm, and ss ($\underline{p}$s < .05). The differences between Group Mm and Groups Ms and mm on this measure approached significance ($\underline{p}$s = .058 and .11, respectively). It was further revealed that rats in Group Mm displayed a greater mean frequency of both ear wipes and genital licks than did rats in all other groups ($\underline{p}$s < .05).

Insert Figures 9, 10, 11, 12, and 13 here

Days 13 and 15

Subjects in Group Mm, who received 5 mg/kg morphine at test, were administered 5 mg/kg for a further 4 days following the initial test. The mean frequencies of each of the recorded behaviors on alternate test days (Days 11, 13, and 15) are displayed in Figures 14 through 18. As can be seen in these figures, the mean frequency of each of the recorded behaviors decreased across days. In the case of rearing

(Figure 14), this difference was not significant. Analyses of the data summarized in Figures 15 through 18 using Friedman ANOVA by ranks reveal that the difference in mean frequency across days was significant for the behaviors of mouth movements ($\underline{X}^2$ [N = 10, df = 2] = 14.31, $\underline{p}$ = .0008), wet dog shakes ($\underline{X}^2$ [N = 10, df = 2] = 16.53, $\underline{p}$ = .003), and ear wipes ($\underline{X}^2$ [N = 10, df = 2] = 9.45, $\underline{p}$ = .009). The difference in mean frequency of genital licks across days approached significance ($\underline{X}^2$ [N = 10, df = 2] = 5.42, $\underline{p}$ = .067).

Insert Figures 14, 15, 16, 17, and 18 here

Temperature data

Day 1

In view of the clarity of the results when presented as Plot plus Error, and the difficulties inherent in applying statistical analyses to these data (e.g., Groups Mm and Ms are expected to generate similar data over much of the observation period), no statistical analyses were performed on the temperature data. Figure19 displays the mean temperature (± SEM) for all groups on the first conditioning day (Day 1). On this day subjects in Groups Mm and Ms were injected with 50 mg/kg morphine, subjects in Groups mm and ms were injected with 5 mg/kg morphine, and subjects in Groups sm and ss were injected with saline. As can be seen in Figure 19, administration of morphine at either 50 or 5 mg/kg resulted in a hyperthermic response evident for 4.5 to 5 h following

injection. Mean body temperature in these groups peaked at the same level, suggesting a ceiling of about 40 °C for hyperthermic changes in temperature in the rat. Administration of saline resulted in a less extreme and much shorter hyperthermic response, with temperatures returning to baseline levels after about 1 h, which is interpreted as the result of the stress induced by handling and injection procedures, rather than any direct thermoregulatory effect of saline administration.

Insert Figure 19 here

Day 10

Mean temperature (± SEM) for all groups on the tenth consecutive conditioning day (Day 10) is displayed in Figure 20. For the purposes of comparison, data from Day 1 is also plotted. As can be seen in Figures 20a and 20b (Groups Mm and Ms, respectively), the hyperthermic response to the tenth injection on 50 mg/kg of morphine is much like that seen on Day 1. While both maximal hyperthermia and the duration of the hyperthermic effect are similar, latency to maximal hyperthermia is reduced in Group Ms, although this effect is not readily apparent in Group Mm. Groups mm and ms (Figures 20c and 20d, respectively) also show similar temperature profiles in response to injection of 5 mg/kg on Days 1 and 10, although decreased latency to maximal hyperthermia is evident in both of these groups. Figures 20e and 20f reveal that the

hyperthermic response to handling and saline injection in Groups sm and ss is attenuated by the tenth day of conditioning.

Insert Figure 20 here

Day 11

Mean temperature (± SEM) for all groups on the first test day (Day 11) is shown in Figure 21. For purposes of comparison, data from Day 1 and Day 10 are also plotted. Figure 21a (Group Mm) shows that subjects conditioned with 50 mg/kg on Days 1 through 10 displayed a marked change in temperature profile when administered 5 mg/kg on Day 11. Hyperthermia is evident during the first 2 h following injection, and hypothermia (0.5 to 1.0 °C below baseline body temperature) from 2.5 to 4 h post-injection. When subjects with an identical conditioning history were administered saline on Day 11 (Group Ms, Figure 21b), a brief, mild hyperthermic effect is evident, following which mean temperature returned to baseline for the duration of the test interval. Subjects in Group mm (Figure 21c) display an almost identical hyperthermic response to 5 mg/kg on Day 11 as was seen when the identical dose was administered on Days 1 and 10. Subjects in Group ms (Figure 21d), also conditioned with 5 mg/kg morphine on Days 1 through 10, display a brief, mild hyperthermic response to injection with saline on Day 11, following which temperature returned to baseline. Morphine-naïve subjects administered 5 mg/kg of morphine for the first time on Day 11 (Group sm,

Figure 21e) displayed a robust hyperthermic response that persisted for 5 h, while subjects receiving saline for the eleventh consecutive day (Group ss, Figure 21f) show no deviation from baseline temperature. For purposes of comparison, the mean temperature for all groups on Day 11 is also plotted in Figure 22.

Insert Figures 21 and 22 here

Days 13 and 15

Subjects in Group Mm, conditioned with 50 mg/kg, were administered 5 mg/kg once daily for an additional 4 days following the first test on Day 11, and mean temperature (± SEM) data from Days 11,13, and 15 are included in Figure 23. As can be seen, the hypothermic response observed from 2.5 to 4 h post-injection on Day 11 is not seen on either Day 13 or Day 15. On the subsequent test days subjects displayed a hyperthermic response lasting approximately 3 h, following which temperature returned to baseline level for the duration of the test period.

Insert Figure 23 here

Discussion

The behavioral data collected in this experiment support the hypothesis that morphine withdrawal can be elicited by administration of morphine. Subjects

conditioned with 50 mg/kg and tested with 5 mg/kg showed a greater mean frequency of all the withdrawal measures (with the exception of rearing) than did subjects with an identical conditioning history treated with saline at test. This result suggests that the 5 mg/kg dose of morphine effectively replicated the early effects of the 50 mg/kg dose, and further, that this pharmacological cue had come to control the expression of CCRs in this preparation. Withdrawal behaviors elicited by the 5 mg/kg dose in Group Mm subjects were not observed in other subjects receiving the same dose at test (Groups mm and sm), suggesting that such behaviors are not an unconditional effect of the 5 mg/kg dose, and that the ability of this dose to elicit such behaviors is conditional on a history of substantially higher doses of morphine during the conditioning phase.

The observation that mean frequency of rearing did not differ among groups adds weight to the contention that rearing may not be a reliable or valid index of withdrawal distress (McDonald & Siegel, 1998); subjects never exposed to morphine at any dose (Group Ss) displayed a mean frequency of rearing equivalent to all other groups at test. This lack of difference is particularly striking with respect to subjects in Group Mm, which otherwise displayed higher indices of withdrawal than all other groups on all other behavioral measures. This result suggests that previous studies where rearing provides the most compelling evidence of elicited withdrawal (e.g., Azorlosa et al., 1994), even when subjects are physically restrained during conditioning (Azorlosa & Simmons, 1999), warrant re-evaluation.

The behavior of mouth movements, on the other hand, would appear to provide a highly sensitive index of morphine withdrawal. The results depicted in Figures 9 through 13 indicate that this was the most frequently observed behavior in all groups, and examination of Figures 9 through 13 reveals that frequency of mouth movements is correlated with frequency of other established indices of withdrawal. The criticism raised in Chapter 4, namely that this behavior reflects sensitization of oral stereotypies, is refuted by the data shown in Figure 15. If sensitization were responsible for the high frequency of this behavior in rats in Group Mm at test, additional exposure to the drug should result in further increases in the frequency of this behavior on Days 13 and 15, or, at least, no decrease in this measure. As can be clearly seen, however, this behavior decreased in frequency over repeated administrations of the 5 mg/kg dose, thus rendering a sensitization-based interpretation of these results problematic.

The temperature data collected in this experiment also supports the hypothesis that morphine withdrawal can be elicited by morphine administration. With the exception of a decrease in latency to peak hyperthermia across conditioning days, an effect also noted by Broadbent and Cunningham (1996), the hyperthermic response to morphine at both the 50 mg/kg and 5 mg/kg doses remained relatively unchanged across conditioning days. As noted by Eikelboom and Stewart (1979), morphine-induced hypothermia is, in some cases, an observed consequence of morphine administration, but this effect appears early and disappears over the course of repeated administrations. No evidence of hypothermia was observed in the present study on any of the first 10 days of

conditioning in any experimental group. The only evidence of a hypothermic response is seen in Group Mm subjects on the first day that they received the small dose of the drug. While hypothermic responses to small doses of morphine have been reported (Eikelboom & Stewart, 1979), these effects were only observed on early trials and disappeared after repeated exposure to morphine. The fact that the response appeared for the first time in the present experiment only after a substantial exposure to the drug over several days suggests that the hypothermic effect observed here was produced by mechanisms different from those responsible for the transitory hypothermic effects reported by Eikelboom and Stewart (1979).

One of the purposes of recording body temperature in the present study was to demonstrate morphine-precipitated morphine withdrawal using a dependent variable that ruled out sensitization-based alternative interpretations of the data. Sensitization may play a role in morphine's thermoregulatory effects, as suggested by Broadbent and Cunningham (1996). Broadbent and Cunningham report that the hyperthermic response shows a decreased latency to maximal hyperthermia over repeated administrations, and therefore the clearly opposite hypothermic effect of morphine withdrawal should not be confounded by such sensitization. That is, if a small dose of morphine elicits hypothermia in rats previously conditioned with repeated large doses, this cannot be confused with the increasingly rapid hyperthermic effects of the large dose or the consistently reported hyperthermic effects of small doses of morphine. Therefore, the hypothermic response to the small dose in Group Mm subjects is unlikely to be the result

of sensitization. The facts that both physiological and behavioral evidence of withdrawal were obtained simultaneously, and further, that both the hypothermia and frequency of mouth movements decreased over repeated testing, suggest that neither of these indices is confounded by sensitization effects.

The hypothermic response observed in Group Mm subjects on Day 11 would appear to be the result of a CCR acting to oppose the anticipated unconditional hyperthermic effects of the 50 mg/kg dose. The nature of the temperature profile for these subjects on Day 11 suggests an initial hyperthermic effect of the 5 mg/kg dose, which is eventually counteracted by a strong, opposing, hypothermic CR. The initial hyperthermic effect observed in Group Mm subjects on Day 11 is evident only for the first 2.5 h following injection, following which a period of hypothermia is observed; administration of the same dose in any other group on any conditioning or test day produced hyperthermia lasting over 4 h, and no evidence of hypothermia at any point during the observed interval.

CHAPTER 6

General Discussion And Conclusion

As discussed in Chapter 1, both external, environmental cues (e.g., Siegel, 1978) and internal, pharmacological cues (e.g., Cepeda-Benito & Short, 1997) have been shown to acquire control over the expression of tolerance to the effects of morphine. Furthermore, other, more recent, studies have suggested a number of factors that may influence the relative ability of these different types of cues to acquire such control (Grisel et al., 1994; Kim et al., 1999). The experiments reported in this thesis demonstrate that both external, environmental cues (Chapters 2 and 3), and internal, pharmacological cues (Chapters 4 and 5) can come to serve as drug-predictive CSs. When these drug-predictive CSs are presented in the absence of the drug (or the absence of the subsequent peak effects of a larger dose of the drug, in the case of the pharmacological cues), they elicit CCRs. These CCRs, expressed in the absence of the unconditional effects of the drug, are evident as withdrawal behaviors. In all the experiments described above, subjects for whom these cues were reliable predictors of the drug effect during conditioning showed more evidence of withdrawal in the presence of drug-paired cues than did controls with an equivalent history of morphine administration.

The results of these experiments provide support for the Pavlovian conditioning model of drug tolerance and withdrawal. This model predicts that cues reliably paired

with the effects of morphine will come to elicit CCRs that are similar to the unconditional homeostatic responses to the effects of the drug (Siegel et al., 2000). It further predicts that when previously drug-paired CSs are presented in the absence of the usual unconditional effects of the drug, the CCRs, having nothing now to oppose them, will be observable at the behavioral level; these CCRs are frequently overpowered and obscured by the unconditional effects of the drug during normal administration, and are evident only as an attenuation of these effects (i.e., tolerance). Each of the experiments described in earlier chapters involved testing subjects in the presence of drug-predictive cues and the absence of the predicted drug effect. In all such cases, CCRs were clearly evident in the form of drug-opposite withdrawal behaviors. These results support the suggestion made by several authors that, in many cases, administration of a drug in effect constitutes a Pavlovian conditioning trial (e.g., Dworkin, 1993; Ramsay & Woods, 1997; Subkov & Zilov, 1937), and that the application of a Pavlovian conditioning analysis to the phenomena of drug tolerance and withdrawal is relevant to a wide range of issues surrounding problems associated with drug use (see Siegel et al., 2000).

A substantial portion of this thesis was devoted to investigating the relative merit of several potential behavioral indices of morphine withdrawal. One of the major contributions of this thesis to the literature is identification and validation of mouth movements as a behavioral index of withdrawal. While the initial observations of this behavior in an experimental setting were somewhat fortuitous, it has proved to be correlated with other established behavioral indices of morphine withdrawal. In addition,

the behavior of mouth movements occurred with a much greater frequency than did other measured behaviors in almost all cases, making it a somewhat more sensitive indicator. The higher frequency of this behavior, as well as the fact that rats in almost all conditions exhibit the behavior to some extent, also make it amenable to more powerful (i.e., parametric) statistical analyses than other behaviors, which often occur with zero frequency in many subjects. In fact, the only subjects to display a zero frequency of mouth movements were occasional subjects getting morphine for the fist time on test day; these subjects were generally so heavily narcotized that they exhibited a zero frequency of all recorded behaviors. Furthermore, to the extent that these mouth movements are reflective of excessive salivation, they are predicted by a Pavlovian conditioning analysis, in that excess salivation is opposite in direction to the unconditional drying effects of morphine on secretory membranes (Jaffe & Martin, 1990). In other words, cues that have reliably preceded the onset of the drying effects of morphine will come to elicit a compensatory response, in this case increased salivation. While this salivary CCR is normally overwhelmed by the unconditional drying effects of the drug (Jaffe & Martin, 1990), when the drug-predictive cues are presented in the absence of the drug's unconditional drying effects, the CCR is evident as increased salivary activity.

A major difficulty faced in the course of the research reported here was designing an experiment that would distinguish between mouth movements as a CCR elicited by drug-paired cues and mouth movements as a sensitized oral stereotypy in subjects with a substantial history of morphine exposure (e.g., Kraus et al., 1997). The inclusion of

extinction trials in the experiment described in Chapter 5, however, provide data that argue heavily against a sensitization-based explanation of the results, while conforming to the predictions of a Pavlovian conditioning-based model. The observation that other indices of withdrawal, both behavioral and physiological, are also attenuated over repeated test trials (i.e., subject to extinction) provides further validation of the use of mouth movements as a measure of morphine withdrawal.

An additional contribution of this thesis to the behavioral assessment of morphine withdrawal in rat models is the suggestion that rearing, a commonly used behavioral index of withdrawal distress, may not be a particularly valid indicator of withdrawal in some cases. The experiment described in Chapter 2 suggests that the behavior of rearing may, in fact, represent a response to a functionally novel environment in many past assessments of context-elicited withdrawal; this experiment shows that both novel and drug-paired environments elicit equivalent frequencies of this behavior. The experiment described in Chapter 3, however, demonstrates a methodological correction for this potential confound, and further demonstrates that context-elicited morphine withdrawal may be observed in the absence of any possible effects of stimulus novelty associated with the testing environment.

The temperature data collected in the experiment described in Chapter 5 provide another interesting window onto the phenomenon of elicited morphine withdrawal. While the initial hypothermic effects of morphine observed in some studies (e.g., Paolino & Bernard, 1968) were not observed in this study, hypothermia was observed for a period

of time in subjects given a small dose of morphine at test. While the initial hypothermic effects of morphine, when they are observed, are generally attenuated over repeated administration (Eikelboom & Stewart, 1979), that same effect would not seem to be responsible for the hypothermia observed in this study. The hypothermic response here appeared only after a history of extensive exposure to high doses of morphine, and is more likely to represent an instance of the hypothermia associated with morphine withdrawal (e.g., Gianuttsos et al., 1975; Lal, Puri, & Karkalas, 1971). It should also be noted that collection of body temperature data provides a powerful tool for the analysis of conditioned drug effects in this preparation. The relatively low variability among subjects on this measure, as indicated by the Standard Error of the Mean shown on Figures 19 through 23, suggests that body temperature may be a more reliable index of morphine withdrawal than most commonly used behavioral measures.

The results of the experiments reported in this thesis are also relevant to attempts to develop effective treatments for drug dependence. The finding that morphine withdrawal may be elicited by administration of the drug in subjects with an extensive history of morphine exposure supports the suggestion that pharmacological cues may play an important role in conditioned drug effects. If a drug is capable of acting as a cue for itself within the course of a given administration, this may help explain some of the contradictory results reported in the literature with regard to the presence or absence of conditioned drug effects in various preparations (see Kim et al., 1999). The importance of pharmacological cues would not appear to be limited to opiates, as similar data has

been collected in studies using ethanol; a small dose of alcohol will augment the craving for additional alcohol and enhance subsequent alcohol consumption (see Siegel, 1986). Goddard (1999) reports similar results, and suggests that "the signal value of a small drug dose may make a contribution to 'binge' drinking and drug 'priming' effects in humans" (p.418). In fact, the potential role of pharmacological cues in the expression of drug tolerance and withdrawal may have been long recognized 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 observation that craving for a drug may be more intense, and withdrawal more severe, following administration of a relatively small quantity of the drug than in the complete absence of the drug, while somewhat counterintuitive, is in accordance with the predictions of a Pavlovian model of conditioned drug effects.

The inclusion of pharmacological cues among the spectrum of stimuli that may come to serve as CSs following repeated pairings with the unconditional effects of a drug may provide insight into a wide range of drug-related phenomena. While the methodology described in Chapters 4 and 5 represents a potentially useful preparation in such investigations, it does have one serious limitation. It has been demonstrated that a drug can serve as a cue for a subsequent administration of a different drug (e.g., Krank & Bennett, 1987; Taukulis, 1986), and also that a drug can serve as a signal for a

subsequent administration of the same drug (e.g., Cepeda-Benito & Short, 1997; Greeley et al., 1984). However, in both the above cases, the putative CS and US are discretely manipulable, in that they consist of discrete drug administrations. In the present preparation, discrete manipulation of the CS and US is not possible, in that both are the product of a single drug administration. Thus, it is not possible to expose subjects to the CS and US in an unpaired manner. While a method for discretely producing the early effects of a large dose (the CS) in intravenously cannulated rats has been developed (Kim et al., 1999), the data described in Chapters 4 and 5 suggest that similar results can be obtained using the simpler IP preparation; the early effects of a large IP dose of morphine (the CS) are effectively reproduced by a small IP dose of morphine. It would seem impossible, on the other hand, to present the US independently of the CS when IP administration is used.

Another difficulty faced by Pavlovian analysis of intra-administration associations is that it may not be readily disconfirmed. As suggested by other research into the role of pharmacological cues in conditioned drug effects, as well as the present experiments, the pharmacological cues inherent in the early effects of an administered drug may provide the most reliable predictor of the later peak drug effect (Kim et al., 1999; Siegel et al., 2000). These reports further suggest that these pharmacological cues may overshadow other external, environmental, cues, particularly when slower, less efficient, routes of drug administration are employed (i.e., intraperitoneal, subcutaneous, or oral administration, as opposed to intravenous or intracerebroventricular administration). The

idea that pharmacological cues may overshadow explicitly manipulated environmental cues, leading to the failure of those environmental cues to acquire control over the expression of tolerance and withdrawal, is appealing in its ability to serve as a possible explanation of the occasional failure to demonstrate the context-specificity of drug tolerance and withdrawal (e.g., King et al., 1987; Mackintosh, 1987; Tiffany et al., 1983). While such failures have been cited as evidence arguing against the Pavlovian conditioning model of drug tolerance and withdrawal, it may be that in such cases the explicitly manipulated external cues are overshadowed by the more salient internal, pharmacological cues. Overshadowing has been demonstrated with respect to conditioned drug tolerance (e.g., Dafters & Bach, 1985; Walter & Riccio, 1983). This explanation, however, may not be easily disconfirmed, in that the early effects of a drug (the putative overshadowing pharmacological CS) are inherent in many instances of drug administration. That is, any instance of failure to demonstrate associative control over the expression of tolerance and withdrawal by explicitly manipulated external environmental cues can be attributed to the presence of other, more salient, stimuli and the formation of intra-administration associations. It may be difficult, or impossible, to discretely manipulate the pharmacological CS and US in many preparations where intra-administration associations may form between early and later drug effects. Thus, explanations of failures to demonstrate associative control over the expression of tolerance and withdrawal that cite overshadowing pharmacological cues, thereby rendering such results consistent with a Pavlovian model of conditioned drug effects,

may not be subject to disconfirmation. It should be noted, however, that some preparations will allow discrete manipulation of these pharmacological stimuli (e.g., Kim et al., 1999), and these techniques can be employed in conjunction with a variety of behavioral assessments. Further studies in this area can be expected to resolve the role of relative stimulus salience in preparations where both pharmacological and environmental cues are paired with the unconditioned effects of a drug.

There are also demonstrations in the literature that overshadowing can be attenuated if the overshadowing CS is repeatedly presented alone (Matzel, Schachtman, & Miller, 1985; Matzel, Shuster, & Miller, 1987). In light of the fact that the experiments described in Chapters 4 and 5 show that IP injection of a small dose of morphine can effectively mimic the early effects of a larger IP dose, it should be possible to repeatedly present the pharmacological CS in the absence of both the unconditioned effects of the drug and the overshadowed environmental CS by repeatedly administering the small dose. Under such circumstances, a Pavlovian analysis of conditioned drug effects would predict that the previously overshadowed environmental CS will come to acquire control over expression of the CR.

The results reported in this thesis also suggest other future studies. In particular, it would be of interest to see if the pattern of results reported in Chapter 5 would be evident when other doses of morphine were employed. The doses of morphine used in this experiment, 50 and 5 mg/kg respectively, are relatively large in comparison to those used in many other investigations of conditioned morphine effects. The decision to select

these particular doses was informed by two factors: First, a substantial amount of pilot work suggested that the small dose should be 10% of the large dose (unpublished data), and that both larger and smaller doses were less effective in producing conditioned withdrawal. Second, demonstrations of drug-opposite conditioned responses to administration of a drug are more convincing if the small dose is relatively large; the 5 mg/kg dose has morphine-opposite effects in subjects with a history of 50 mg/kg administrations, while clearly morphine-like effects were observed in both drug-naive subjects and subjects with a history of 5 mg/kg administrations. It would be of interest to examine the range of values for the dose parameters that would allow this phenomenon to be demonstrated.

In conclusion, the experiments described in this thesis support a Pavlovian conditioning-based analysis of morphine tolerance and withdrawal in the rat model. Greater behavioral indices of morphine withdrawal were consistently observed in subjects presented with drug-predictive cues than were observed in similar subjects in the absence of those cues. It has further been demonstrated that associative control of morphine tolerance and withdrawal can be acquired by both environmental and pharmacological cues. In addition, the results of these experiments have implications with regard to the use of various behavioral indices of morphine withdrawal; the use of some traditional behavioral indices is supported, while the use of another commonly reported behavioral index of morphine withdrawal is shown to be problematic, and the proposal of a new, sensitive behavioral index of morphine withdrawal is supported. The

results of these experiments should inform both future studies of drug tolerance and withdrawal, and the development of effective clinical treatments for drug dependence.

References

Adams, J. U., & Holtzman, S. G. (1990). Tolerance and dependence after continuous morphine infusion from osmotic pumps measured by operant responding in rats. Psyschopharmacology, 100, 451-458.

Akil, H., Watson, S. J., Young, E., Lewis, M., Khatchaturian, H., & Walker, J. M. (1984). Endogenous opioids: Biology and function. Annual Review of Neuroscience, 7, 223-255.

Anonymous. (1939). Alcoholics Anonymous: The story of how many thousands of men and women have recovered from alcoholism. New York: Works.

Archer, J. (1973). Tests for emotionality in rats and mice: A review. Animal Behavior, 21, 205-235.

Azorlosa, J. L., Hartley, N. E., & Deffner-Rappold, C. (1994). Context-specific morphine tolerance and withdrawal: The effects of interdose interval. Psychobiology, 22, 304-311.

Azorlosa, J. L., & Simmons, E. L. (1999). Context-specific morphine withdrawal: Evidence that rearing reflects withdrawal, not exploration. Psychobiology, 27, 557-560.

Barr, G. A., & Wang, S. (1992). Tolerance and withdrawal to chronic morphine treatment in the week-old rat pup. European Journal of Pharmacology, 215, 35-42.

Berlyne, D. E. (1955). The arousal and satiation of perceptual curiosity in the rat. Journal of Comparative and Physiological Psychology, 48, 238-246.

Berlyne, D. E. (1960). Conflict, Arousal, and Curiosity. New York: McGraw-Hill.

Brady, L. S., & Holtzman, S. G. (1980). Schedule-controlled behavior in the morphine-dependent and post-dependent rat. Psychopharmacology, 70, 11-18.

Broadbent, J., & Cunningham, C. L. (1996). Pavlovian conditioning of morphine hyperthermia: Assessment of the interstimulus interval and CS-US overlap. Psychopharmacology, 126, 156-164.

Buccafusco, J. J. (1983). Cardiovascular changes during morphine withdrawal in the rat: Effects of clonidine. Pharmacology, Biochemistry, and Behavior, 18, 209-215.

Cappell, H., Roach, C., & Poulos, C. X. (1981). Pavlovian control of cross-tolerance between pentobarbital and ethanol. Psychopharmacology, 74, 54-57.

Carr, G. D., Fibiger, H. C., & Phillips, A. G. (1989). Conditioned place preference as a measure of drug reward. In J. M. Liebman & S. J. Cooper (Eds.), The Neuropharmacological Basis of Reward (pp. 264-319). Oxford: Clarendon.

Cepeda-Benito, A., & Short, P. (1997). Morphine's interoceptive stimuli as cues

for the development of associative morphine tolerance in the rat. Psychobiology, 25, 236-240.

Cepeda-Benito, A., & Tiffany, S. T. (1993). Morphine as a cue in associative tolerance to morphine's analgesic effects. Pharmacology, Biochemistry, and Behavior, 46, 149-152.

Clark, W. G. (1979). Influence of opioids on central thermoregulatory mechanisms. Pharmacology, Biochemistry, and Behavior, 10, 609-613.

Dafters, R., & Anderson, G. (1982). Conditioned tolerance to the tachycardia effect of ethanol in humans. Psychopharmacology, 78, 365-367.

Dafters, R., & Bach, L. (1985). Absence of environment-specificity in morphine tolerance acquired in nondistinctive environments: Habituation or stimulus overshadowing? Psychopharmacology, 87, 101-106.

Deffner-Rappold, C., Azorlosa, J. L., & Baker, J. D. (1996). Acquisition and extinction of context-specific morphine withdrawal. Psychobiology, 24, 219-226.

Dooley, C. P., Saad, C., & Valenzuela, J. E. (1988). Studies of the role of opioids in control of human pancreatic secretion. Digestive Diseases and Science, 33, 598-604.

DuMez, A. G. (1919). Increased tolerance and withdrawal phenomena in chronic morphinism: A review of the literature. Journal of the American Medical Association, 72, 1069-1072.

Duthie, D. J. R., & Nimmo, W. S. (1987). Adverse effects of opioid analgesic drugs. British Journal of Anaesthesiology, 59, 61-77.

Dworkin, B. R. (1993). Learning and Physiological Regulation. Chicago: University of Chicago Press.

Dyck, D. G., Driedger, S. M., Nemeth, R., Osachuk, T. A. G., & Greenberg, A. H. (1987). Conditioned tolerance to drug-induced (poly I:C) natural killer cell activation: Effects of drug dosage and context-specificity parameters. Brain, Behavior, and Immunity, 1, 251-266.

Eikelboom, R., & Stewart, J. (1979). Conditioned temperature effects using morphine as the unconditioned stimulus. Psychopharmacology, 61, 31-38.

Eikelboom, R., & Stewart, J. (1981). Temporal and environmental cues in conditioned hypothermia and hyperthermia associated with morphine. Psychopharmacology, 72, 147-153.

Eikelboom, R., & Stewart. J. (1982). Conditioning of drug-induced physiological responses. Psychological Review, 89, 507-528.

Emmett-Oglesby, M. W., Mathis, D. A., Moon, R. T. Y., & Lal, H. (1990). Animal models of drug withdrawal symptoms. Psychopharmacology, 101, 292-309.

Epstein, L. H., Caggiula, A. R., & Stiller, R. L. (1989). Environment-specific tolerance to nicotine. Psychopharmacology, 97, 235-237.

Falls, W. A., & Kelsey, J. E. (1989). Procedures that produce context-specific

tolerance to morphine in rats also produce context-specific withdrawal. Behavioral Neuroscience, 103, 842-849.

Faneslow, M. S., & German, C. (1982). Explicitly unpaired delivery of morphine and the test situation: Extinction and retardation of tolerance to the suppressing effects of morphine on locomotor activity. Behavioral and Neural Biology, 35, 231-241.

Geller, E. B., Hawk, C., Keinath, S. H., Tallarida, R. J., & Adler, M. W. (1983). Subclasses of opioids based on body temperature change in rats: Acute subcutaneous administration. Journal of Pharmacology and Experimental Therapeutics, 225, 391-398.

Gianuttsos, G., Drawbaugh, R., Hynes, M., & Lal, H. (1975). The narcotic withdrawal syndrome in the rat. In S. Ehrenpreis & A. Neidle (Eds.), Methods in Narcotics Research (pp. 293-309). New York: Marcel Dekker.

Goddard, M. J. (1999). The role of US signal value in contingency, drug conditioning, and learned helplessness. Psychonomic Bulletin and Review, 6, 412-423.

Goldstein, A. (1984). Opioid peptides: Function and significance. In H. O. J. Collier, J. Hughes, M. J. Rance, & M. B. Tyers (Eds.), Opioids: Past, Present, and Future. London: Tayler and Frances.

Greeley, J., Le, D. A., Poulos, C. X., & Cappell, H. (1984). Alcohol is an

effective cue in the conditional control of tolerance to alcohol. Psychopharmacology, 83, 159-162.

Greeley, J., & Ryan, C. (1995). The role of interoceptive cues for drug delivery in conditioning models of drug dependence. In: D. C. Drummond, S. T. Tiffany, S. Glautier, & B. Remington (eds.), Addictive behaviour: Cue exposure theory and practice. (pp. 121-136), Chichester, England: Wiley.

Grisel, J. E., Wiertelak, E. P., Watkins, L. R., & Maier, S. F. (1994). Route of morphine administration modulates conditioned analgesic tolerance and hyperalgesia. Pharmacology, Biochemistry, and Behavior, 49, 1029-1035.

Jaffe, J. H. (1990). Drug addiction and drug abuse. In A. G. Gilman, T. W. Rall, A. S. Nies, & P. Taylor (Eds.), The Pharmacological Basis of Therapeutics (8th ed.). New York: Pergamon.

Jaffe, J. H., & Martin, W. R. (1990). Opioid analgesics and antagonists. In A. G. Gilman, T. W. Rall, A. S. Nies, & P. Taylor (Eds.), The Pharmacological Basis of Therapeutics (8th ed.). New York: Pergamon.

Jorenby, D. E., Keesey, R. E., & Baker, T. B. (1989). Effects of dose on effector mechanisms in morphine-induced hyperthermia and poikilothermia. Psychopharmacology, 98, 269-274.

Kavaliers, M., & Hirst, M. (1986). Environmental specificity of tolerance

to morphine-induced analgesia in a terrestrial snail: Generalization of a behavioral model of tolerance. Pharmacology, Biochemistry, and Behavior, 25, 1201-1206.

Kavaliers, M., & Ossenkopp, K.-P. (1985). Tolerance to morphine-induced analgesia in mice: Magnetic fields function as environmental specific cues and reduce tolerance development. Life Science, 37, 1125-1135.

Kayan, S., Ferguson, R. K., & Mitchell, C. L. (1973). An investigation of the pharmacologic and behavioral tolerance to morphine in rats. Journal of Pharmacology and Experimental Therapeutics, 185, 300-306.

Kayan, S., Woods, L. A., & Mitchell, C. L. (1969). Experience as a factor in the development of tolerance to the analgesic effect of morphine. European Journal of Pharmacology, 6, 333-339.

Kelsey, J. E., Aranow, J. S., & Matthews, R. T. (1990). Context-specific morphine withdrawal in rats: Duration and effects of clonidine. Behavioral Neuroscience, 104, 704-710.

Kim, J. A., & Siegel, S. (in press). The role of cholesystokinin in conditional compensatory responding and morphine tolerance. Behavioral Neuroscience.

Kim, J. A., Siegel, S., & Patenall, V. R. A. (1999). Drug-onset cues as signals: Intra- administration associations and tolerance. Journal of Experimental Psychology: Animal Behavior Processes, 25, 491-504

King, D. A., Bouton, M. E., & Musty, R. E. (1987). Associative control of tolerance to the sedative effect of a short-acting benzodiazepine. Behavioral Neuroscience, 101, 104-114.

Krank, M. D. (1987). Conditioned hyperalgesia depends on the pain sensitivity measure. Behavioral Neuroscience, 101, 854-857.

Krank, M. D., & Bennett, D. (1987). Conditioned activity and the interaction of amphetamine experience with morphine's activity effects. Behavioral and Neural Biology, 48, 422-433.

Kraus, M. A., Piper, J. M., & Kornetsky, C. (1997). Persistent increases in basal cerebral metabolic activity induced by morphine sensitization. Pharmacology, Biochemistry, and Behavior, 57, 89-100.

Lal, H., Puri, S. K., & Karkalas, Y. (1971). Blockade of opioid withdrawal symptoms by haloperidol in rats and humans. Pharmacologist, 13, 263.

Lat, J., & Gollova-Hemon, E. (1969). Permanent effects of nutritional and endocrinological intervention in early ontogeny on the level of non-specific excitability and on lability (emotionality). Annals of the New York Academy of Science, 159, 710-719.

Mackintosh, N. J. (1987). Neurobiology, psychology and habituation. Behaviour Research and Therapy, 25, 81-97.

MacQueen, G. M., & Siegel, S. (1989). Conditional immunomodulation following training with cyclophosphamide. Behavioral Neuroscience,

103, 638-647.

MacRae, J. R., & Siegel, S. (1997). The role of self administration in morphine withdrawal in rats. Psychobiology, 25, 77-82.

Mansbach, R. S., Gold, L. H., & Harris, L. S. (1992). The acoustic startle response as a measure of behavioral dependence in rats. Psychopharmacology, 108, 40-46.

Martin, W. R. (1983). Pharmacology of opioids. Pharmacology Reviews, 35, 283-323.

Martin, W. R., Wikler, A., Eades, C. G., & Pescor, F. T. (1963). Tolerance to and physical dependence on morphine in rats. Psychopharmacologia, 4, 247-260.

Matzel, L. D., Schachtman, T. R., & Miller, R. R. (1985). Recovery of an overshadowed association achieved by extinction of the overshadowing stimulus. Learning and Motivation, 16, 398-412.

Matzel, L. D., Shuster, K., & Miller, R. R. (1987). Covariation in conditioned response strength between elements trained in compound. Animal Learning and Behavior, 15, 439-447.

McDonald, R. V., Parker, L. A., & Siegel, S. (1997). Conditioned sucrose aversions produced by naloxone-precipitated withdrawal from acutely administered morphine. Pharmacology, Biochemistry, and Behavior, 58, 1003-1008.

McDonald, R. V. & Siegel, S. (1998). Environmental control of morphine withdrawal: Context specificity or stimulus novelty? Psychobiology, 26, 53-56.

Mucha, R. F., Kalant, H., & Birbaumer, N. (1996). Loss of tolerance to morphine after a change in route of administration: Control of within-session tolerance by interoceptive conditioned stimuli. Psychopharmacology, 124, 365-372.

Mucha, R. F., Kalant, H., & Kim, C. (1987). Tolerance to hyperthermia produced by morphine in the rat. Psychopharmacology, 92, 452-458.

Nakamura, H., Ishii, K., & Shimizu, M. (1978). Some altered responses in rats formerly dependent on morphine. Psychopharmacology, 56, 269-277.

Numan, R., & Lal, H. (1981). Effect of morphine on rectal temperature after acute and chronic treatment in the rat. Progress in Neuropsychopharmacology, 5, 363-371.

Obál, F., Vicsay, M. S., & Madaràsz, I. (1965). Role of a central nervous mechanism in the acquired tolerance to the temperature decreasing effect of histamine. Acta Physiologica Academiae Scientiarum Hungaricae, 30, 15-29.

O'Brien, C. P., Ehrman, R. N., & Ternes, J. W. (1986). Classical conditioning in human opioid dependence. In S. R. Goldberg & I. P. Stolerman (Eds.), Behavioral Analysis of Drug Dependence (pp. 329-356). Orlando, FL:

Academic Press.

Paolino, R. M., & Bernard, B. K. (1968). Environmental temperature effects on the thermoregulatory response to systemic and hypothalamic administration of morphine. Life Science, 7, 857-863.

Pavlov, I. P. (1927). Conditioned Reflexes (G. V. Anrep, trans.). London: Oxford University Press.

Pollock, J., & Kornetsky, C. (1996). Reexpression of morphine-induced oral stereotypy six months after last morphine sensitizing dose. Pharmacology, Biochemistry, and Behavior, 53, 67-71.

Poulos, C. X., & Cappell, H. (1991). Homeostatic theory of drug tolerance: A general model of physiological adaptations. Psychological Review, 98, 390-408.

Poulos, C. X., & Hinson, R. E. (1982). Pavlovian conditional tolerance to haloperidol catalepsy: Evidence of dynamic adaptation in the dopaminergic system. Science, 218, 491-492.

Ramsay, D. S., & Woods, S. C. (1997). Biological consequences of drug administration: Implications for acute and chronic tolerance. Psychological Review, 104, 170-193.

Scoles, M. T., & Siegel, S. (1986). The potential role of saline trials in morphine-induced place-preference conditioning. Pharmacology, Biochemistry, and Behavior, 25, 1169-1173.

Schachter, S. (1977). Nicotine regulation in heavy and light smokers. Journal of Experimental Psychology (General), 106, 5-12.

Schwarz, K. S., & Cunningham, C. L. (1990). Conditioned stimulus control of morphine hyperthermia. Psychopharmacology, 101, 77-84.

Seeley, R. J., Hawkins, M. H., Ramsay, D. S., Wilkinson, C. W., & Woods, S. C. (1996). Learned tolerance to the corticosterone-increasing action of ethanol in rats. Pharmacology, Biochemistry, and Behavior, 55, 269-273.

Siegel, S. (1975). Evidence from rats that morphine tolerance is a learned response. Journal of Comparative & Physiological Psychology, 89, 498-506.

Siegel, S. (1978). Reply to Hayes and Mayer's Technical Comment ("Morphine tolerance: Is there evidence for a conditioning model?"), Science, 200, 344-345.

Siegel, S. (1983). Classical conditioning, drug tolerance, and drug dependence. In Y. Israel, F. B. Glaser, H. Kalant, R. E. Popham, W. Schmidt, & R. G. Smart (Eds.), Research Advances in Alcohol and Drug Problems: Vol. 7. (pp. 207-246). New York: Plenum Press

Siegel, S. (1986). Environmental modulation of tolerance: Evidence from benzodiazepine research. In H. H. Frey, W. P. Koella, W. Froscher, & H. Meinardi (eds.), Tolerance to Beneficial and Adverse Effects of Antiepileptic Drugs, pp. 89-100, New York: Raven.

Siegel, S. (1988). State-dependent learning and morphine tolerance. Behavioral

Neuroscience, 102, 228-232.

Siegel, S. (1989). Pharmacological conditioning and drug effects. In A. J. Goudie & M. Emmett-Oglesby (Eds.), Psychoactive Drugs, (pp. 115-180). Clifton, NJ: Humana.

Siegel, S. (1991). Feedforward processes in drug tolerance. In R. G. Lister & H. J. Weingartner (Eds.), Perspectives in Cognitive Neuroscience, pp. 405-416. New York: Oxford.

Siegel, S., & Allan, L. G. (1998). Learning and homeostasis: Drug addiction and the McCollough effect. Psychological Bulletin, 124, 230-239.

Siegel, S., Baptista, M. A. S., Kim, J. A., McDonald, R. V., & Weise-Kelly, L. A. (2000). Pavlovian psychopharmacology: The associative basis of tolerance. Experimental and Clinical Therapeutics, 8, 276-293.

Siegel, S., Hinson, R. E., & Krank, M. D. (1978). The role of predrug signals in morphine analgesic tolerance: Support for a Pavlovian conditioning model of tolerance. Journal of Experimental Psychology (Animal Behavior Processes), 4, 188-196.

Siegel, S., Sherman, J. E., & Mitchell, D. (1980). Extinction of morphine analgesic tolerance. Learning and Motivation, 11, 289-301.

Smith, J. B. (1991). Situational specificity of tolerance to decreased operant responding by morphine and l-nantradol. Psychopharmacology, 103, 115-120.

Steinfels, G. F., & Young, G. A. (1981). Effect of narcotic abstinence on schedule-controlled behavior in dependent rats. Pharmacology, Biochemistry, and Behavior , 14, 393-395.

Stinus, L., Robert, C., Karasinski, P., & Limoge, A. (1998). Continuous quantitative monitoring of spontaneous opiate withdrawal: Locomotor activity and sleep disorders. Pharmacology, Biochemistry, and Behavior, 59, 83-89.

Subkov, A. A., & Zilov, G. N. (1937). The role of conditioned reflex adaptation in the origins of hyperergic reactions. Bulletin de Biologie et de Médicine Expérimentale, 4, 294-296.

Suzuki, T., Fukagawa, Y., & Misawa, M. (1990). Enhancement of morphine withdrawal signs in the rat after chronic treatment with naloxone. European Journal of Pharmacology, 178, 239-242.

Taukulis, H. K. (1986). Conditional hyperthermia in response to atropine associated with a hypothermic drug. Psychopharmacology, 90, 327-331.

Tiffany, S. T., Petrie, E. C., Baker, T. B., & Dahl, J. (1983). Conditioned morphine tolerance in the rat: Absence of a compensatory response and cross-tolerance with stress. Behavioral Neuroscience, 97, 335-353.

Tilson, H. A., Rech, R. H., & Stolman, S. (1973). Hyperalgesia during withdrawal as a means of measuring the degree of dependence in morphine dependent rats. Psychopharmacologia, 28, 287-300.

Walter, T. A. & Riccio, D. C. (1983). Overshadowing effects in the stimulus control of morphine analgesic tolerance. Behavioral Neuroscience, 97, 658-662.

Wei, E., & Way, E. L. (1975). Application of the pellet implantation technique for the assessment of tolerance and physical dependence in the rodent. In S. Ehrenpreis & A. Neidle (Eds.), Methods in Narcotics Research (pp. 243-259). New York: Marcel Dekker.

Wikler, A., & Pescor, F. T. (1967). Classical conditioning of a morphine abstinence phenomenon, reinforcement of opioid-drinking behavior and "relapse" in morphine-addicted rats. Psychopharmacologia, 10, 255-284.

Young, G. A., Steinfels, G. F., & Khazan, N. (1980). Narcotic abstinence in dependent rats: EEG and behavioral correlates. Pharmacology, Biochemistry, and Behavior, 13, 115-119.

Figure Captions

Figure 1. Mean frequency (± SEM) of rearing at test.

Figure 2. Mean frequency (± SEM) of rearing at test.

Figure 3. Mean frequency (± SEM) of mouth movements at test.

Figure 4. Mean frequency (± SEM) of wet dog shakes at test.

Figure 5. Mean frequency (± SEM) of ear wipes at test.

Figure 6. Mean frequency (± SEM) of rearing at test.

Figure 7. Mean frequency (± SEM) of mouth movements at test.

Figure 8. Mean frequency (± SEM) of wet dog shakes at test.

Figure 9. Mean frequency (± SEM) of rearing on the first test day (Day 11).

Figure 10. Mean frequency (± SEM) of mouth movements on the first test day (Day 11).

Figure 11. Mean frequency (± SEM) of wet dog shakes on the first test day (Day 11).

Figure 12. Mean frequency (± SEM) of ear wipes on the first test day (Day 11).

Figure 13. Mean frequency (± SEM) of genital licks on the first test day (Day 11).

Figure 14. Mean frequency (± SEM) of rearing across the first, third, and fifth test days (Days 11, 13, and 15) for subjects in Group Mm.

Figure 15. Mean frequency (± SEM) of mouth movements across the first, third, and fifth test days (Days 11, 13, and 15) for subjects in Group Mm.

Figure 16. Mean frequency (± SEM) of wet dog shakes across the first, third, and fifth test days (Days 11, 13, and 15) for subjects in Group Mm.

Figure 1

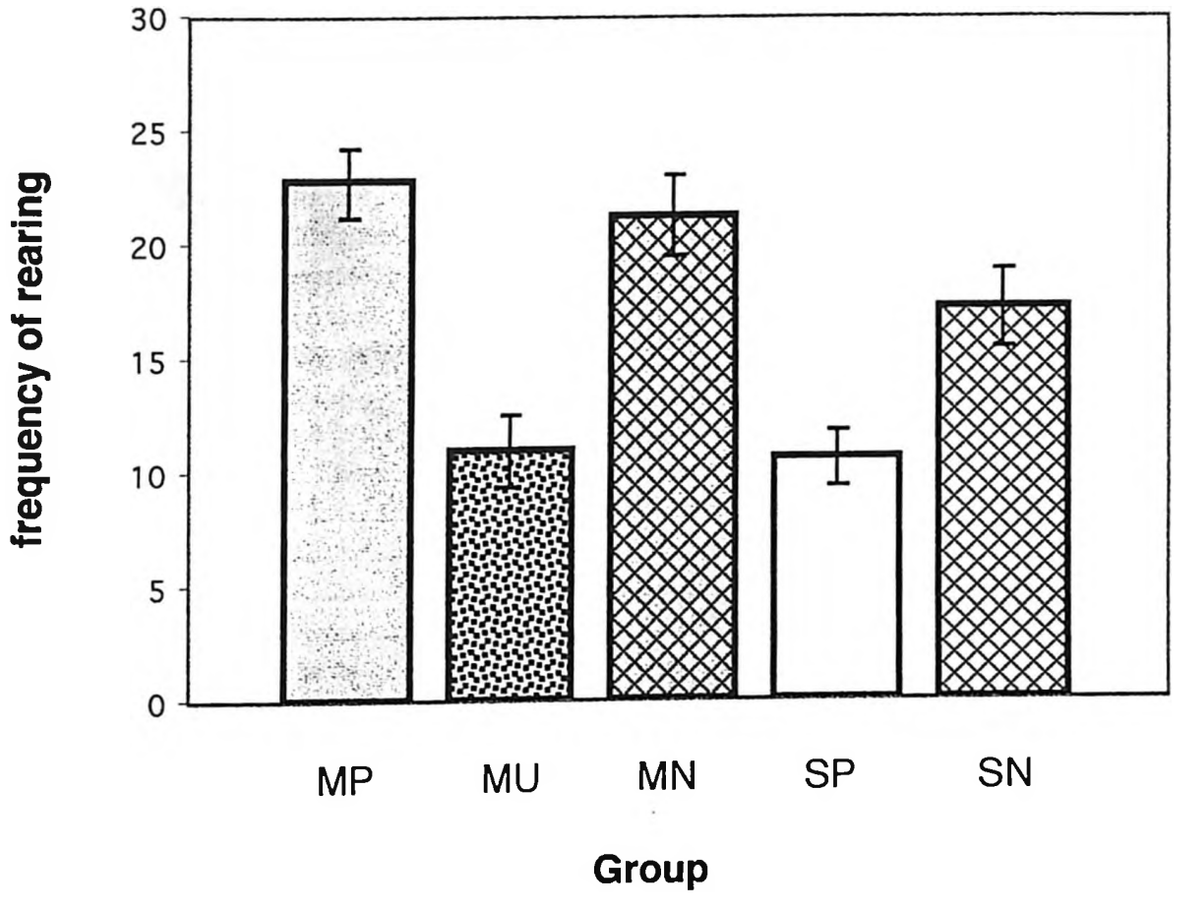
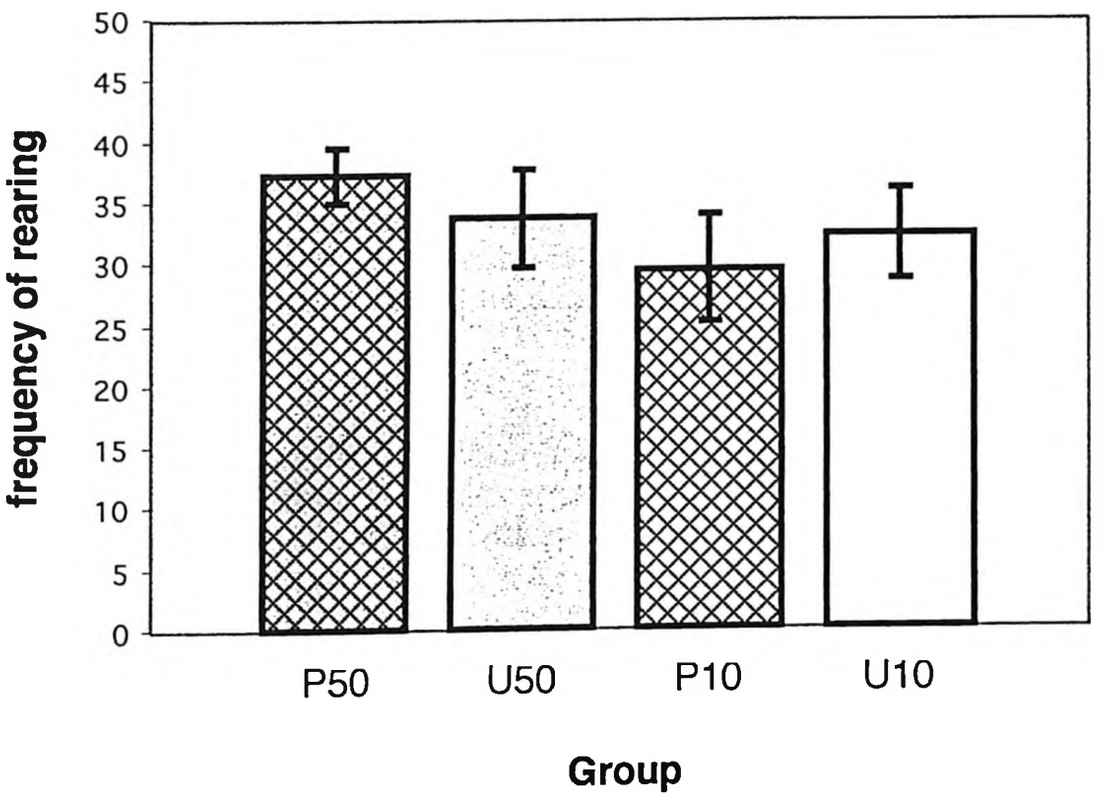


Figure 2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frequency of rearing
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Figure 3

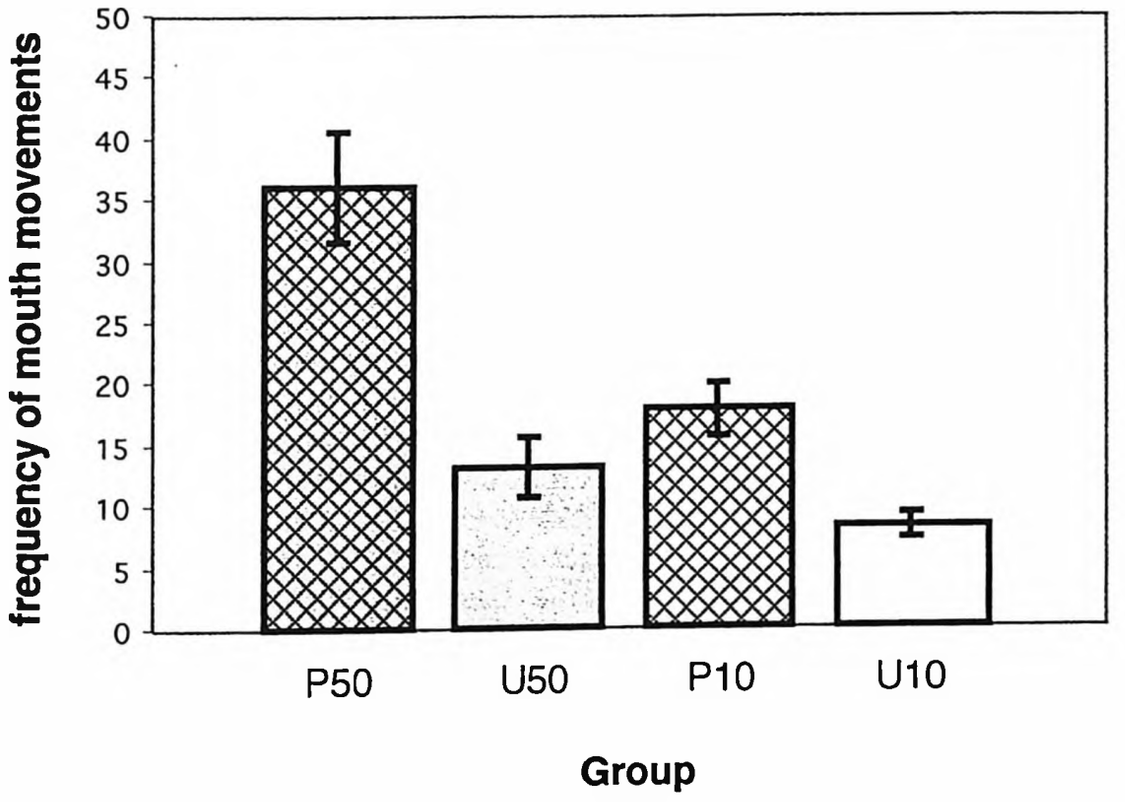
frequency of mouth movements
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Figure 4

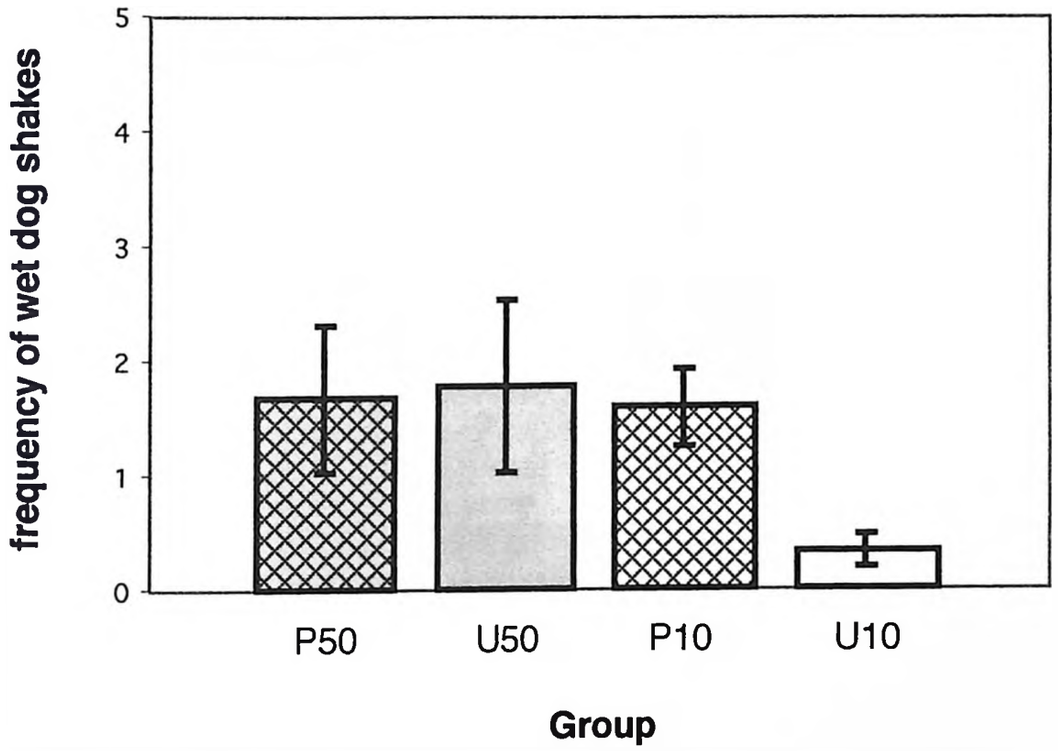

Figure 5

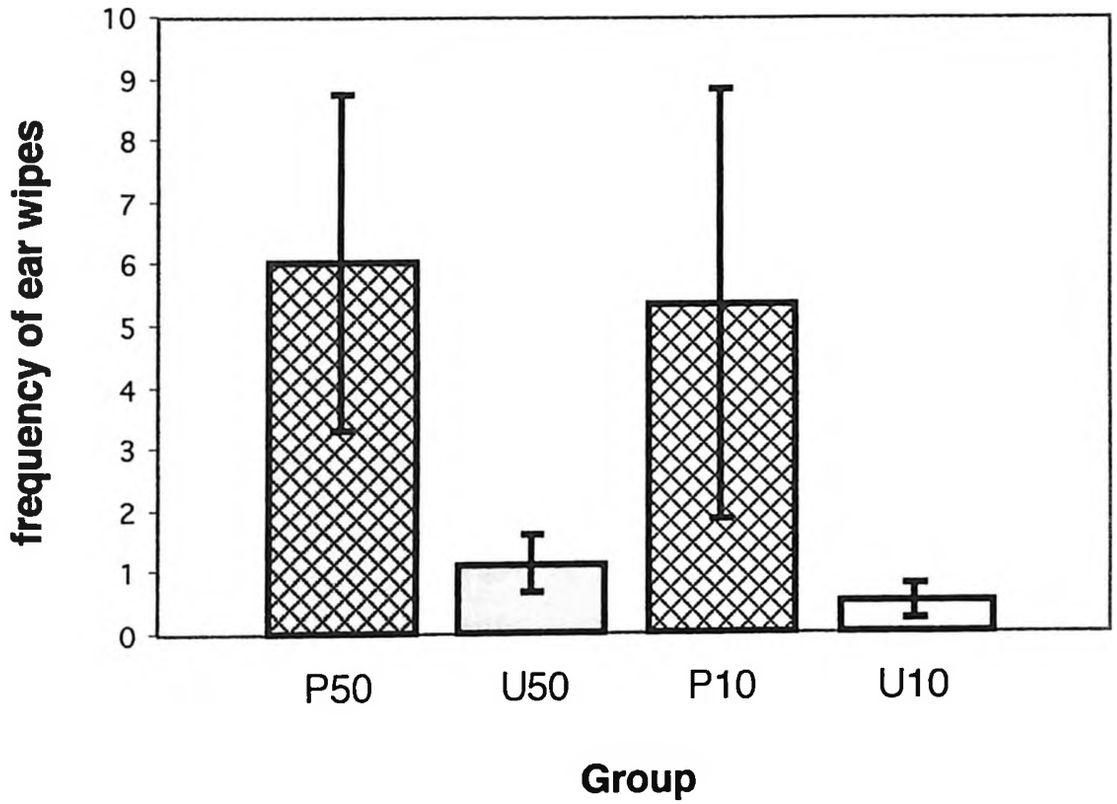

Figure 6

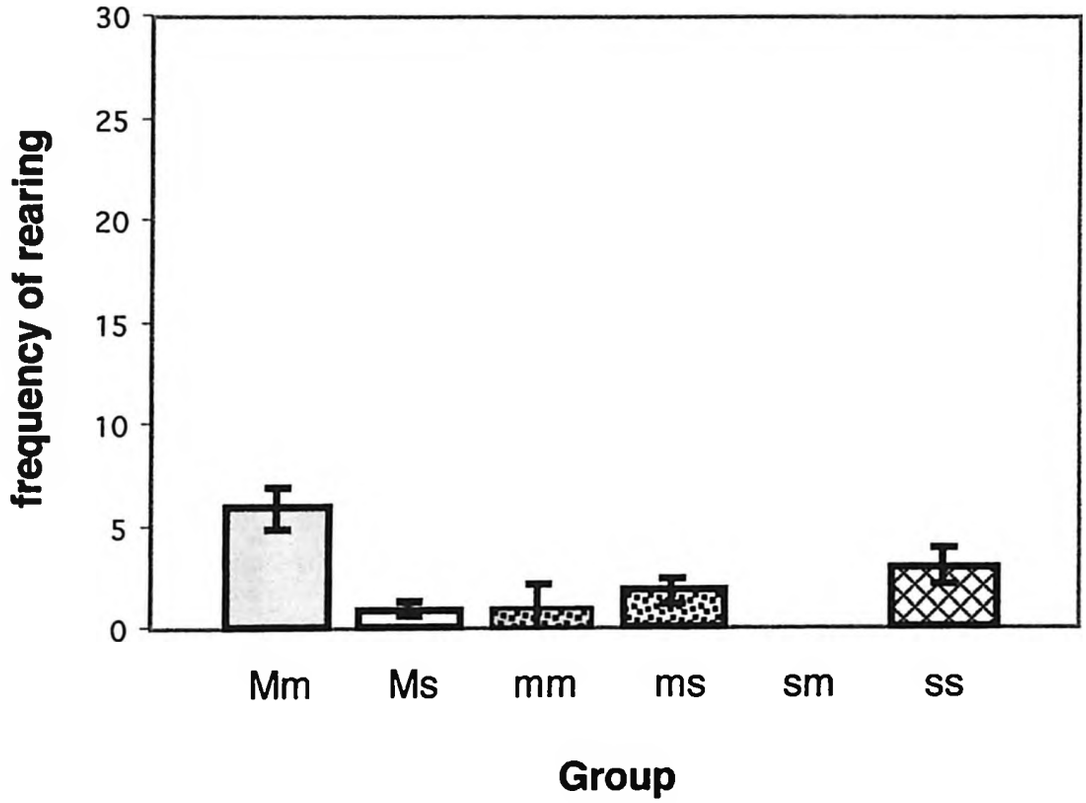

Figure 7

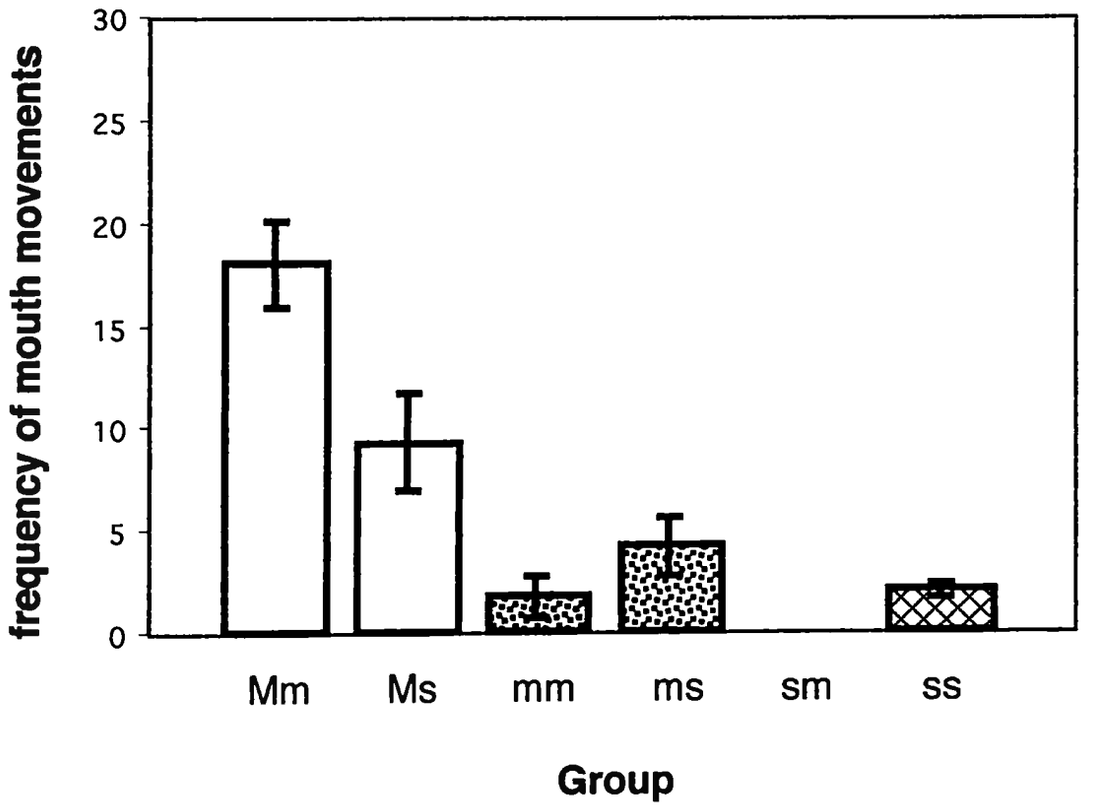
frequency of mouth movements
30
25
20
15
10
5
0
Mm
Ms
mm
ms
sm
ss
Group

Figure 8

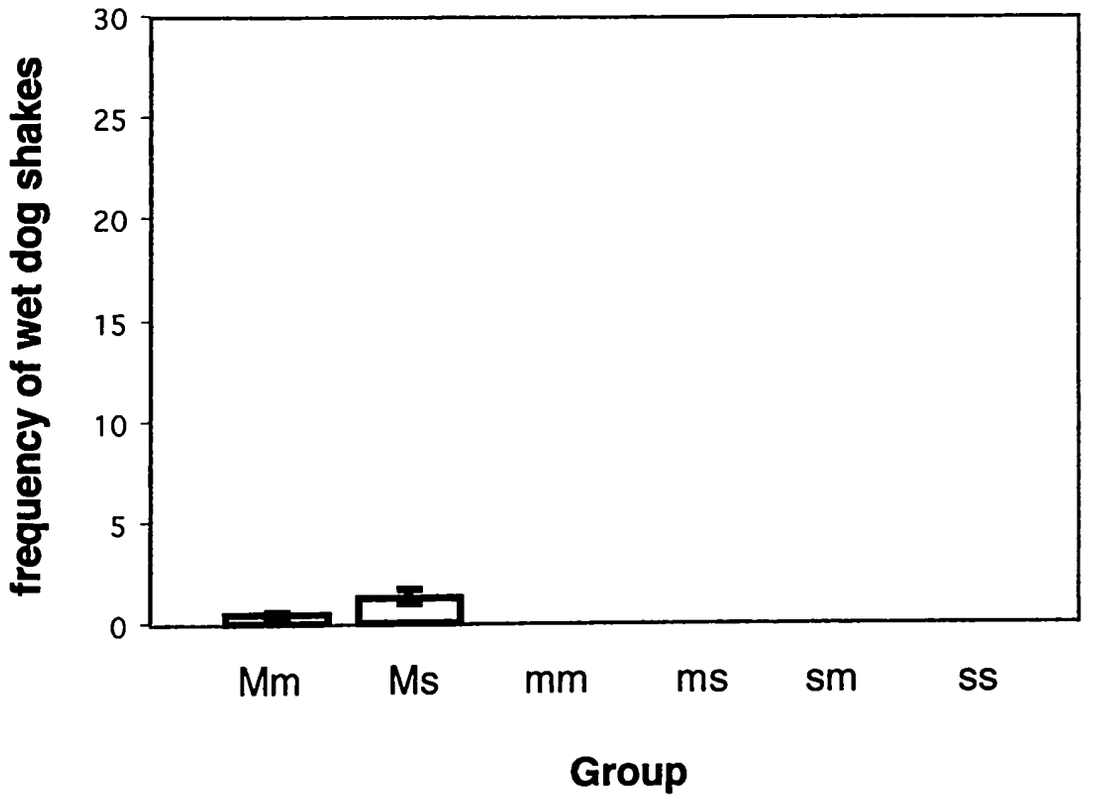
frequency of wet dog shakes
30
25
20
15
10
5
0
Mm
Ms
mm
ms
sm
ss
Group

Figure 9

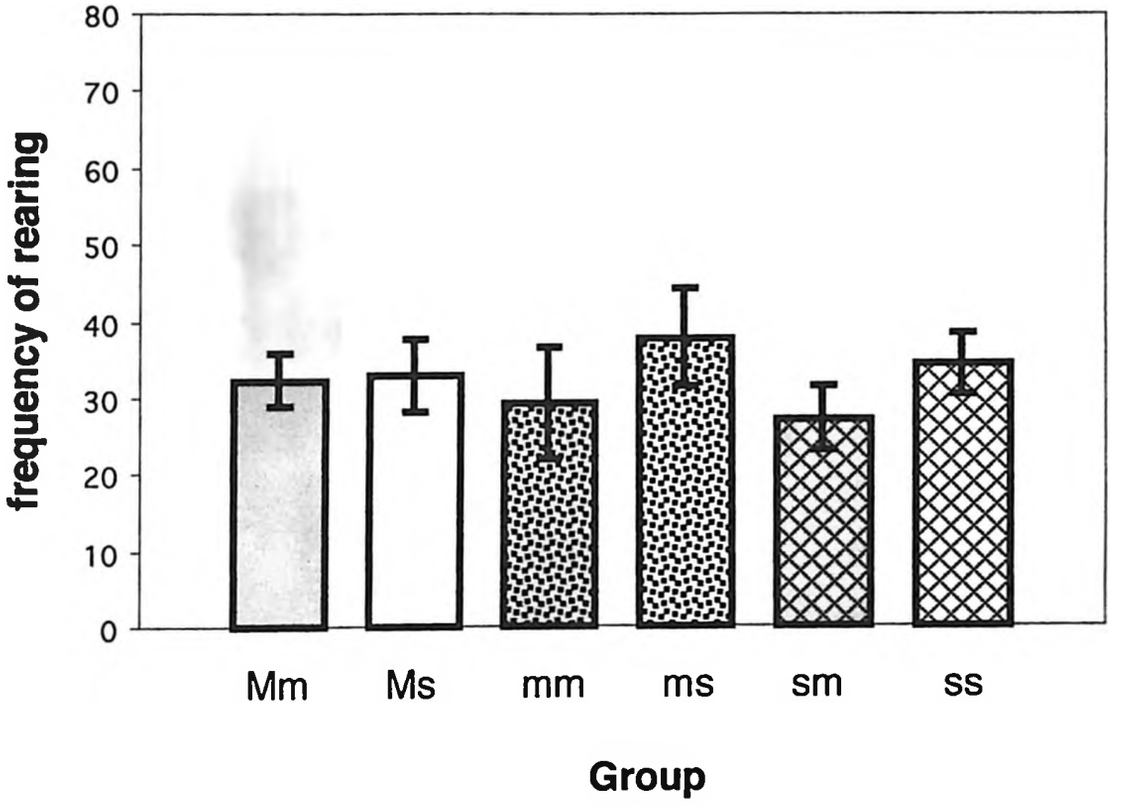
80
70
60
50
40
30
20
10
0
frequency of rearing
Mm
Ms
mm
ms
sm
ss
Group

Figure 10

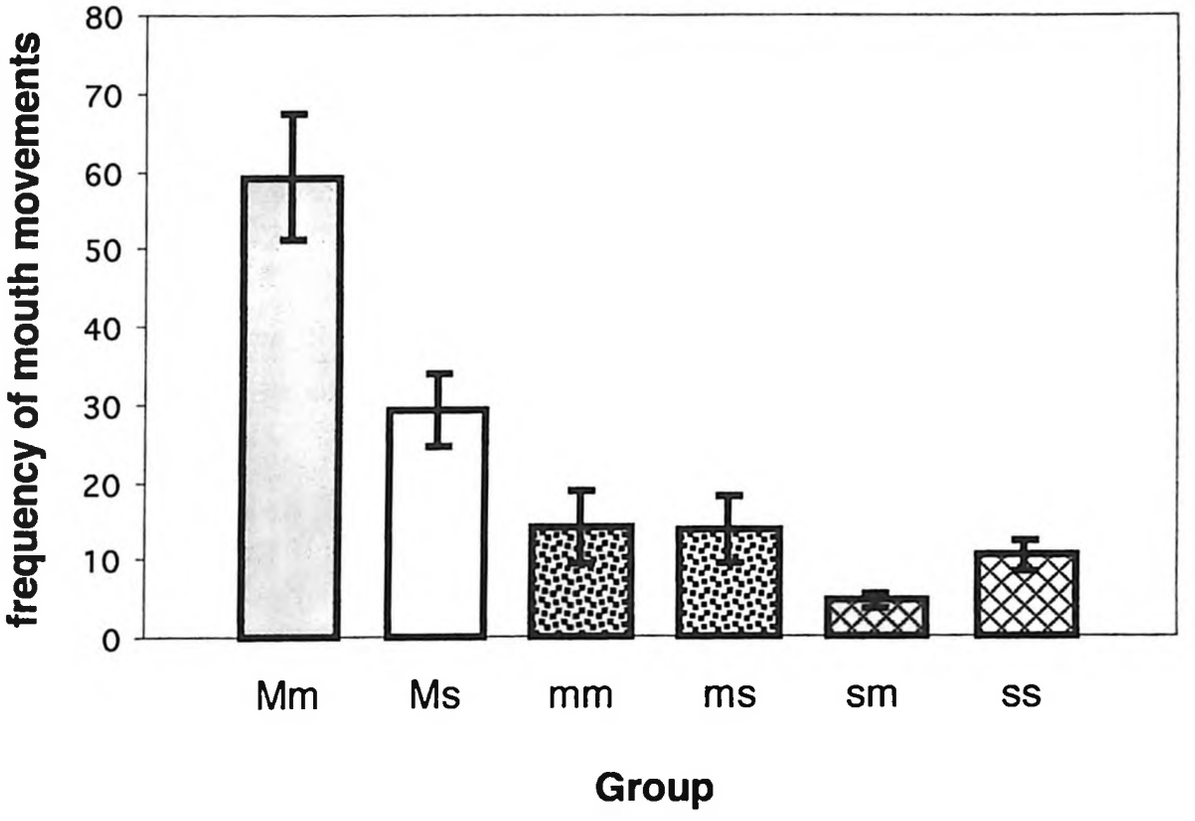
frequency of mouth movements
80
70
60
50
40
30
20
10
0
Mm
Ms
mm
ms
sm
ss
Group

Figure 11

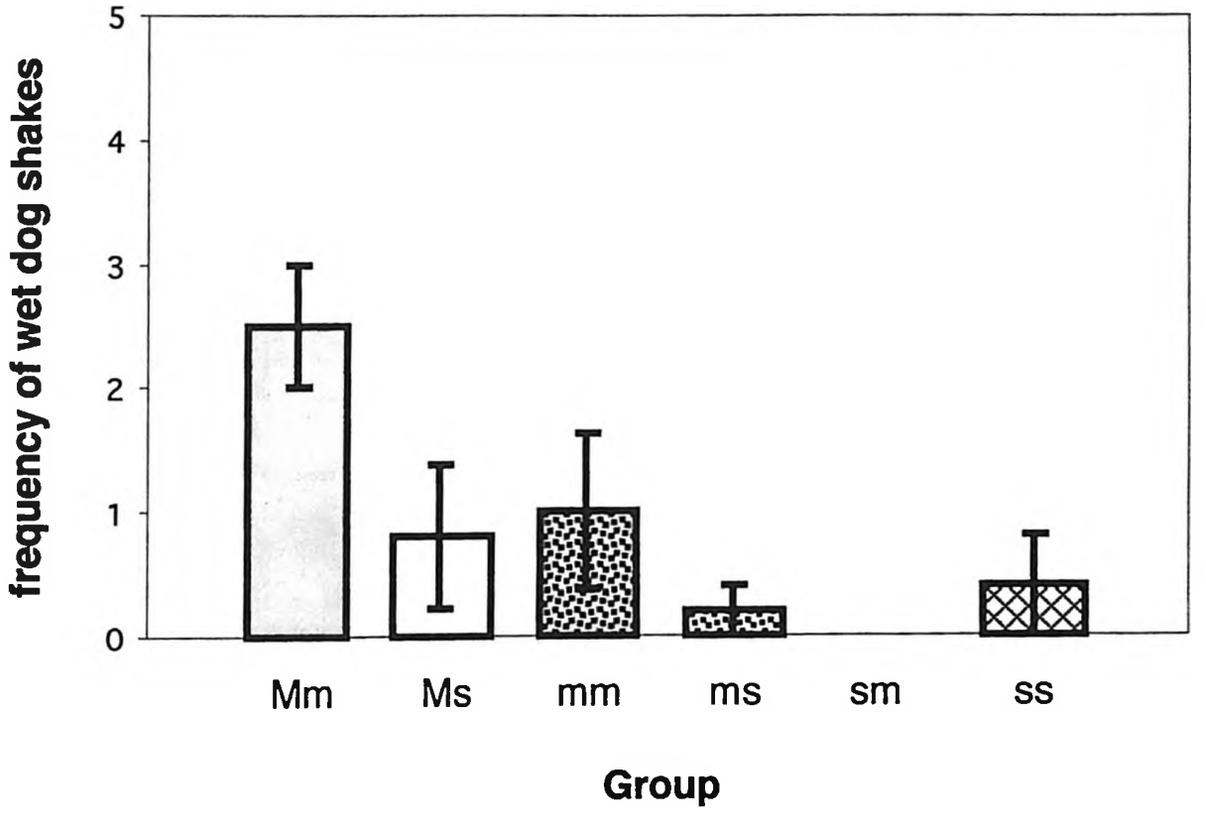

Figure 12

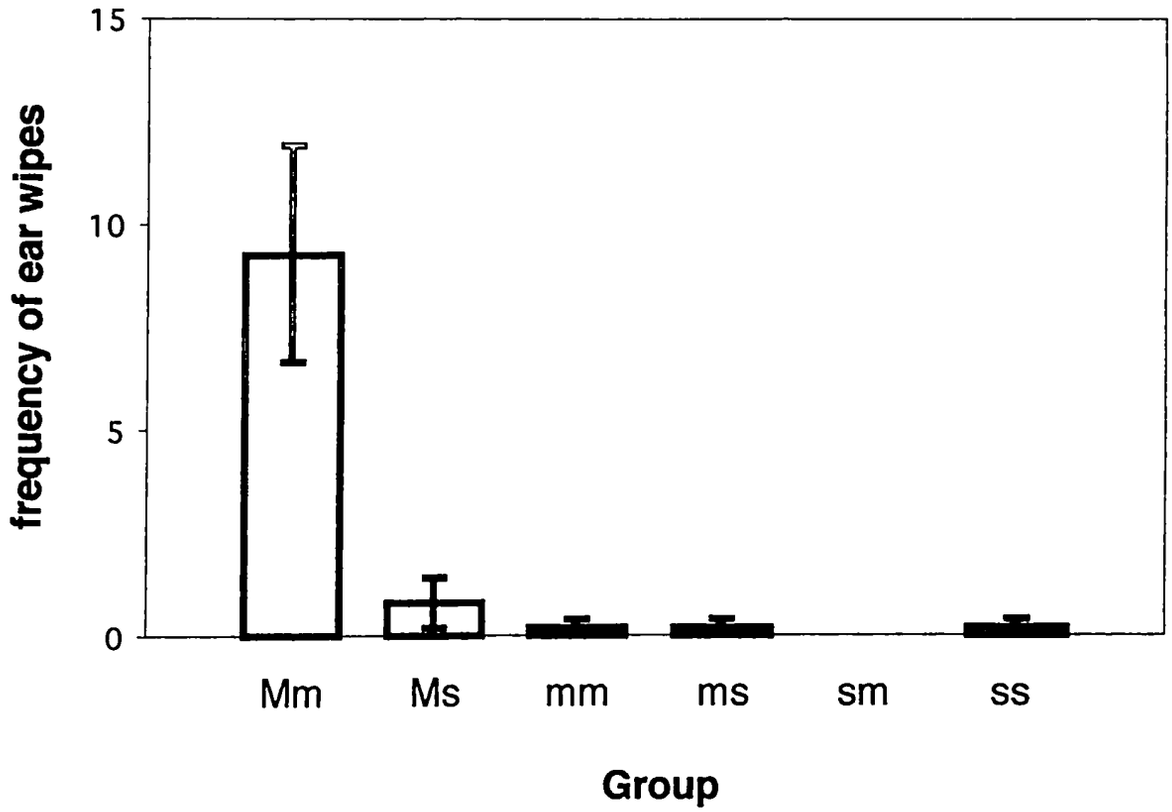

Figure 13

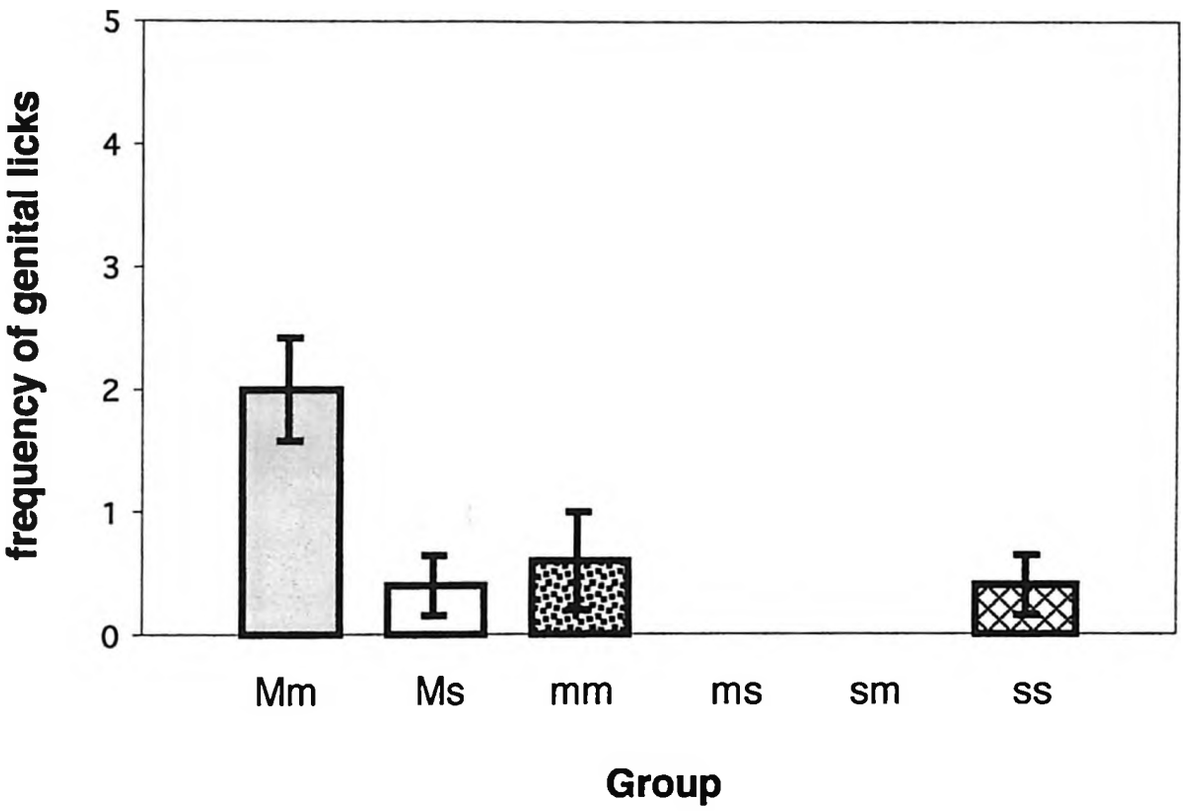

Figure 14

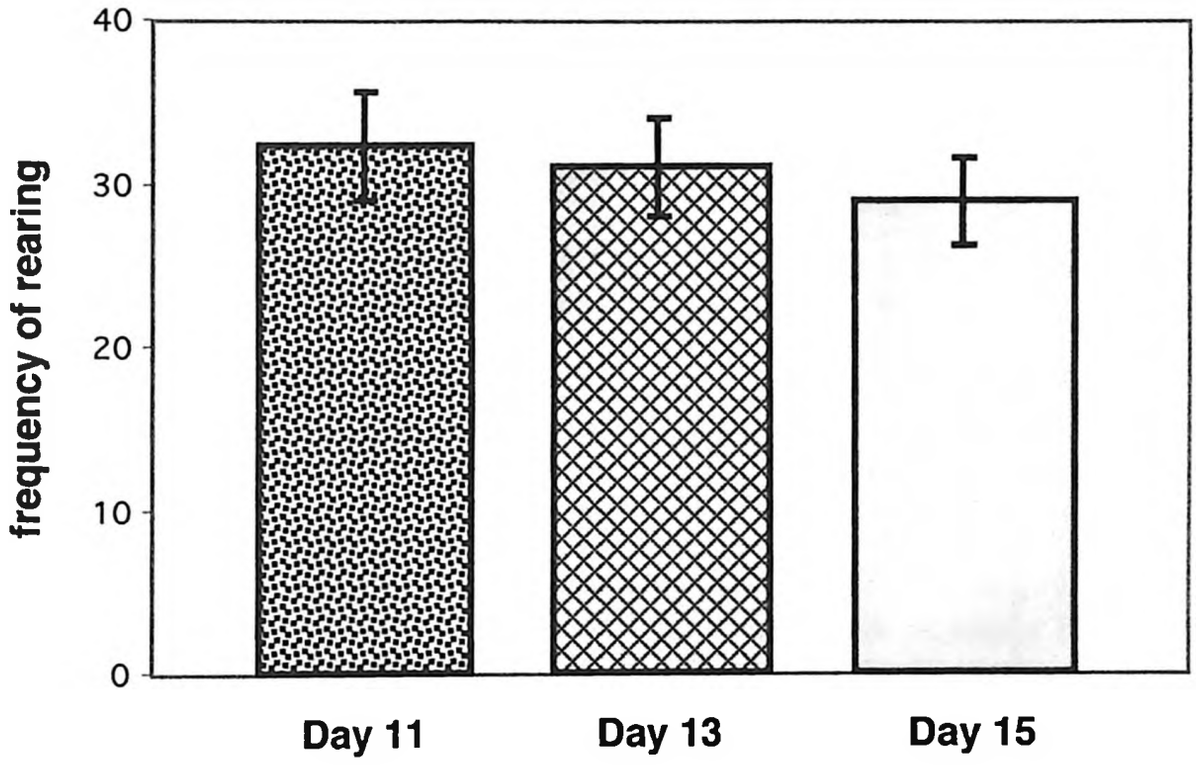

Figure 15

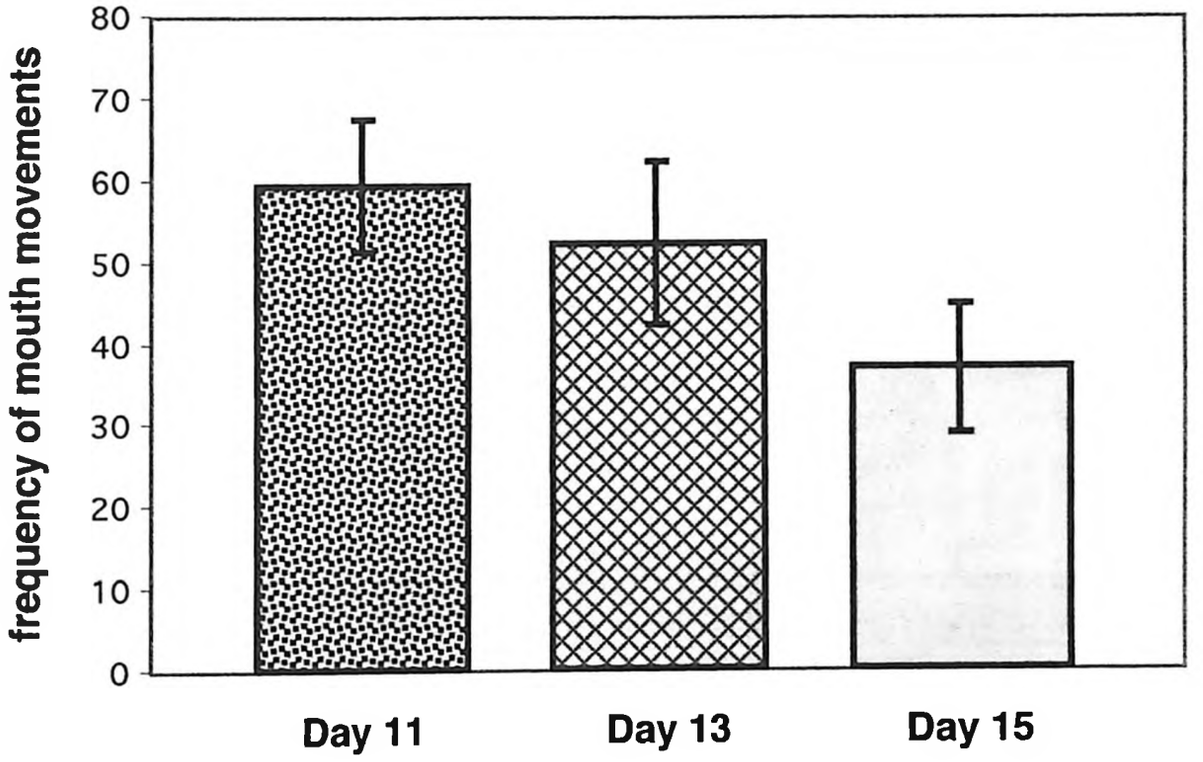
frequency of mouth movements
80
70
60
50
40
30
20
10
0
Day 11
Day 13
Day 15

Figure 16

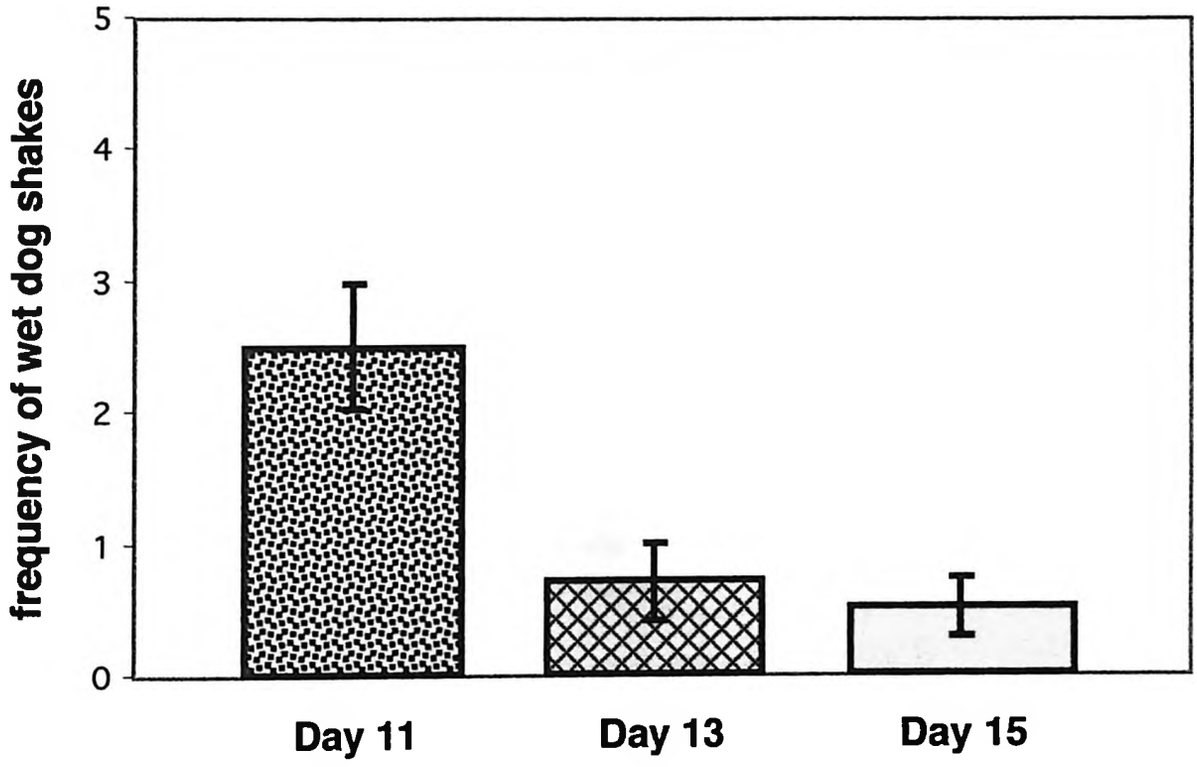

Figure 17

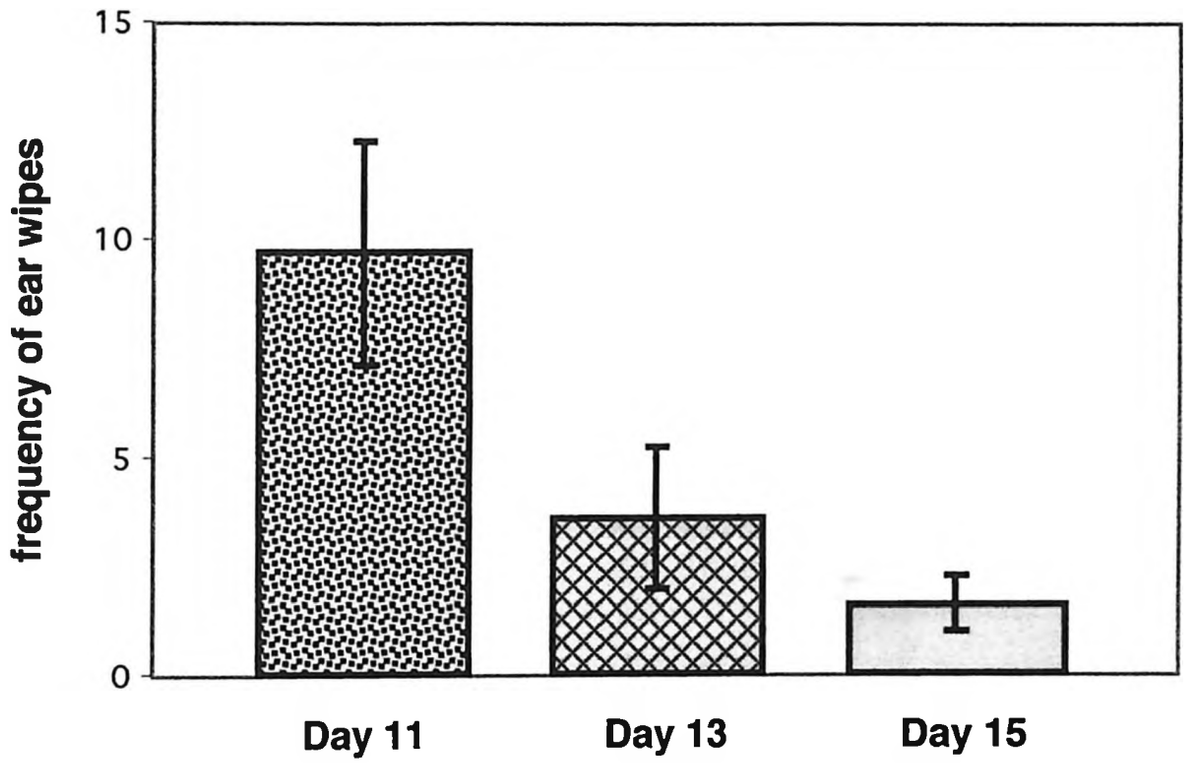

Figure 18

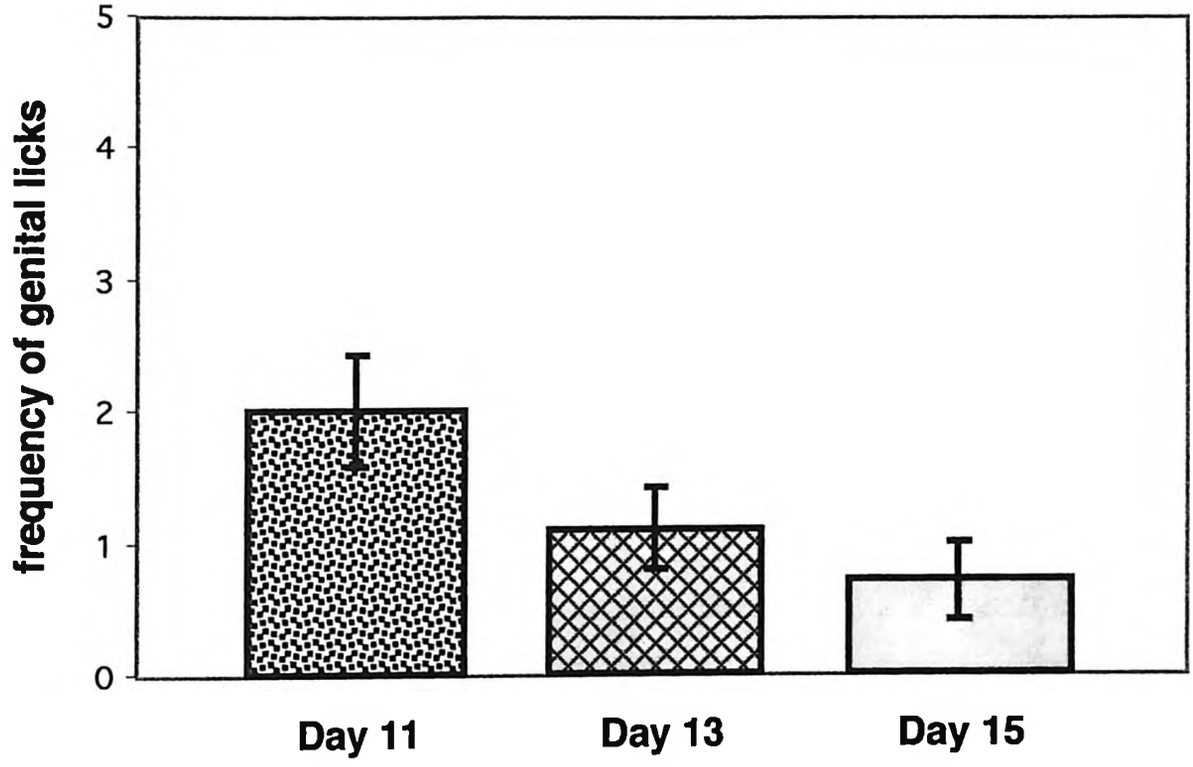

Figure 19

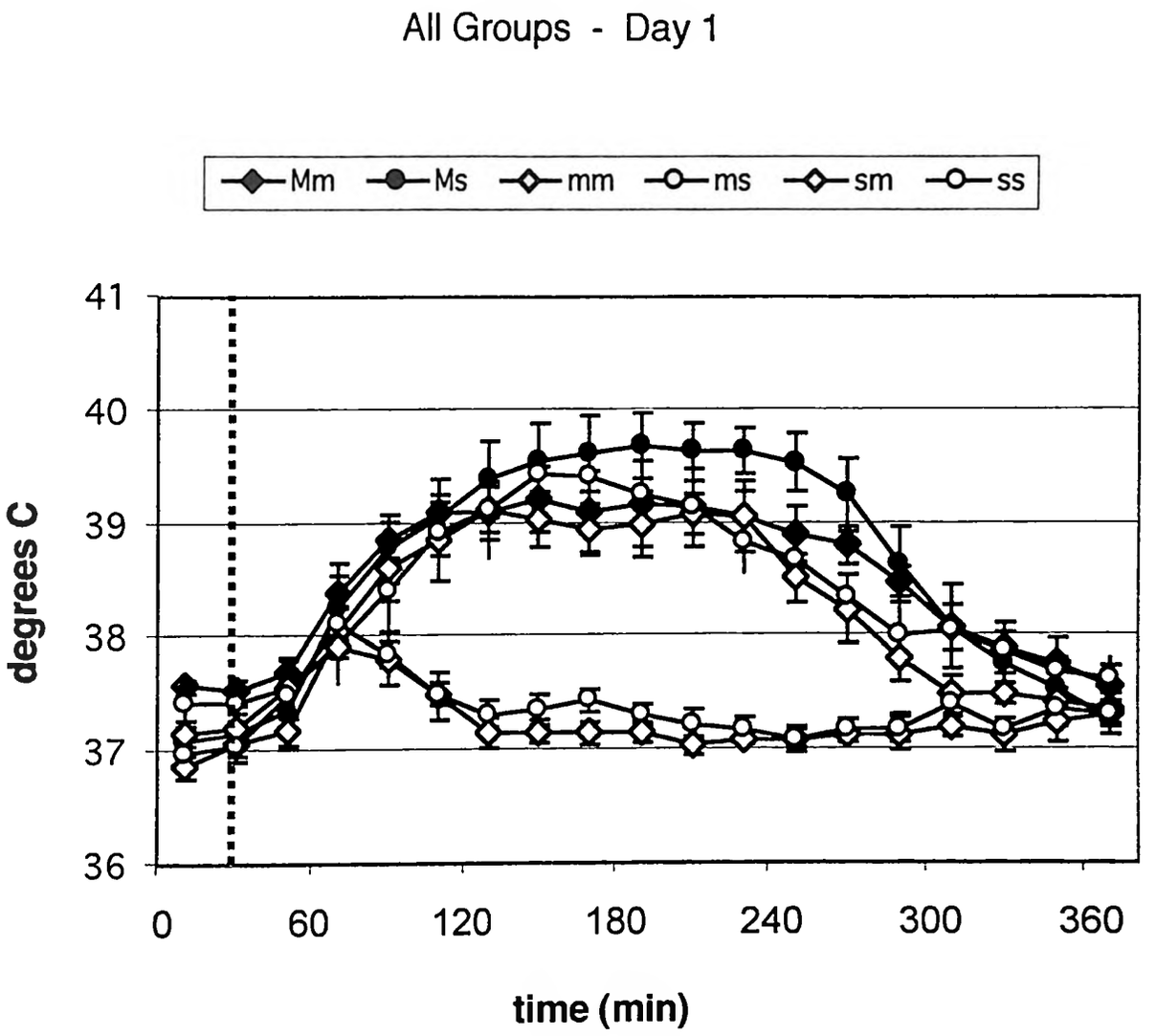
All Groups - Day 1
Mm
Ms
mm
ms
sm
ss
41
40
39
38
37
36
degrees C
0
60
120
180
240
300
360
time (min)

Figure 20

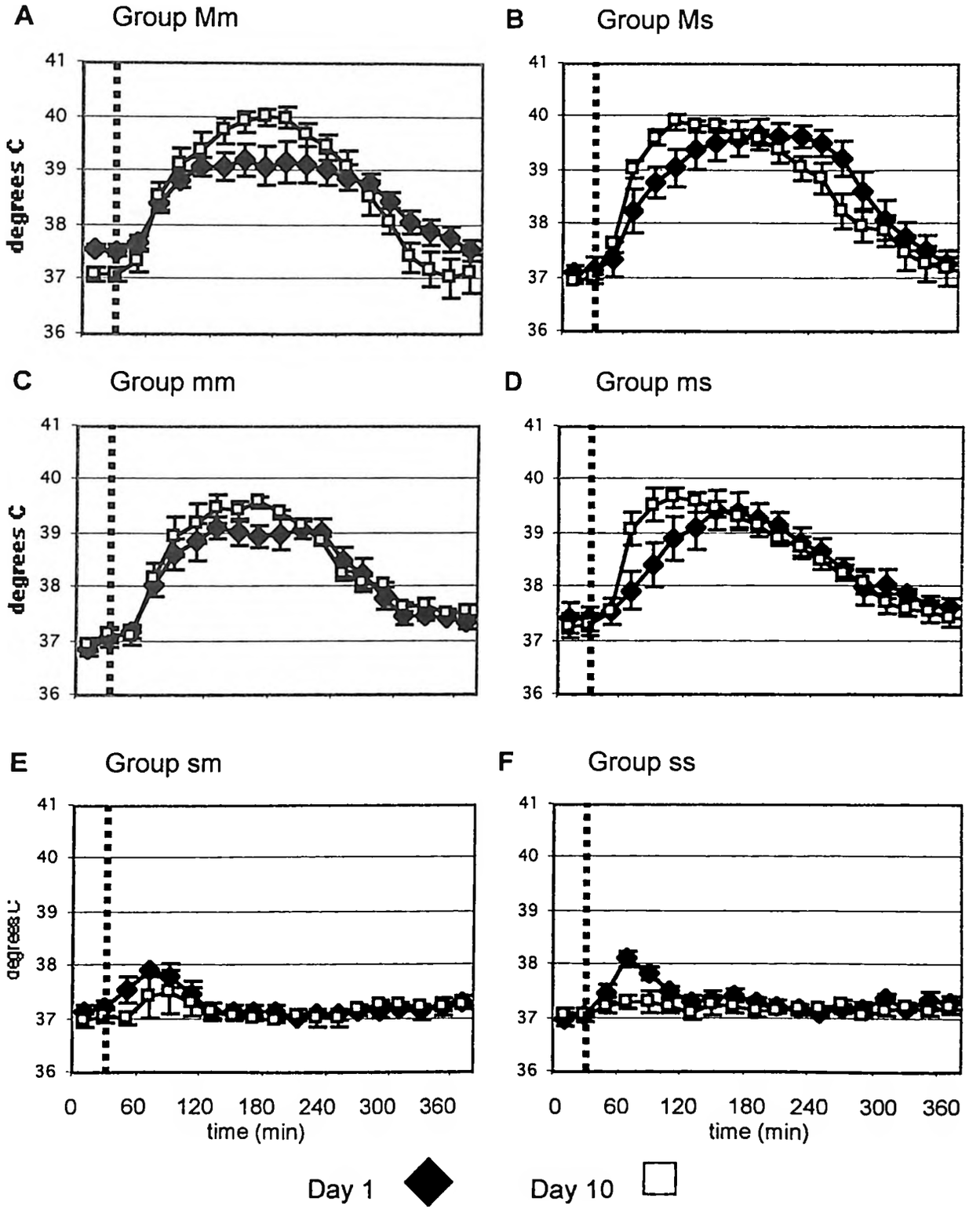
A
Group Mm
B
Group Ms
C
Group mm
D
Group ms
E
Group sm
F
Group ss
degrees C
41
40
39
38
37
36
0
60
120
180
240
300
360
time (min)
Day 1
Day 10

Figure 21

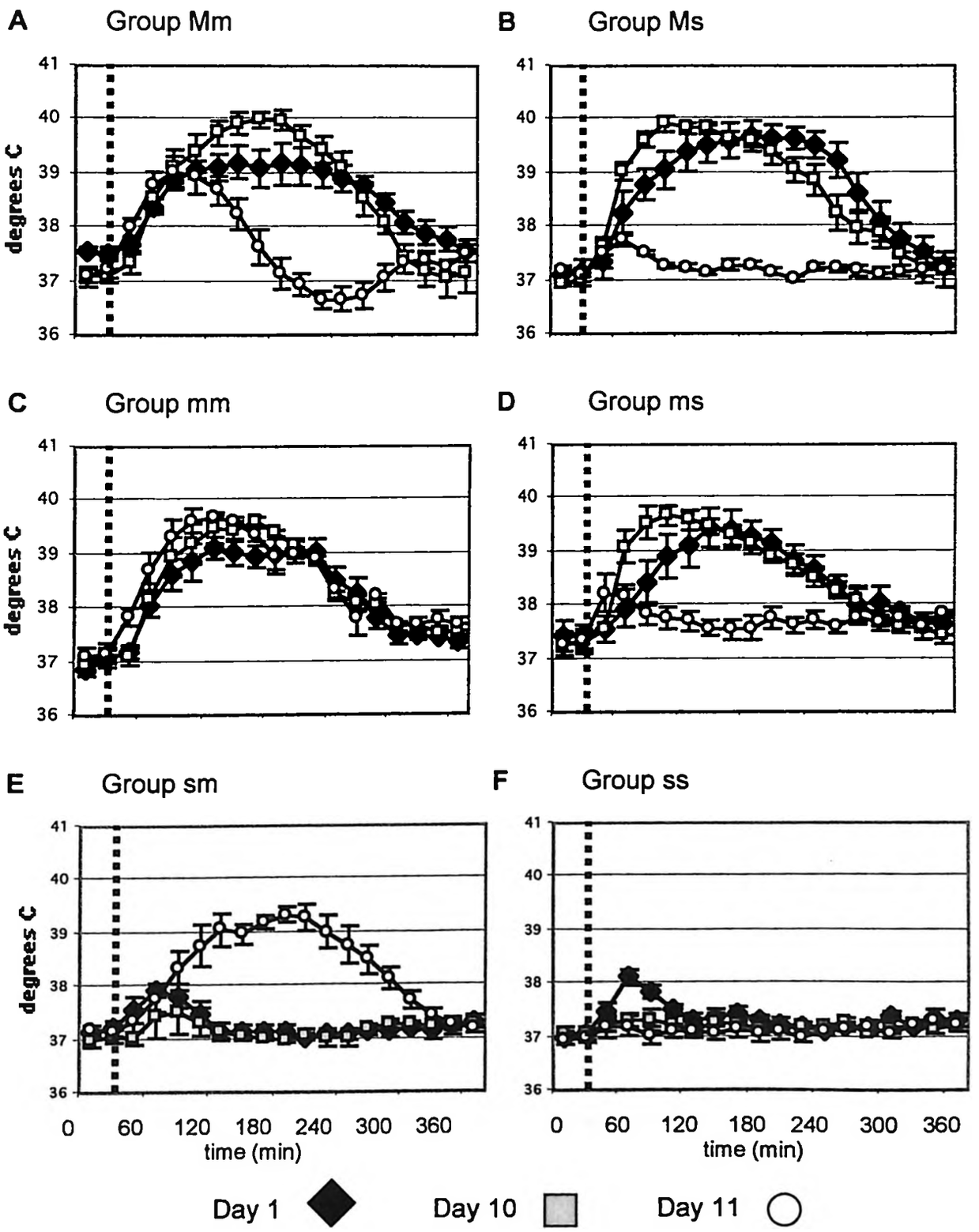
A
Group Mm
B
Group Ms
C
Group mm
D
Group ms
E
Group sm
F
Group ss
degrees C
41
40
39
38
37
36
0
60
120
180
240
300
360
time (min)
Day 1
Day 10
Day 11

Figure 22

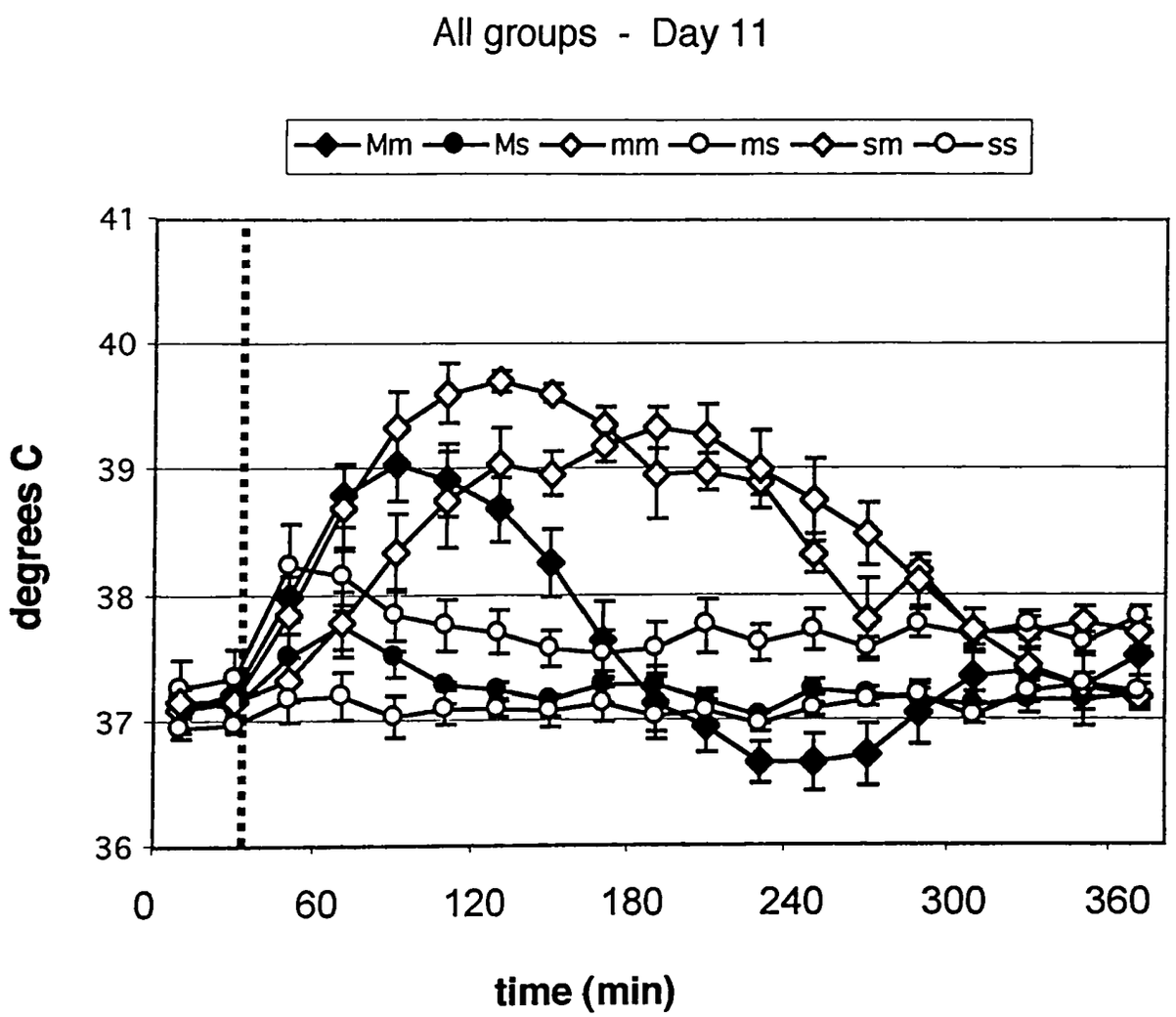
All groups - Day 11
Mm
Ms
mm
ms
sm
ss
41
40
39
38
37
36
degrees C
0
60
120
180
240
300
360
time (min)

Figure 23

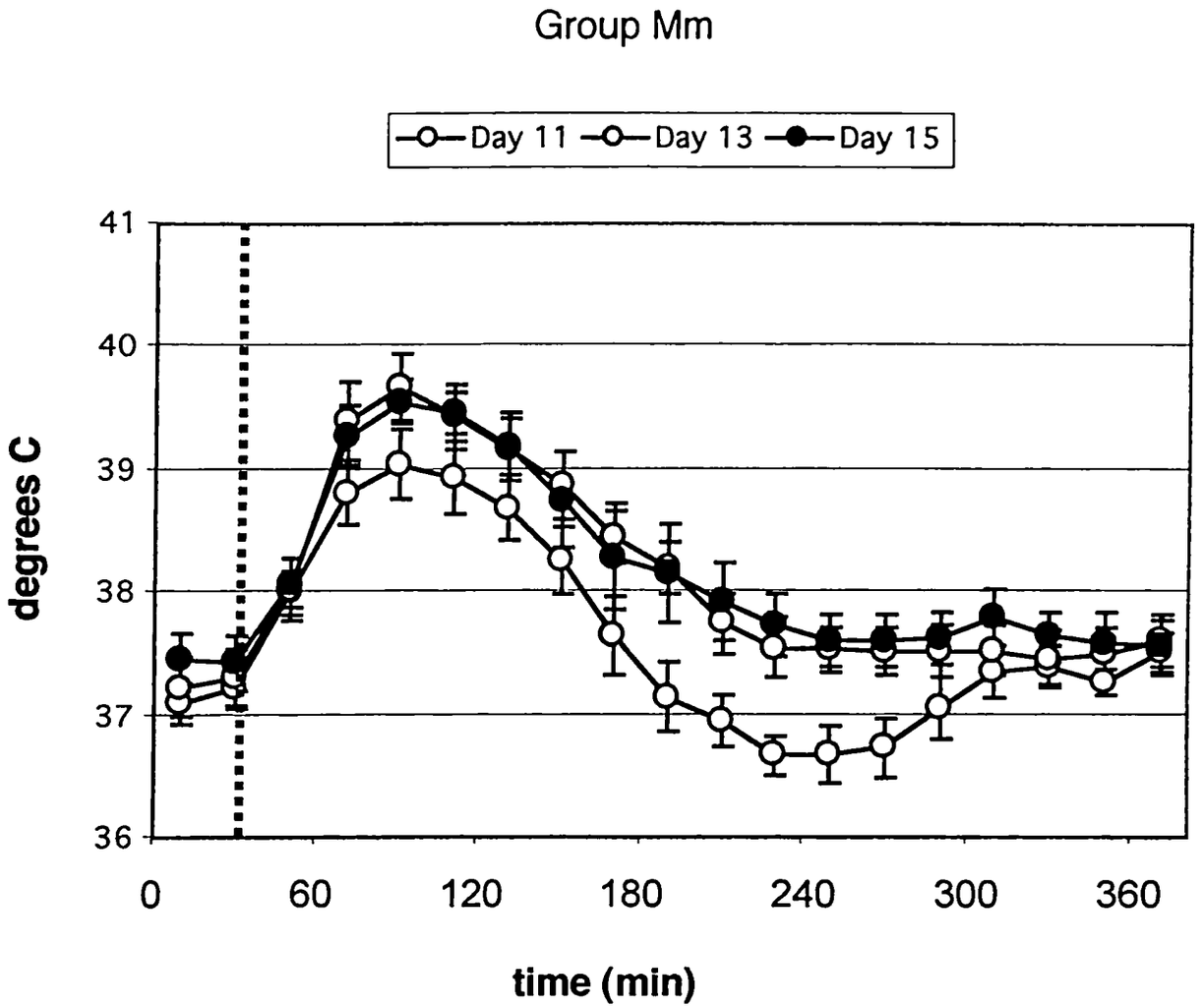
Group Mm
Day 11
Day 13
Day 15
41
40
39
38
37
36
degrees C
0
60
120
180
240
300
360
time (min)

Table 1

Schedule of Injections and Environments in Experiment 2

	Odd-numbered Days		Even-numbered Days	
Group	a.m.	p.m.	a.m.	p.m.
P50	SAL [E_1]	50 mg/kg MOR [E_1]	SAL [E_2]	50 mg/kg MOR [E_1]
P10	SAL [E_1]	10 mg/kg MOR [E_1]	SAL [E_2]	10 mg/kg MOR [E_1]
U50	SAL [E_1]	50 mg/kg MOR [E_2]	SAL [E_2]	50 mg/kg MOR [E_2]
U10	SAL [E_1]	10 mg/kg MOR [E_2]	SAL [E_2]	10 mg/kg MOR [E_2]

Note. The term in brackets represents the environment in which the injection was administered. SAL = saline and MOR = morphine.

Appendix A

Data from Chapter 2

Rat	Group	Rearing	Genital Licks	Ear Wipes	Jumps	Feces	Total
A11	MN	14	0	0	0	0	14
A20	MN	18	0	0	0	0	18
A24	MN	11	1	0	0	0	12
A28	MN	24	0	0	0	0	24
A3	MN	17	0	0	0	0	17
A7	MN	25	0	0	0	0	25
B11	MN	25	0	0	0	0	25
B20	MN	26	0	0	0	0	26
B24	MN	16	1	0	0	0	17
B28	MN	32	0	2	0	0	34
B3	MN	22	2	0	0	0	24
B7	MN	19	0	0	0	0	19
A1	MP	16	1	1	0	0	18
A10	MP	20	0	0	0	0	20
A14	MP	26	0	0	0	0	26
A18	MP	22	0	0	1	0	23
A22	MP	12	0	2	0	0	14

Rat	Group	Rearing	Genital Licks	Ear Wipes	Jumps	Feces	Total
A26	MP	22	0	0	0	0	22
B1	MP	22	0	0	0	0	22
B10	MP	26	0	0	0	0	26
B14	MP	25	0	1	0	0	26
B18	MP	15	0	0	0	0	15
B22	MP	32	0	0	0	5	37
B26	MP	29	0	0	0	0	29
A15	MU	9	0	0	0	0	9
A19	MU	19	0	0	0	0	19
A2	MU	11	0	0	0	1	12
A23	MU	8	2	0	0	5	15
A27	MU	12	1	0	0	0	13
A6	MU	0	0	0	0	2	2
B15	MU	13	0	0	0	0	13
B19	MU	12	0	0	0	0	12
B2	MU	14	1	0	0	7	22
B23	MU	7	0	0	0	0	7
B27	MU	19	0	0	0	0	19
B6	MU	4	0	0	0	0	4

Rat	Group	Rearing	Genital Licks	Ear Wipes	Jumps	Feces	Total
A13	SN	9	0	0	0	0	9
A17	SN	6	1	1	0	0	8
A21	SN	10	1	0	0	0	11
A30	SN	16	0	0	0	0	16
A5	SN	21	0	0	0	0	21
A9	SN	14	2	0	0	0	16
B13	SN	24	0	0	0	0	24
B17	SN	25	0	0	0	0	25
B21	SN	22	0	0	0	0	22
B30	SN	22	0	0	0	0	22
B5	SN	11	0	3	0	0	14
B9	SN	18	0	0	0	0	18
A12	SP	3	0	0	0	0	3
A16	SP	6	0	0	0	0	6
A25	SP	14	0	0	0	0	14
A29	SP	8	0	0	0	0	8
A4	SP	11	1	0	0	0	12
A8	SP	12	0	0	0	0	12
B12	SP	9	0	0	0	0	9

Rat	Group	Rearing	Genital Licks	Ear Wipes	Jumps	Feces	Total
B16	SP	8	2	8	0	0	18
B25	SP	16	0	0	0	0	16
B29	SP	11	0	1	0	0	12
B4	SP	10	0	0	0	0	10
B8	SP	5	1	1	0	0	7

Appendix B

Data from Chapter 3

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes
A1	PP10	49	21	0	0
A2	PP10	10	13	2	6
A3	PP10	7	16	2	43
B1	PP10	20	19	1	4
B2	PP10	30	16	4	0
B3	PP10	30	21	3	6
C1	PP10	63	20	2	0
C2	PP10	26	22	1	2
C3	PP10	27	8	0	0
D1	PP10	34	32	1	1
D2	PP10	24	2	2	0
D3	PP10	34	23	1	2
A4	PU10	20	9	0	0
A5	PU10	29	8	0	0
A6	PU10	10	13	1	3
B4	PU10	28	12	0	1
B5	PU10	44	9	1	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes
B6	PU10	44	12	0	0
C4	PU10	35	9	0	0
C5	PU10	53	2	0	0
C6	PU10	28	5	1	0
D4	PU10	34	11	1	2
D5	PU10	45	3	0	0
D6	PU10	17	5	0	0
A7	PP50	35	21	6	22
A8	PP50	27	24	2	2
B7	PP50	44	29	0	18
B8	PP50	34	29	0	0
C7	PP50	30	45	2	5
C8	PP50	48	28	1	1
C9	PP50	41	37	1	2
D7	PP50	36	51	3	4
D8	PP50	40	61	0	0
A9	PU50	22	9	5	4
A10	PU50	24	15	0	1
B9	PU50	19	10	1	2

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes
B10	PU50	29	12	0	0
C10	PU50	47	13	2	1
C11	PU50	50	4	6	0
D9	PU50	39	22	2	2
D10	PU50	46	27	0	0
D11	PU50	27	6	0	0

Appendix C

Data from Chapter 4

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes
A1	Mm	7	21	3
F8	Mm	12	17	0
D9	Mm	11	21	0
A11	Mm	5	24	0
G1	Mm	5	21	0
A2	Mm	5	6	1
E6	Mm	1	29	0
B8	Mm	9	23	1
A10	Mm	0	1	0
F11	Mm	2	32	0
A3	Mm	0	9	0
A4	Mm	13	23	0
B10	Mm	8	13	0
C8	Mm	10	6	0
G9	Mm	1	20	0
C1	Mm	4	13	1
B3	Mm	6	27	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes
D1	mm	0	4	0
B2	mm	8	8	0
B6	mm	0	1	0
D8	mm	0	1	0
C9	mm	0	0	0
F10	mm	0	0	0
C12	mm	0	0	0
C14	mm	0	0	0
C6	Ms	0	4	1
C7	Ms	0	9	3
D10	Ms	0	1	0
D12	Ms	0	9	2
D3	Ms	0	35	0
C4	Ms	0	26	2
C5	Ms	0	4	2
D7	Ms	3	15	3
A9	Ms	0	4	3
B12	Ms	0	2	1
E9	Ms	0	2	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes
A12	Ms	1	4	0
B5	Ms	1	22	0
G11	Ms	0	1	0
E1	Ms	4	7	3
D4	Ms	2	9	0
D6	Ms	4	3	2
E3	ms	2	10	0
B4	ms	4	7	0
A5	ms	2	3	0
A7	ms	0	0	0
D11	ms	0	0	0
C13	ms	1	6	0
C15	ms	3	3	0
F1	Sm	0	0	0
C2	Sm	0	0	0
D5	Sm	0	0	0
A6	Sm	0	0	0
E8	Sm	0	0	0
E11	Sm	0	0	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes
B1	Sm	0	0	0
D2	Sm	0	0	0
F3	Sm	0	0	0
F4	Sm	0	0	0
F6	Sm	0	0	0
B7	Sm	0	0	0
E10	Sm	0	0	0
E13	Sm	0	0	0
E14	Sm	0	0	0
F9	Sm	0	0	0
G3	Ss	0	1	0
E4	Ss	8	3	0
E5	Ss	12	1	0
G6	Ss	1	3	0
A8	Ss	3	0	0
C10	Ss	3	3	0
C11	Ss	4	1	0
F12	Ss	1	3	0
E2	Ss	7	1	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes
F5	Ss	0	5	0
G7	Ss	1	2	0
G8	Ss	4	2	0
B9	Ss	2	1	0
G10	Ss	0	1	0
C11	Ss	1	3	0
G12	Ss	0	2	0

Appendix D

Behavioral Data from Chapter 5, Day 11

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes	Genital Licks
E5	Mm	16	40	2	20	3
E6	Mm	25	42	1	16	3
E7	Mm	31	28	2	0	1
F1	Mm	25	100	3	8	1
F5	Mm	42	62	2	14	2
F7	Mm	50	56	4	4	1
F8	Mm	36	35	2	2	1
F9	Mm	45	50	5	0	1
F11	Mm	25	92	4	11	5
F12	Mm	28	87	0	22	2
D4	Ms	43	46	0	0	0
D5	Ms	15	23	3	0	0
E9	Ms	36	25	0	0	0
G1	Ms	34	31	1	3	1
G4	Ms	36	21	0	1	1
B1	mm	24	10	0	0	1
B2	mm	11	5	0	0	0

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes	Genital Licks
B3	mm	52	9	0	1	0
B4	mm	40	32	3	0	0
G7	mm	19	14	2	0	2
D7	ms	35	10	1	1	0
D8	ms	41	12	0	0	0
D9	ms	25	28	0	0	0
D12	ms	61	2	0	0	0
G8	ms	27	17	0	0	0
C2	sm	17	4	0	0	0
C4	sm	34	4	0	0	0
C6	sm	38	2	0	0	0
G9	sm	29	6	0	0	0
G11	sm	18	7	0	0	0
C1	ss	29	13	0	0	0
C3	ss	46	7	0	0	1
C5	ss	33	16	2	0	0
G10	ss	40	11	0	0	1
G12	ss	23	5	0	1	0

Appendix E

Behavioral Data from Chapter 5, Day 13

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes	Genital Licks
E5	Mm	26	44	0	8	1
E6	Mm	25	42	1	16	3
E7	Mm	38	22	1	0	2
F1	Mm	19	127	0	0	0
F5	Mm	45	48	0	0	1
F7	Mm	26	34	3	0	1
F8	Mm	21	29	0	0	2
F9	Mm	45	31	1	2	0
F11	Mm	27	67	1	4	1
F12	Mm	38	78	0	6	0

Appendix F

Behavioral Data from Chapter 5, Day 15

Rat	Group	Rearing	Mouth Movements	Wet Dog Shakes	Ear Wipes	Genital Licks
E5	Mm	21	31	0	1	0
E6	Mm	32	24	0	0	0
E7	Mm	27	8	0	0	0
F1	Mm	27	83	0	4	0
F5	Mm	28	23	3	4	3
F7	Mm	25	30	1	2	1
F8	Mm	31	21	1	0	1
F9	Mm	49	19	0	0	0
F11	Mm	17	73	1	0	1
F12	Mm	32	56	1	5	1

Appendix G

Temperature Data from Chapter 5, Day 1

Group Mm

Rat	E5	E6	E7	F1	F5	F7	F8	F9	F11	F12
Time (min)										
0	37.24	37.52	37.78	37.73	37.63	37.34	37.61	37.68	37.37	37.84
20	37.18	37.61	37.56	37.46	37.29	37.05	37.90	37.74	38.12	37.44
40	38.10	38.41	37.65	38.05	37.46	36.91	37.24	37.66	37.94	37.51
60	38.92	39.10	37.87	38.88	38.14	38.23	37.77	38.13	38.72	38.19
80	39.40	39.51	38.49	39.37	38.41	38.85	38.24	38.34	39.01	38.92
100	39.50	39.51	38.47	39.99	38.60	38.51	38.84	38.81	39.40	39.29
120	39.41	39.28	38.35	40.07	38.84	37.92	39.33	38.00	39.95	39.93
140	39.47	39.64	38.24	39.67	39.02	37.53	39.74	38.25	40.16	40.41
160	39.44	39.65	38.09	38.46	39.26	37.44	40.06	37.65	40.54	40.45
180	39.31	39.91	38.07	38.52	39.60	37.76	40.13	37.27	40.75	40.45
200	38.99	39.30	38.15	39.15	39.83	37.93	40.16	37.12	40.50	40.27
220	38.81	39.55	38.10	39.37	39.50	38.19	40.07	37.15	39.99	39.83
240	38.78	39.29	38.18	39.32	38.56	38.80	39.75	37.30	39.67	39.35
260	38.62	38.83	38.46	39.15	38.68	38.41	39.55	37.87	39.33	39.14
280	38.23	38.65	39.26	38.67	38.25	37.71	38.05	38.57	38.58	38.80
300	37.69	38.65	39.28	38.15	37.24	37.22	37.69	38.37	38.08	38.40
320	37.41	38.32	39.28	37.24	37.09	37.27	37.73	38.63	38.15	37.81
340	37.64	38.41	39.33	37.02	37.32	36.99	37.36	38.19	37.74	37.70
360	37.50	38.32	38.80	36.82	37.51	37.00	37.08	37.26	37.68	37.63

Group Ms

Rat	D5	E4	E9	F1	F4
Time (min)					
0	37.49	37.23	36.92	36.67	37.13
20	38.12	37.05	36.79	36.85	36.95
40	38.63	37.45	36.83	36.97	36.91
60	39.20	38.89	37.06	37.56	38.52
80	39.17	39.57	37.81	38.49	38.90
100	39.44	39.89	37.86	39.17	38.92
120	39.74	40.30	38.24	39.41	39.24
140	39.84	40.58	38.50	39.49	39.29
160	39.70	40.76	38.70	39.61	39.31
180	39.59	40.67	38.92	39.69	39.54
200	39.56	40.60	39.24	39.43	39.31
220	39.66	40.42	39.38	39.33	39.43
240	39.49	40.41	39.64	38.88	39.26
260	39.25	40.29	39.60	38.48	38.63
280	39.11	39.66	38.53	37.87	38.02
300	38.49	39.30	37.18	37.61	37.83
320	38.16	38.60	37.36	37.27	37.47
340	37.50	38.56	37.32	37.11	37.19
360	37.32	37.73	37.03	37.13	37.12

Group mm

Rat	B1	B2	B3	B4	F7
Time (min)					
0	36.74	36.52	36.81	37.06	37.24
20	37.06	36.53	37.03	37.42	37.35
40	37.13	36.79	37.07	37.54	37.34
60	37.72	37.38	38.59	38.49	38.11
80	38.22	37.74	38.84	39.38	38.91
100	38.39	38.02	38.65	40.01	39.20
120	38.70	38.73	39.08	39.84	39.27
140	38.65	39.00	38.48	39.82	39.24
160	38.67	39.18	38.27	39.52	39.08
180	38.81	38.52	38.54	40.08	39.03
200	39.00	38.95	38.62	39.76	39.09
220	38.68	39.18	38.44	39.75	39.20
240	37.82	38.50	38.58	39.14	38.55
260	37.46	38.07	37.97	39.30	38.42
280	37.10	37.93	37.81	38.36	37.88
300	36.85	37.58	37.73	37.84	37.44
320	37.24	37.67	37.59	37.60	37.37
340	37.31	37.51	37.51	37.59	37.28
360	37.82	37.22	37.22	37.46	37.07

Group ms

Rat	D7	D8	D9	D12	F8
Time (min)					
0	37.50	37.32	36.95	36.96	38.43
20	37.57	37.24	37.13	36.91	38.26
40	37.76	37.31	37.31	37.05	38.41
60	38.35	37.73	37.60	37.05	39.00
80	38.96	38.23	37.80	37.37	39.69
100	39.59	38.68	38.14	38.01	40.18
120	40.00	38.98	38.29	38.17	40.21
140	40.00	39.38	38.49	38.77	40.56
160	39.78	39.67	38.73	38.49	40.36
180	39.29	39.64	38.70	38.44	40.24
200	38.85	39.19	38.78	38.75	40.17
220	38.38	38.54	38.79	38.60	39.88
240	38.31	38.78	38.58	38.21	39.49
260	37.84	39.02	38.42	38.05	38.42
280	37.01	39.01	37.67	38.05	38.32
300	37.61	38.98	37.75	37.64	38.30
320	37.97	37.77	37.54	37.95	38.11
340	37.34	37.64	37.53	37.64	38.31
360	37.25	37.46	37.32	37.82	38.28

Group sm

Rat	C2	C4	C6	F9	F11
Time (min)					
0	37.46	36.73	37.27	37.21	37.14
20	37.68	37.01	37.12	37.30	36.94
40	38.48	37.35	37.10	37.31	37.61
60	37.96	37.97	37.82	37.85	37.96
80	37.65	37.26	37.43	38.42	38.32
100	37.53	36.79	37.34	38.12	37.67
120	36.85	36.79	37.39	37.45	37.32
140	36.90	36.97	37.20	37.29	37.45
160	36.97	36.90	37.23	37.18	37.49
180	36.90	37.13	37.50	37.14	37.14
200	36.84	36.91	37.24	37.07	37.14
220	36.93	37.04	37.24	37.15	37.16
240	36.76	37.01	37.41	37.16	37.14
260	37.01	37.03	37.18	37.15	37.27
280	36.61	37.34	37.27	37.11	37.41
300	36.94	37.40	37.22	37.14	37.34
320	36.58	37.28	37.35	37.14	37.29
340	36.60	37.28	37.62	37.39	37.32
360	36.93	37.31	37.60	37.30	37.47

Group ss

Rat	C1	C3	C5	F10	F12
Time (min)					
0	37.07	36.73	37.22	37.24	36.70
20	37.12	36.87	37.27	37.26	36.78
40	37.91	37.03	37.73	37.53	37.25
60	38.39	37.71	38.36	38.30	37.84
80	38.24	37.71	37.64	37.94	37.71
100	37.84	37.28	37.48	37.51	37.42
120	37.40	37.04	37.62	37.55	37.03
140	37.23	37.15	37.71	37.69	37.05
160	37.49	37.23	37.68	37.65	37.16
180	37.48	37.18	37.35	37.53	37.03
200	37.72	36.93	37.27	37.22	37.00
220	37.32	36.89	37.48	37.18	37.06
240	37.15	36.84	37.32	37.20	36.99
260	37.45	37.09	37.28	37.17	36.96
280	37.50	36.84	37.44	37.23	36.95
300	37.39	37.23	37.66	37.56	37.15
320	37.19	37.17	37.24	37.32	36.99
340	37.01	37.50	37.82	37.32	37.13
360	37.33	37.27	37.68	37.26	37.02

Appendix H

Temperature Data from Chapter 5, Day 10

Group Mm

Rat	E5	E6	E7	F1	F5	F7	F8	F9	F11	F12
Time (min)										
0	36.98	36.88	36.71	36.99	36.86	37.40	36.94	37.91	37.41	36.91
20	36.85	36.80	36.69	37.19	37.04	37.13	36.91	37.99	37.34	37.15
40	36.81	37.08	36.79	38.02	37.41	37.75	37.02	38.54	37.02	37.04
60	37.82	37.98	38.84	39.38	38.58	39.32	37.18	39.65	38.45	38.15
80	38.02	38.14	40.21	40.13	39.03	39.86	37.60	40.10	39.15	39.00
100	38.09	38.54	40.25	39.95	39.24	40.10	37.55	41.01	39.48	39.67
120	38.54	39.09	40.12	39.91	39.73	40.10	39.03	41.20	39.66	40.14
140	39.47	39.40	40.03	40.18	39.96	40.12	39.04	41.32	39.73	40.06
160	39.64	39.26	40.15	40.13	40.07	40.02	39.75	41.08	39.96	40.06
180	40.04	38.62	40.02	40.33	39.96	39.66	40.18	41.19	40.03	39.89
200	39.82	38.27	39.94	39.57	39.50	39.38	40.19	40.93	39.73	39.56
220	39.70	37.61	39.92	39.20	39.29	39.43	39.98	40.66	39.52	39.24
240	39.36	36.85	39.33	38.92	39.02	39.20	39.76	40.51	39.26	38.93
260	39.38	36.80	38.91	38.79	38.10	38.40	39.16	40.15	38.45	37.11
280	38.88	37.00	38.99	38.48	38.15	38.17	38.27	38.01	38.19	36.79
300	38.66	36.49	38.63	37.99	37.39	37.79	36.58	37.21	37.12	36.39
320	38.74	36.18	38.73	37.70	37.13	37.71	36.15	36.92	36.81	35.99
340	38.61	35.04	38.57	37.56	37.12	37.77	36.12	36.90	36.79	36.03
360	38.42	34.54	38.21	37.54	37.35	37.15	37.75	37.17	37.51	35.71

Group Ms

Rat	D5	E4	E9	G1	G4
Time (min)					
0	37.04	36.91	36.85	36.86	37.02
20	37.19	36.98	37.01	36.94	36.95
40	37.81	38.07	37.46	37.61	37.18
60	39.62	39.13	38.65	38.78	38.95
80	39.53	39.75	39.18	39.59	40.04
100	39.53	40.26	40.02	39.71	40.05
120	39.70	40.17	39.80	39.85	39.66
140	39.64	40.16	40.01	39.80	39.62
160	39.41	39.95	39.91	39.45	39.53
180	39.30	40.02	39.82	39.57	39.38
200	38.67	39.87	39.80	39.52	39.10
220	38.69	39.67	39.44	39.03	38.46
240	38.02	39.35	39.53	38.62	38.82
260	37.79	37.33	39.28	38.44	38.46
280	37.69	37.00	38.58	38.27	38.37
300	37.79	36.96	38.59	37.87	38.20
320	37.39	36.52	38.46	37.26	37.79
340	37.67	36.30	38.21	36.92	37.17
360	37.57	36.11	37.98	37.14	37.16

Group mm

Rat	B1	B2	B3	B4	G7
Time (min)					
0	36.90	36.52	37.03	37.03	37.22
20	36.96	36.65	37.30	37.53	37.20
40	36.97	36.48	37.23	37.46	37.40
60	38.46	37.10	37.91	38.78	38.58
80	39.09	37.66	38.83	40.00	39.22
100	39.27	37.91	39.25	40.13	39.48
120	39.44	38.46	39.69	40.02	39.71
140	39.41	38.85	39.47	39.98	39.43
160	39.63	39.31	39.58	39.54	39.88
180	39.34	39.35	39.46	39.25	39.59
200	39.09	38.90	39.11	39.19	39.43
220	38.81	38.66	38.82	38.88	39.14
240	38.35	38.26	37.76	38.35	38.64
260	37.93	37.94	38.26	37.83	38.47
280	38.10	37.95	37.91	38.17	38.13
300	37.76	37.86	37.68	37.57	37.33
320	37.87	37.85	37.67	37.81	37.26
340	37.52	37.67	37.61	37.64	37.21
360	37.89	37.44	37.66	37.64	37.16

Group ms

Rat	D7	D8	D9	D12	G8
Time (min)					
0	36.90	37.44	37.19	36.79	38.11
20	36.88	37.22	37.44	36.96	38.02
40	37.14	37.73	37.61	36.99	38.37
60	38.83	39.67	38.88	38.17	39.92
80	39.15	40.00	39.34	38.81	40.41
100	39.25	40.00	39.53	39.42	40.20
120	39.15	40.00	39.45	39.38	39.97
140	38.97	39.82	39.56	39.36	39.61
160	38.95	39.59	39.36	38.80	39.82
180	38.85	39.11	39.51	39.06	39.21
200	38.51	38.94	39.24	38.70	39.20
220	38.40	38.63	39.28	38.25	39.11
240	38.33	38.41	39.03	38.01	38.81
260	38.12	38.20	38.44	37.83	38.78
280	38.13	37.91	38.47	37.46	38.51
300	37.79	37.58	37.87	37.06	38.23
320	37.56	37.59	37.64	37.17	38.10
340	37.65	37.23	37.60	37.08	38.20
360	37.18	37.11	37.63	37.21	38.12

Group sm

Rat	C2	C4	C6	G9	G11
Time (min)					
0	36.55	37.00	36.95	37.32	37.05
20	36.71	37.01	37.38	37.22	37.00
40	36.74	36.79	37.25	37.18	37.17
60	36.47	36.99	37.02	38.09	38.56
80	36.66	37.13	36.89	38.65	38.25
100	36.78	37.01	36.94	37.96	37.96
120	36.85	36.96	36.91	37.60	37.48
140	36.93	37.03	36.96	37.27	37.14
160	37.06	37.09	37.07	37.09	36.86
180	36.97	37.10	37.10	37.07	36.76
200	37.03	36.90	37.16	37.41	36.89
220	36.96	36.63	36.97	37.63	36.90
240	36.90	36.70	37.08	37.61	36.78
260	37.01	37.43	36.94	37.43	37.06
280	37.10	37.46	37.48	37.25	37.14
300	37.21	37.18	37.37	37.39	37.29
320	37.31	37.07	37.27	37.16	37.32
340	37.46	36.99	37.32	37.32	37.13
360	37.25	37.30	37.06	37.44	37.24

Group ss

Rat	C1	C3	C5	G10	G12
Time (min)					
0	36.89	37.04	36.92	37.48	37.10
20	37.12	36.89	37.01	37.31	37.05
40	37.33	36.77	37.02	37.49	37.57
60	37.30	37.15	37.00	37.78	37.36
80	36.85	37.13	37.08	38.13	37.35
100	37.00	37.25	36.97	37.75	37.24
120	36.79	37.00	37.12	37.52	37.14
140	36.94	36.95	37.88	37.33	37.18
160	36.99	37.01	37.36	37.48	37.28
180	37.12	36.76	37.15	37.42	37.23
200	37.28	36.99	37.07	37.27	37.21
220	37.25	37.06	37.16	37.19	37.20
240	37.29	37.24	37.14	37.17	37.20
260	37.44	37.03	37.13	37.38	37.32
280	37.37	37.01	36.98	36.95	37.10
300	37.32	37.04	37.03	37.10	37.15
320	37.30	36.99	37.51	37.25	37.15
340	37.23	37.02	37.30	36.99	37.17
360	37.17	37.70	37.25	36.95	37.12

Appendix I

Temperature Data from Chapter 5, Day 11

Group Mm

Rat	E5	E6	E7	F1	F5	F7	F8	F9	F11	F12
Time (min)										
0	36.20	36.60	37.44	37.07	36.84	37.27	37.00	38.23	37.48	36.83
20	36.69	36.65	37.21	37.17	36.96	37.26	36.86	38.43	37.66	37.29
40	37.90	37.22	37.70	37.92	37.61	38.15	37.52	39.01	38.69	38.22
60	37.15	38.28	38.61	39.17	38.70	38.88	38.64	40.07	39.38	39.14
80	36.73	38.64	39.06	39.15	39.67	39.18	39.05	40.14	39.60	39.24
100	36.62	38.65	38.73	38.94	39.49	39.33	38.93	40.06	39.38	39.19
120	36.67	38.80	38.37	38.63	38.97	38.80	38.84	39.79	39.27	38.75
140	36.27	37.85	37.91	38.39	39.08	38.75	37.82	39.31	38.72	38.47
160	36.08	35.70	37.95	37.97	38.53	38.25	37.26	38.36	38.31	37.99
180	35.94	35.45	37.45	37.65	37.73	38.01	36.22	37.52	38.01	37.49
200	35.69	37.18	36.46	37.16	37.46	37.52	36.12	37.03	37.90	37.01
220	35.58	37.20	36.65	36.57	36.95	37.05	36.05	36.99	36.98	36.63
240	35.05	37.01	36.67	36.41	36.78	36.98	36.20	36.54	37.87	37.15
260	34.77	36.98	36.65	36.71	36.84	36.72	36.61	36.94	37.65	37.42
280	35.05	37.12	37.93	37.22	36.81	37.40	36.68	37.28	37.65	37.40
300	36.72	37.14	37.88	37.38	37.25	37.30	35.95	38.29	37.84	37.76
320	36.97	37.04	37.97	37.48	37.16	36.97	36.94	38.53	37.86	36.99
340	36.87	36.99	37.17	37.24	37.29	37.21	37.13	37.82	37.83	37.13
360	36.70	37.05	37.93	37.60	37.25	37.61	37.37	38.52	37.72	37.40

Group Ms

Rat	D5	E4	E9	G1	G4
Time (min)					
0	37.14	37.08	37.56	37.16	37.01
20	36.90	37.09	37.59	36.96	37.01
40	37.57	36.47	38.09	37.88	37.59
60	37.79	37.71	38.15	37.34	37.83
80	37.57	37.61	37.57	37.35	37.44
100	37.31	37.35	37.16	37.33	37.23
120	37.35	37.45	37.09	37.17	37.16
140	37.19	37.25	37.26	37.07	37.05
160	37.34	37.36	37.60	37.09	37.01
180	37.17	37.43	37.40	37.21	37.24
200	37.02	37.39	37.13	37.09	37.13
220	37.16	36.95	37.08	36.88	37.10
240	37.31	37.48	37.06	37.14	37.26
260	37.38	37.29	37.11	37.16	37.11
280	37.41	37.32	37.25	36.79	37.10
300	37.16	37.47	37.10	36.80	37.09
320	37.28	37.07	37.45	36.82	37.17
340	37.38	37.21	37.48	36.83	36.96
360	37.37	37.37	37.25	37.06	36.98

Group mm

Rat	B1	B2	B3	B4	G7
Time (min)					
0	37.10	36.76	36.76	37.52	37.40
20	37.08	36.70	36.92	37.65	37.46
40	37.82	37.56	37.46	38.28	38.14
60	37.93	37.95	38.98	39.70	38.91
80	38.85	38.45	39.58	40.18	39.61
100	39.47	38.89	39.50	40.27	39.96
120	39.83	39.46	39.59	39.77	39.89
140	39.77	39.71	39.51	39.41	39.68
160	39.71	39.53	39.00	39.11	39.50
180	39.49	39.45	37.65	38.94	39.31
200	39.17	39.36	38.55	38.78	39.06
220	39.10	39.14	38.69	38.66	38.97
240	38.48	38.44	37.70	38.48	38.57
260	38.35	37.92	36.52	37.96	38.25
280	38.00	38.26	38.24	38.30	38.24
300	37.77	37.66	37.74	37.70	37.63
320	37.76	37.89	37.67	37.93	37.23
340	38.03	37.73	38.06	37.77	37.29
360	38.04	37.73	37.64	37.77	37.28

Group ms

Rat	D7	D8	D9	D12	G8
Time (min)					
0	37.00	36.98	36.92	37.30	38.12
20	37.10	37.07	36.95	37.51	38.13
40	37.84	38.62	37.58	37.71	39.41
60	37.73	38.14	38.11	37.81	38.99
80	37.59	37.92	37.74	37.39	38.57
100	37.42	38.09	37.51	37.38	38.40
120	37.45	37.70	37.59	37.43	38.38
140	37.35	37.62	37.41	37.34	38.14
160	37.11	37.58	37.39	37.41	38.23
180	37.15	37.49	37.51	37.33	38.39
200	37.73	37.92	37.47	37.21	38.46
220	37.41	37.74	37.57	37.25	38.13
240	37.88	37.71	37.37	37.42	38.23
260	37.57	37.48	37.70	37.31	37.83
280	37.77	37.74	37.91	37.36	38.08
300	37.59	37.90	37.55	37.50	37.84
320	37.99	37.66	37.59	37.49	38.07
340	37.66	37.68	37.62	37.32	37.81
360	37.83	37.79	37.55	37.90	38.05

Group sm

Rat	C2	C4	C6	G9	G11
Time (min)					
0	36.86	37.18	37.09	37.41	37.32
20	37.03	37.22	36.82	37.46	37.28
40	37.24	37.16	36.84	37.75	37.70
60	37.81	37.40	37.16	38.67	37.85
80	38.00	37.97	37.76	39.43	38.57
100	38.06	38.26	38.17	39.92	39.42
120	38.65	38.70	38.46	39.99	39.47
140	38.50	38.89	38.76	39.47	39.24
160	38.97	39.66	38.98	39.21	39.13
180	39.39	39.84	39.47	38.95	39.04
200	39.84	39.68	39.50	38.88	38.48
220	39.66	39.62	39.10	38.69	37.97
240	39.36	39.21	39.11	38.60	37.53
260	38.94	38.96	38.67	38.30	37.58
280	38.38	38.38	38.36	38.16	37.33
300	37.84	38.27	37.50	37.71	37.25
320	37.70	37.78	37.18	37.32	37.19
340	37.51	37.32	37.09	37.26	37.11
360	37.34	37.10	37.16	37.23	37.05

Group ss

Rat	C1	C3	C5	G10	G12
Time (min)					
0	36.71	36.99	36.79	37.10	37.22
20	36.92	36.89	36.82	37.07	37.15
40	36.85	36.86	36.91	37.78	37.49
60	36.79	36.86	37.11	37.78	37.49
80	36.74	36.71	36.88	37.56	37.31
100	36.71	37.05	37.09	37.54	37.16
120	36.95	37.04	37.18	37.31	37.04
140	36.70	37.08	37.49	37.13	36.97
160	36.82	37.18	37.64	37.05	36.99
180	36.68	37.26	37.39	36.96	36.94
200	36.64	37.20	37.54	37.09	36.98
220	36.99	36.73	37.11	37.03	37.05
240	36.98	37.09	37.28	37.06	37.05
260	37.21	37.31	37.07	37.10	37.15
280	37.13	37.19	37.30	37.06	37.30
300	36.84	37.21	37.03	36.92	37.24
320	37.01	37.37	37.51	37.06	37.19
340	36.85	38.10	37.30	37.06	37.15
360	36.97	37.70	37.25	37.17	37.01

Appendix J

Temperature Data from Chapter 5, Day 13

Rat	E5	E6	E7	F1	F5	F7	F8	F9	F11	F12
Time (min)										
0	37.69	36.94	37.17	37.33	36.91	37.13	36.72	38.44	37.37	36.64
20	37.77	36.89	37.21	37.30	36.83	36.84	36.80	38.38	37.51	37.51
40	38.31	37.71	37.65	37.78	37.48	38.15	37.34	39.27	38.46	38.02
60	39.07	39.74	38.37	39.20	38.85	39.29	39.15	41.02	39.78	39.60
80	40.01	39.72	38.92	39.44	39.19	39.52	39.25	41.20	39.82	39.65
100	39.10	39.57	39.00	39.02	39.15	39.16	39.15	41.00	39.59	39.59
120	38.90	39.24	39.00	38.98	38.74	39.24	38.36	40.50	39.45	39.33
140	38.45	39.39	38.89	38.79	38.14	39.05	37.86	39.95	39.01	39.13
160	38.25	38.92	38.26	38.60	37.36	38.56	38.72	38.76	38.08	38.94
180	38.58	38.48	38.43	38.11	37.23	38.62	37.57	38.51	37.86	38.56
200	37.73	38.14	37.68	37.70	37.11	38.09	36.92	38.78	37.23	38.03
220	37.97	37.66	37.24	37.41	37.18	37.81	36.66	38.55	37.16	37.83
240	37.69	37.33	37.13	37.44	37.19	37.58	37.31	38.58	37.56	37.54
260	37.74	37.31	37.19	37.39	37.04	37.54	37.19	38.62	37.68	37.49
280	37.76	37.12	37.09	37.44	37.05	37.49	37.44	38.56	38.01	37.25
300	38.16	36.97	37.15	37.47	37.16	37.75	37.22	38.22	37.85	37.34
320	37.69	37.05	37.20	37.48	37.14	37.71	36.72	38.54	37.56	37.52
340	38.20	37.21	37.25	37.22	37.27	37.86	37.26	38.29	37.40	37.07
360	38.11	37.62	37.30	37.30	37.30	38.11	36.86	38.34	37.52	37.53

Appendix K

Temperature Data from Chapter 5, Day 15

Rat	E5	E6	E7	F1	F5	F7	F8	F9	F11	F12
Time (min)										
0	37.86	37.48	37.10	37.12	36.84	37.41	37.89	38.28	37.61	36.88
20	37.52	37.10	37.05	36.99	36.76	37.12	37.73	38.48	37.75	37.75
40	38.17	37.83	37.48	37.80	37.41	38.43	38.65	38.04	38.70	38.26
60	39.06	39.33	38.68	39.11	38.78	39.57	39.92	38.34	40.02	39.84
80	39.51	39.32	39.21	39.38	39.12	39.80	40.20	39.05	40.06	39.89
100	39.82	39.30	38.93	39.11	39.08	39.44	39.98	39.34	39.83	39.83
120	39.76	37.87	38.76	38.99	38.67	39.52	39.83	39.24	39.69	39.57
140	39.68	36.80	38.01	38.66	38.07	39.33	39.46	38.89	39.25	39.37
160	39.59	36.27	37.95	38.17	37.29	38.84	38.87	38.38	38.32	39.18
180	39.40	36.35	38.11	37.99	37.16	38.90	38.79	37.89	38.10	38.80
200	39.10	36.74	38.38	37.63	37.04	38.37	38.45	37.49	37.77	38.18
220	38.54	36.81	37.95	37.47	37.11	38.09	38.33	37.52	37.40	38.07
240	38.22	36.63	37.52	37.40	37.12	37.86	38.23	37.47	37.80	37.78
260	38.47	37.21	37.21	37.32	36.97	37.82	38.14	37.26	37.92	37.73
280	38.29	37.48	37.11	37.33	36.98	37.77	38.13	37.44	38.25	37.49
300	38.74	37.55	37.11	37.46	37.09	38.03	38.30	37.81	38.09	37.78
320	38.47	37.59	37.18	37.44	37.07	37.77	38.20	37.52	37.80	37.36
340	38.85	37.83	36.75	37.45	37.20	37.44	38.13	37.55	37.34	37.21
360	38.73	37.28	37.36	37.57	37.23	37.29	38.13	37.55	37.12	37.25