AUGMENTED LEVELS OF TOLERANCE AND PHYSICAL DEPENDENCE IN RATS SELF-ADMINISTERING MORPHINE COMPARED TO THOSE PASSIVELY RECEIVING THE DRUG

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

INTRAVENOUS SELF-ADMINISTRATION OF MORPHINE BY RATS

DOCTOR OF PHILOSOPHY (1995) McMASTER UNIVERSITY
(Psychology) Hamilton, Ontario

TITLE: Augmented levels of tolerance and physical dependence in rats self-administering morphine compared to those passively receiving the drug.

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NUMBER OF PAGES: ix, 269

ABSTRACT

Two physiological changes (namely tolerance and physical dependence) reliably emerge following repeated exposure to the opiate drugs and are considered to be important contributors to the behavioural state of addiction. As such, they are often induced in experimental subjects prior to examining addictive behaviour. An assumption implicit in such work is that the physiological effects of passively-administered drugs are equivalent to those of actively self-administered drugs. Two experiments are presented which demonstrate that the development of both tolerance and physical dependence is attenuated in rats passively receiving morphine compared to those self-administering identical doses of the drug. An additional experiment found evidence for the ability of a schedule of passive drug delivery to attenuate physical dependence and tolerance established through drug self-administration. Furthermore, in all three experiments, tolerance and physical dependence were found to develop rapidly and reliably following the self-administration of surprisingly small doses of morphine. One means by which self-administration might facilitate the acquisition of tolerance and physical dependence is through the inherently greater predictability of self-administered drug doses. An experiment designed to assess whether increased signalling of impending drug doses would likewise facilitate the acquisition of tolerance and physical dependence failed to demonstrate any such effect.

Acknowledgements

I am indebted to the members of my supervisory committee (Drs. John Platt, Ron Racine, Shep Siegel, and Harvey Weingarten) for their insight, assistance, and support over the years. Many people assisted in one way or another with the work reported herein. These include: Doreen Mitchell, Mitch Mitchell, Lianne Cooper, Mike Scoles, Rolfe Morrison, Faye Prawdzik, Glenda MacQueen, Mike Bader, Bruce Lidsten, Alex Dzubin, Al Lewis, Doug Scott, Steve Dworkin, Lucille Hoover, and Fred LePiane. My sincere thanks to all.

The contribution of my wife, Dr. Megan Jane Rutherford, has been enormous. Without her persistence and encouragement over the past three years, this thesis would not have been completed. Thank-you, darling.

My gratitude goes out to the numerous members of the Department of Psychology, both faculty and students, who helped to make my years at McMaster so gratifying. I would particularly like to point out the significant roles played by April Takeda, John & Bev Platt, Eric Schaller, Bill Lambos, Lee Brooks, Steve Link and Don Maxwell.

Finally, I would like to thank my parents, Emma and Sandy MacRae. Thank-you for giving me life, thank-you for preparing me for this world, and thank-you for being so patient over the years.

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CHAPTER 1: INTRODUCTION

> "As far back as recorded history, every society has used drugs that produce effects on mood, thought, and feeling. Moreover, there were always a few individuals who digressed from custom with respect to the time, the amount, and the situation in which these drugs were to be used. Thus, both the non-medical use of drugs and the problem of drug abuse are as old as civilization itself." (Jaffe, 1990, p.522)

Overview

Non-medical drug use is viewed by many as one of the primary social and medical problems we face today. Similar concerns have been voiced repeatedly in the past over the non-medical use of many different psychoactive drugs encompassing agents from many different pharmacological classes. The drug of primary concern today is cocaine (particularly in it's smokeable form "crack"). Over the past century, there have been periods during which the non-medical use of opium, morphine, heroin, ethanol, marijuana and nicotine has evoked similar concern and outcry. Excessive non-medical use of a psychoactive drug (drug abuse) creates numerous issues and problems for both the abuser and for the society in which he or she resides. The widespread abuse of ethanol and nicotine, for example, is generally accepted as resulting directly in the largest group of avoidable social and medical problems faced by modern society. The chronic abuser of opiate drugs (opium

and its various derivatives, e.g., morphine and heroin) typifies to many the picture of the "drug addict," whose life revolves around the acquisition of the next dose. The abuser of "crack" is viewed as a sorry (and unpredictably dangerous) individual who, like the heroin "addict," has become a slave to the drug.

A large body of evidence has accumulated which addresses various issues pertaining to how and why an individual becomes so involved with the use of a given drug as to earn the label of "drug abuser" or "addict". Such research generally focuses on one or more of the following issues:

-types of individuals predisposed to drug use
-intrinsic properties of the drug
-changes in the user following short-term drug use
-changes in the user following long-term drug use
-the initiation of drug use
-the transition from drug use to drug abuse
-the treatment of established patterns of drug use
-relapse to drug use following a period of abstinence.

Focus/Goals of Dissertation

This dissertation will focus on research and issues that relate to the opiate drugs (collectively referred to as the opiates). While research on other drug classes will be noted where appropriate, the opiates are among the most

thoroughly studied of the "drugs of abuse." Much is known regarding the mechanisms of action of the opiates, and the effects of repeated exposure to the class are well documented. The discovery of the endogenous opioid peptides and their receptors leads to the likelihood that major advances in knowledge will be made regarding the physiological mechanisms underlying various opiate effects. Further specification of the behavioural and psychological factors influencing opiate effects will aid in the ultimate synthesis of behavioural, psychological, and physiological evidence as it pertains to the question of opiate abuse. The goal of this thesis is to contribute new information to the field of addiction research regarding some of the psychological and behavioural dimensions which impinge on the effects of exposure to opiate drugs. Specifically, this thesis addresses the hypothesis that the effects of exposure to opiate drugs depend, in part, on the level of control an organism has over drug administration.

Structure of Thesis

Chapter 1 introduces some of the relevant aspects of investigations into the nature of the non-medical use of drugs, beginning with the definition of terms commonly employed in such work and ending in the role traditionally played by psychologists in examining various aspects of drug use. Chapter 2 outlines the main empirical findings to date

and introduces the hypotheses about the role of control in the development and expression of the effects of chronic drug use that motivated the present research. Chapters 3, 4, 5 and 6 present four experiments that tested these hypotheses, and Chapters 7 and 8 summarize and elaborate upon their implications for current theory and both experimental and clinical practice.

Definition of Terms

> "When I use a word, it means just what I choose it to mean-neither more nor less." (Lewis Carroll, 1871, p. 188)

Many of the terms used in discussions concerning the non-medical use of psychoactive drugs are inadequately defined. There is a lack of agreement amongst both the public and drug-abuse investigators as to the meaning of the terms commonly employed in describing drug abuse. This contributes to the lack of understanding that often occurs in attempts to communicate ideas relating to this field. Many of the terms used to describe different aspects of the non-medical use of psychoactive drugs are not simply descriptive in nature. A good deal of meaning and value is often communicated through the use of one term vs. another that superficially describes the same phenomenon. For example, a heavy chronic non-medical user of psychoactive drugs might be referred to as a "chronic drug user," a "drug abuser," a "drug addict," or a "drug-dependent individual."

In the discussion that follows, an attempt will be made to employ a consistent set of terminology in describing the effects of chronic drug use. To that end, definitions of the more important terms to be employed follow. These terms include euphoria, dysphoria, tolerance, physical dependence, psychological dependence, craving, drug abuse, and addiction.

Drug Effects

It is generally agreed that the primary motivation underlying the use of a drug is for the subjective effects of the drug. Other consequences of drug use may be important in determining the initiation or continuance of its use, but are beyond the scope of this investigation. These include various social attributions concerning the act of drug administration, a desire to act in a manner inconsistent with current laws, etc.

The subjective effects of a drug can be dichotomized on a scale based on the affective (hedonic) state imposed by their administration. Those drug effects that "feel good" or "are desirable" are termed the hedonically positive effects of the drug. The hedonically positive effects of such drugs will be referred to as their reinforcing effects throughout this thesis. One of the distinguishing features of drugs of abuse is their ability to induce some hedonically positive state in the user. Such

positive reactions to administration of a drug can take on many forms. The opiates, being an extremely effective group of analgesics, can lead to the rapid relief of pre-existing pain in users. They can also lead to a powerful feeling of well-being, pleasure and contentment which is commonly referred to as euphoria.

Such euphoria is often only one of a constellation of effects that first-time users of a drug experience. This group of effects need not all be hedonically positive in nature. The emphasis of theoreticians attempting to explain the non-medical use of drugs is, however, commonly placed on the euphoric components of drug action.

Those drug effects that "feel bad" or "are undesirable" are considered the dysphoric, or hedonically negative effects of the drug. For example, first-time opiate users often report such unpleasant feelings as nausea following drug administration. The hedonically negative effects of such drugs will be referred to as their punishing effects throughout this thesis. While such dysphoric drug effects are interesting in terms of the initiation of chronic drug-seeking behaviour, they are generally ignored by theoreticians attempting to explain the non-medical use of drugs.

Tolerance

Following repeated exposure to many drugs, users begin to experience lessened effects to a given dose of drug. In order to attain the same levels of drug effect as previously experienced, larger doses of the drug must be administered. Tolerance refers to a decrease in the effectiveness of a given dose of a drug following repeated exposure to the drug. Alternatively, tolerance may be defined as an increase in the dose of a drug required to attain effects similar to those previously attained. The meaning of these two definitions is the same: tolerance implies a decreased impact on the system of the drug user following repeated exposure to the drug. Tolerance develops rapidly and reliably following repeated exposure to opiates.

Dependence

Physical Dependence

Another phenomenon that develops following repeated exposure to any of a number of drugs is physical dependence. Users feel physical discomfort following a sufficient drug-free interval. This discomfort is rapidly relieved through subsequent administration of the drug. Physical dependence develops reliably and markedly following repeated use of opiates. The behavioural syndrome evidenced in an opiate-dependent individual for whom sufficient time has elapsed

since the last drug administration is called withdrawal (or withdrawal distress or the abstinence syndrome or the withdrawal syndrome). It is the abstinence syndrome evidenced by physically-dependent individuals that is, to many, the defining characteristic of the opiate addict.

Psychological Dependence

Psychological dependence upon a drug is manifested in the presence of a craving for the drug. This craving stems from a desire to experience the drug's positive effects. The meaning of the term craving, itself, has been the subject of controversy (see Kozlowski & Wilkinson, 1987). Craving, in the current context, will be defined as a desire to use the drug. Such desire is often inferred from the presence of behaviour that results in the acquisition or use of the drug, particularly when physical dependence upon the drug is not apparent.

Addiction

The various terms used to describe the chronic non-medical use of psychoactive drugs are amongst the least precise in the meaning(s) they convey. The various terms used have included addiction, abuse, drug habit, drug dependence, the non-medical use of drugs, sustained opiate-directed behaviour, drug appetite, and morphinomania. Early controversy surrounding the use of such terminology in the

study of drug addiction is outlined in Terry and Pellens' (1928) review of the literature concerning the "Opium Problem". These authors noted that:

> "As elsewhere, here also controversial subjects are involved, for different writers have used terms and definitions which in themselves have indicated particular attitudes toward the nature of the problem. Thus habit, craving, appetite, mania, addiction, addiction-disease, all may be interpreted significantly if desired by those using or reading them. We have tried to select some name for the condition of chronic opium-using that would not carry a partisan, restricted or incomplete implication as far as the nature of the condition is concerned. The phrase chronic opium intoxication seems to fulfill this purpose better than any other but it has the disadvantage of length and is at times an awkward expression." (Terry & Pellens, 1928 p. XX)

These authors subsequently chose to use whichever of the terms seemed to best suit the needs of the moment. They did, however, emphasise that "adherence to no school of thought or theory of the nature of the condition under consideration is to be inferred from the terminology employed in the present volume" (p.XX).

The use of the various terms describing the non-medical use of psychoactive drugs has been inconsistent over the years. Nichols (1965, p.80) goes so far as to suggest that the term "addiction" "is used in such an arbitrary and loose manner, even by investigators, that it has little real meaning". He suggests, instead, that "it is better to use the more descriptive term "sustained opiate-directed behavior," which focuses attention on the important part of

the problem: the change in behavior that is brought about by opiates." (Nichols, 1965 p.80).

Historically, there has been a tendency to equate the state of physical dependence upon a drug with addiction to that drug. For the purposes of this exposition, these two terms will be used with their more commonly accepted scientific meanings. Dependence will be used to refer to the state of physical dependence defined earlier as consisting of the manifestion of withdrawal distress in the absence of drug. The terms addiction and abuse will be used interchangeably in this thesis to refer to the behavioural syndrome of compulsive drug use operationally defined by Nichols as "sustained opiate-directed behaviour".

Psychology and Drug Effects

Early Interest Regarding Drug Effects and Addiction

Early psychological interest in the field of drug addiction focused on the question of which individual characteristics (such as the possession of a weak will or a lack of moral values, [Terry & Pellens, 1928, p.1]) set the drug abuser apart from other people. These characteristics were generally viewed as being of a psychological nature (personality, moral character, strength of will, etc.). The commonly observed fact that not all individuals that were exposed to a given "addictive" drug became "addicted" to it

led to the hypothesis that an "addictive personality" might exist, pre-disposed to the harmful potential of such drugs.

Over a century of effort has been expended in pursuit of such a set of characteristics which would identify individuals at risk of developing a compulsive addiction to drugs. In summarizing such attempts, Brecher warns "against placing excessive reliance on studies equating particular personality characteristics with a tendency to use a particular drug." (Brecher, 1972, p.19). This warning stems from the premise that numerous factors which vary substantially across time contribute to the attraction of different "types" of individuals (which vary with changes in these factors) to the use of drugs. These factors include popular social attitudes toward drugs, the legal climate surrounding the possession, use and/or sale of drugs, and numerous other unspecified social and/or enviromental influences peculiar to a particular time and place.

In the late 19th century, the view arose that, in addition to the psychological nature of addiction, there was a physiological component as well. With this recognition, it became more common to view addiction as a disease-like state. The addiction-as-disease viewpoint remains clouded, however, by the fact that continued drug use is, at least on the surface, a matter of choice. The active decision must

be made to administer the drug. That the action of drug self-administration occasionally becomes so regular as to be viewed as a habit further enhanced the interest of early experimental psychologists in this dramatic class of behaviours.

Experimental Psychology and Drug Effects

Classical Conditioning and Tolerance/Dependence

Over the past 70 years, the Pavlovian (or classical) school of experimental psychology has explored the effects of pairing biologicallyrelevant stimuli with relatively neutral stimuli on organisms' subsequent reactions to these neutral stimuli (Pavlov, 1927). Changes in an organism's reactions to such neutral stimuli following pairings with biologicallyrelevant stimuli is said to reflect the presence of classical (or Pavlovian) conditioning.

The prototypical classical conditioning experiment presents an animal with a portion of food, coupled with the sounding of a tone. While the tone initially results in little reaction from the animal, following numerous such pairings it will be reliably followed by increased salivation.

As early as 1925, it was shown that certain drugs would function as biologically relevant stimuli in a manner consistent with the classical conditioning model (Collins

and Tatum, 1925). In the mid-1970s, it was shown that previously neutral stimuli which had been repeatedly paired with drug administrations came to reliably influence the subsequent expression of tolerance to that drug's effects (Siegel, 1975). Subsequent work has shown that such stimuli act in a manner consistent with those employed in traditional classical conditioning experiments. Today, the evidence for a role of classical conditioning mechanisms in the development, expression, and loss of tolerance is substantial (e.g., Poulos and Cappell, 1991; Siegel, 1989).

A role for classical conditioning mechanisms has been postulated in the development and expression of physical dependence. This postulation does not enjoy as much experimental support as does the contribution of classical conditioning mechanisms to tolerance (but see O'Brien, Testa, O'Brien, Brady, & Wells, 1977, for an example). The possibility of a role played by classical conditioning mechanisms in the development, maintenance, expression and loss of physical dependence does, however, exist.

Operant Conditioning and Drug Self-Administration

Over the past 50 years, the operant (or instrumental) school of experimental psychology has explored numerous situations in which organisms are allowed to operate upon their environment (Skinner, 1938). If

environmental change is made contingent on some aspect of an organism's behaviour, the frequency of that behaviour is often seen to change. Changes in the frequency of behaviour reliably correlated with such contingent environmental change are said to reflect the presence of operant conditioning.

The prototypical operant conditioning experiment allows a hungry animal the opportunity to acquire food through the emission of a specified response (e.g., lever-pressing). When the delivery of food is made contingent upon lever-pressing, the frequency of lever-pressing will typically increase. Generally, the result is that more food is delivered to the animal.

By the mid-1950s, similar experimental preparations were being utilized in an attempt to model human drug addiction (Headlee, Coppock & Nichols, 1955; Nichols, Headlee & Coppock, 1956). By the early 1960s, preparations similar to that described above were developed which allowed an animal to deliver a dose of a drug to itself (Weeks, 1961; Davis & Nichols, 1962). The self-administration of drugs was found to result in patterns of operant responding similar to those observed for such standard reinforcers as food and water. In addition, it has been shown that animals will, in general, self-administer drugs that have been shown

to be abused by humans (Griffiths & Balster, 1979; Griffiths & Bigelow, 1978).

Over the past 30 years, the drug self-administration procedure has proven extremely useful in modelling the behavioural aspects of addiction. In addition, the validity of the technique is supported by the use of self-administration procedures to screen for the abuse potential of newly developed drugs. It has been found that behavioural tests in which animals are given an opportunity to self-administer such new compounds provide valuable information concerning the likelihood that humans will abuse such substances.

Addiction

As mentioned above, early psychological interest in the study of addiction centred primarily on the identification of characteristics which defined individuals susceptible to the addictive process. Only over the past 40 years has the emphasis in research on addiction shifted to the behavioural features of the state.

The fact that addiction is defined in terms of an organism dosing itself with a drug has led to an emphasis on experiments employing self-administration techniques in attempts to model addictive behaviour. This thesis will concentrate on the literature surrounding the self-administration of drugs in attempts to model human drug

addiction. The contributions of tolerance and physical dependence to the addictive process will be discussed. A previously unappreciated role of the act of self-administration in the genesis of addictive behaviour will be described, and theoretical and practical issues stemming from this finding will be presented.

CHAPTER 2: LITERATURE REVIEW

> "In the human use of opiates, S-initiated-response versus passivity gives strikingly different probabilities for the occurrence of addiction (sustained opiate-directed behavior). Passive recipients of opiates, such as hospital patients, may show tolerance, physical dependence, and even a classical withdrawal syndrome; but when released from the hospital, they do not rob drug stores, forge prescriptions, or indeed, show any sign of "craving" opiates. Despite the enormous number of patients who receive opiates and the claims of addicts, only a few addicts are created by medical treatment.
>
> In contrast, illegal users emit opiate-directed responses. The physician who gives himself an injection to relieve the symptoms of illness or fatigue and the delinquent who tries heroin on a dare both emit the drug-taking action, and exactly that action rapidly becomes a chronic obsessive compulsion for both of them. In contrast to the extremely low addiction rate for passive hospital patients, the addiction rate for the response-emitting users, whether physicians or delinquents, is quite high.
>
> In animal experiments, Ss usually are passive recipients of opiates on a schedule established by E. These passive animals, like the passive hospital patients, show all of the physical changes brought about by opiates but do not show sustained opiate-directed behavior--they do not relapse." (Nichols, 1963, pp. 896-897)

History of Opiate Use

Drawn from Terry and Pellens, (1928) Chs 1,2

Human knowledge of the medical usefulness of the opiates goes back at least as far as the Sumerian culture. Medical writings from that time and from the days of the

ancient Greeks, Romans, and Egyptians included treatments for a vast number of illnesses and complaints that included the extract of the opium poppy (papaver somniferum).

It must be remembered that the primary emphasis in the practice of medicine until the last century was the treatment of symptoms. The causes of disease were not well understood and were, as a result, generally not treated. Treatment was focused on alleviating the complaints (symptoms) of patients. The opiates constitute one of the most potent analgesic drug classes in existence and are also extremely effective in suppressing coughing and diarrhea. It is not surprising, then, that opiates were such a popular ingredient in medicines throughout recorded history.

The early unrestricted use of opiates must have resulted in cases of physically-dependent individuals. However, little mention (yet alone warning) of this "danger" is evident in the medical literature until the mid- to late 19th century. Terry and Pellens mention numerous early warnings of the dangers of indiscriminate opiate use (as early as J. Jones' book, "The mysteries of opium revealed" published in 1700: see Terry & Pellens, 1928, p.58). It is apparent that these early warnings did not filter through to the medical community.

The increasing awareness of the dangers of indiscriminate opiate use stemmed, in large part, from the

rapid growth in the use of these drugs during the latter part of the 19th century. A number of factors which have been invoked to explain the dramatic increase in use of the opiates are:

-the wide-spread use of opium amongst migrant Chinese workers

-the isolation and purification of morphine in 1804

-the widespread use of morphine as an analgesic in the U.S. Civil War

-the introduction of the hypodermic syringe as a new means of drug delivery in the 1840s

-the growth of social sanctions against the use of alcohol (re: the "gin problem"), coupled with the effective substitution of morphine for alcohol in alcoholics

-the growth and popularity of patent medicines

-the synthesis of heroin in 1898.

With this dramatic increase in the use of opiates came increasing opportunity for physicians to observe physically-dependent individuals. It is ironic that some of the early treatments advocated for the treatment of opiate dependence involved the administration of other opiates. Initially, morphine was used to treat opium dependence, followed by the use of heroin to treat morphine dependence. A good portion of the opiate addiction problem at the turn

of this century was, either directly or indirectly, iatrogenic in nature.

By the second decade of this century, the dangers of indiscriminate opiate use were well known. The passage of the Harrison Narcotic Act in 1914 restricted access to these drugs and introduced sanctions against physicians found to be abusing their right of prescription. After this point, the distribution of opiates in support of the addict population moved "underground" and has remained so until the present day.

Early Addiction Research and Theory

Human work

Early research on addiction centred on the effects of protracted exposure to the opiates. The stage for much of the subsequent work on opiates was set in 1925 by Lawrence Kolb. In describing the opiate user, Kolb emphasized that initial opiate use stemmed from a "mental pleasure" caused by drug use. Such pleasure, the description follows, occurs only in "unstable persons" and is rapidly decreased as tolerance develops. Once tolerance to the "mental pleasure" of opiates has developed, subsequent use is maintained primarily through the relief of the pain associated with opiate withdrawal.

In a series of papers published in 1929, Light, Torrance, and Karr describe the physiological impact of the administration of morphine to addicts and the emotional and physiological reactions to the withdrawal and subsequent re-administration of morphine. These investigators noted both the lack of any physical deterioration in the addicts and normal levels of physiological function observed following morphine administration. In examining the addicts throughout a 48-hour drug-free period, Light and Torrance noted both an emotional and a physiological component in the ensuing withdrawal syndrome. These authors were struck by the intensity of the emotional component, particularly since this "mental suffering" clearly preceded any observable physiological withdrawal symptoms. This mental suffering was presented as an extremely powerful motivating force in the addict. In the first of this series of papers, Light and Torrance observe that:

> "After a person has become firmly addicted to the use of opium or one of its derivatives, we have reason to believe that the problem of securing and maintaining an adequate supply of the drug comes to be the major purpose of his existence. To an extraordinary degree, he develops a sagacity and persistence in this direction which may out-match the abilities of those who are conducting the investigation. The ingenuity that is displayed in maintaining channels of supply is amazing. We have come to believe that practically every word which the addict utters and every deed which he may perform are based directly or indirectly on a motive concerned with the maintenance of these channels of supply." (pp.206-207).

It is clear that these investigators held concerns over the effectiveness of their efforts to control drug dosing on the part of the addicts. They also held concerns over the veracity of self-report information from the addicts relating to their emotional and physical state. These concerns are ultimately voiced both as a disclaimer and as an explicit warning to future investigators attempting to evaluate human drug addicts:

> "Without this introductory paper, the reader might fail to appreciate in the papers which are to follow, the deceptiveness of the addict during experimentation." (p.211).

Himmelsbach (1942) examined 21 addicts over a 9-month period of abstinence from opiates. In contrast to the findings of Light and Torrance, he noted that the physiological adaptation to morphine in the addict is "incomplete" (p.772). Physiological function in the face of morphine was observed to be within normal levels prior to the initiation of abstinence. The perturbations in function observed throughout withdrawal had recovered and stabilized following a period of 6 months. Comparison of the levels of physiological function following withdrawal with those observed during addiction led to the conclusion that "adjustment to morphine is not complete during addiction" (p.771). This suggested that the physiological disturbances associated with withdrawal distress could persist for a period on the order of months.

Animal work

The findings of Light and Torrance reviewed above indicated that opiate addicts could tolerate exposure to opiates without negative physiological consequences. This made studying the effects of opiates in humans acceptable on ethical grounds providing, of course, that the humans were already addicted to the drug. However, such restrictions inhibited the ability to examine the development of the effects of chronic exposure to the opiates. As a result, the bulk of such work has been performed on animals.

The animal work on the effects of chronic exposure to the opiates showed that both tolerance and physical dependence developed in a manner similar to that observed clinically in humans (e.g., Schmidt & Livingston, 1933; Spragg, 1940). Two of these early papers are of particular relevance to the current analysis. Collins and Tatum (1925) observed the presence of what appeared to be conditioned salivary and emetic responses in dogs during experiments concerning the effects of chronic morphine exposure. Following as few as seven daily subcutaneous injections of morphine, the sight of the hypodermic syringe came to reliably elicit salivation in the animals. This observation stands as the first indication that drug-related effects could be conditioned in a manner consistent with Pavlovian conditioning (although note that Pavlov mentions a similar

observation of conditioned salivary and emetic responding by one Dr. Krylov as having been observed "quite recently" [Pavlov,1927, pp.35-37]). The importance of this discovery lies in the fact that physiological effects normally associated with the presence of a drug can be evidenced in the absence of that drug. As will be shown later, conditioned pharmacological responses can be either drug-like or withdrawal-like in form.

Spragg (1940) conducted the first experimental evaluation of the ability of animals to develop and display a "desire" for a drug. This investigation was conducted in order to address what Spragg perceived as a deficiency in previous investigations of "addiction" employing animals as experimental subjects. This deficiency was that such investigations, which assumed addiction to be an organic phenomenon, tended to focus exclusively on the physiological and pharmacological effects of acute and chronic morphine administrations. Tolerance and physical dependence were the two aspects of "addiction" most studied and, indeed, addiction came to be defined in terms of the development of physical dependence (pp. 7-8). What was missing from this formulation, in Spragg's reasoning, was "this factor of positive desire and striving for the drug" (p.8). Spragg argued that, if animal models of human addiction are to

contribute further to the knowledge of the human state, they must incorporate this fundamental part of addiction.

Spragg gave four chimpanzees repeated, increasing daily or bi-daily injections of morphine in a specific room. Early indications of the development of physical dependence were apparent following from three to seven weeks of such exposure. Over the course of the next few months, three of the animals had begun to display behaviours which Spragg interpreted as the "first signs of desire for the morphine injection" (p. 59). These behaviours included an eagerness to be removed from the home cage at the regular injection time, struggling to get to the room in which injections were regularly administered, the display of frustration when attempts were made to remove the animal from the injection room without an injection having been supplied, eager cooperation in the injection procedure, and the initiation of the procedure which normally led to injections. While these observations are somewhat compelling, their subjective nature detracts from their value.

Data from a series of choice tests are presented for two of the "morphine-desiring" chimpanzees. On each such test, a choice could be made between opening a box containing food and a box containing a morphine-filled hypodermic syringe. For each of these animals, the box containing the syringe was opened significantly more often

than chance would suggest if the animals had been without an injection of morphine for a period in excess of 16 hours. Conversely, if an injection of morphine had occurred recently, both chimpanzees opened the box containing food significantly more often than chance would suggest. Spragg concluded that chimpanzees can, under favourable circumstances, come to display the desire and striving for the drug that defines human addiction. This desire and striving is thought to come about through the formation of an association between the injection and the alleviation of withdrawal distress. Spragg ultimately speculated that "morphine addiction seems to be fundamentally a phenomenon of learning or perception, which may or may not be socially facilitated." (p. 123).

The importance of Spragg's work is two-fold. The primary theoretical contribution of the work lies in it's redefinition of addiction to include some notion of "striving" to acquire the drug. The primary practical contribution of the work lies in it's realization that research employing animal subjects can be designed in such a way as to address questions about such addiction. With these ideas, the stage was set for the intensive behavioural research effort on the problem of addiction which was to follow.

Current Emphases in Addiction Research and Theory

Over the past 40 years, psychological attention has increasingly shifted to the behavioural aspects of addiction. The sustained or compulsive nature of drug-acquisitory behaviour has, to many, come to define the state of addiction (e.g., Jaffe, 1980; Nichols, 1965). Three predominant theoretical treatments of addiction are those emphasizing the physiological, associative, and adaptive natures of the problem. These are summarized below, followed by a discussion of how the tolerance and dependence associated with chronic drug exposure are felt to contribute to the addictive process. The use of the drug self-administration paradigm to experimentally evaluate factors contributing to the addictive process is then reviewed.

Theories of Addiction

Theoretical Accounts of Addiction

The perception of drug addiction as a major social and health problem in North America has led to a substantial body of research focusing on the etiology, prevention, and treatment of addiction. While a good deal of effort has been expended examining the addictive process, no comprehensive theory or model of addiction has resulted. This is particularly evident when one considers the lack of-

agreement among addiction researchers on the definition of addiction itself.

Numerous interpretations of addictive behaviour have been offered over the years. The definition of addiction in terms of a primary behavioural component of compulsive or sustained drug use is often extended to include the importance of physical dependence. This is a convenient definition of addiction to numerous drugs including the opiates. The existence of addictive behaviour to drugs that do not, apparently, result in physical dependence suggests that physical dependence is not a necessary precursor to addiction. Historically, models of addiction have fallen into three categories: physiological, associative, and adaptive. These categories differ in their specification of the mechanisms which contribute to the addictive process.

Physiological Theories

The physiological theories of addiction stem from the basic supposition that addictive behaviour is simply a symptom of some underlying "disease." This disease is viewed as being of either genetic or experiential origin.

The genetic models posit the presence of a congenital disorder manifested as a homeostatic disturbance which drug use tends to nullify. The discovery of endogenous opiate-like substances in the mid-1970's (Hughes, 1975) has added fuel to genetic theories through the

possibility that congenital deficiencies in endogenous opioid peptides might result in a state which predisposes an individual to subsequent drug use. The primary problem with such theories lies in the difficulty of adequately assessing the contribution of genetic factors to behaviour.

Support for a genetic influence in addiction comes from studies examining differences in the incidence of alcoholism between siblings who have been raised in different environments. The first such study which supported a genetic basis for alcoholism was the adoption study reported in 1974 (Goodwin, Schulsinger, Moller, Hermansen, Winokur & Guze, 1974). These investigators reported similar rates of alcoholism in sons of alcoholics raised by their biological parents and in their brothers, who had been raised by adoptive parents. Another line of research has evaluated the genetic contribution to alcoholism by examining the concordance of alcoholism between both fraternal and identical twins. In a review of research on the genetic basis of alcoholism, Searles (1988) questions the conclusions of such studies on the grounds that serious methodological flaws pervade such research. Searles concludes that the contribution of environmental factors to the etiology of alcoholism may have been systematically underemphasized in such research.

Viewing the physiological "disorder" underlying addiction as being experiential in origin allows for more extensive investigation of the mechanisms which result in the disorder. The bulk of work in this area has emphasized changes in the organism resulting directly from pharmacological stimulation. While the development of physical dependence is the change most often emphasized by physiological theorists, the high correlation between drug tolerance and physical dependence has led to the suggestion that:

> "A satisfactory theory should offer a unitary explanation of the fact that tolerance and dependence apparently develop, persist, and disappear together, as though they were reflections of the same underlying biologic change" (Goldstein, Arnow, & Kalman, 1974, p. 510).

Cochin (1971) suggested that theories positing a physiological change in the development of tolerance can be classified, albeit loosely, into five distinct categories:

-interference with access of the drug to its site of action

-altered metabolic processes

-prolonged receptor occupation

-cellular adaptations based on biochemical change

-some change, posited at the cellular level, which "resembles an immune reaction or reaction analogous to memory" (Cochin, 1971, p. 432).

The theme common to these approaches is that tolerance develops through mechanisms which decrease the pharmacological effectiveness of a drug. In the absence of pharmacological stimulation, the same mechanisms are manifested as withdrawal distress. The argument follows that such withdrawal distress can then only be alleviated through drug administration.

The discovery of endogenous opioid peptides has also influenced the experiential school. It has been proposed that repeated exposure to opiates might lead to an enduring incapacity of the body to synthesize or utilize endogenous opioids. This incapacity is then thought to lead to a homeostatic disturbance in the absence of pharmacological stimulation which is manifest as withdrawal distress (Snyder, 1977).

One criticism of the "addiction as disease" concept rests in the fact that humans abuse (and become addicted to) a wide variety of substances, with greatly different pharmacological effects. Proponents of physiologically-based addiction would, therefore, have to postulate either the vulnerability of many disparate physiological systems to addiction-causing "disease" or, alternatively, a common physiological system underlying all addictions. The finding that rats readily perform an operant response to acquire electrical stimulation of certain brain areas (Olds &

Milner, 1954) has led to a preference for the second of these alternatives. The common physiological system that has been presented as underlying all addictions is that of the "pleasure centres" or other reinforcement-associated systems within the central nervous system (e.g., Wise & Bozarth, 1987). It is, perhaps, the intense, "compulsive" behaviour displayed by animals allowed to self-stimulate these centres that has led to their predominance in the search for the primary physiological mechanism underlying addiction.

In summary, physiological theorists of addiction emphasize the importance of previous exposure to the drug in explaining the addictive process. Such exposure results in the development of both tolerance to the drug's various effects and physical dependence upon the drug. The tolerance that develops results in a decrease, per unit dose, in the effects the drug has on the reinforcement centres of the brain. The physical dependence that develops results in a "need" on the part of the dependent organism for the drug. This need is generally held to reflect an underlying homeostatic imbalance which becomes evident in the absence of the drug.

One of the defining assumptions of physiological theorists is that tolerance and dependence result directly from previous exposure to the drug. The specific conditions

of such exposure, and the specific history of the experimental subjects are given little, if any, attention. The single important variable is previous pharmacological stimulation. Following this emphasis, it is common for investigators looking at the physiological mechanisms involved in the addictive process to employ experimental subjects that have had previous experience with the drug. In the interests of experimental control, such experience is generally experimenter-determined in a manner that equates the amount of drug exposure across experimental subjects.

Associative Theories

The emphasis on the ability of drugs to alleviate withdrawal distress is central to a number of conceptions of addiction but begs the question as to why drug use is initiated in the first place. While this question is far from resolved, it has become apparent in the past three decades that drug use is subject not only to the pharmacological history of the organism but also to the associative history of that organism.

Early associative accounts of drug addiction postulated that initial drug-acquisitive behaviour is maintained by the intrinsic reinforcing properties of drugs (Epling & Bradshaw, 1974; Schuster & Thompson, 1969). From this operant perspective, any behaviour which is followed by a reinforcing agent is more likely to be repeated in the

future. In evaluating such interpretations of addiction, animal models employing the operant self-administration of drugs are commonly used. Oral, inhalatory, intra-gastric, intravenous, and intracerebral self-administration are the techniques that have been employed for this purpose (see Young & Herling, 1986 for a review). Such work has revealed that simple schedules of reinforcement employing drugs as reinforcers lead to patterns of responding typical of such "standard" reinforcers as food for hungry animals (Thompson & Pickens, 1969). For example, under fixed-ratio schedules of reinforcement, increasing the ratio requirement leads to an increase in responding (Weeks & Collins, 1968), suggesting a certain degree of auto-titration of drug intake in self-administering animals.

Following repeated self-administration, the operant model proceeds to the addition of "negative reinforcement" in the maintenance of drug-acquisitive behaviours. Under this conception, the termination of withdrawal distress in the now dependent organism by the administration of the drug is thought to have reinforcing properties, thereby promoting the re-occurrence of drug self-administration. Most drugs which are administered by man serve well as operant reinforcers in laboratory animals (Goldberg & Henningfield, 1988; Griffiths & Balster, 1979; Griffiths & Bigelow, 1978). This finding supports the notion that operant work in drug

addiction employing animal subjects is dealing with a mechanism which relates to human addictive behaviour.

The operant work on addiction does, however, tend to neglect the possibility that the physiological consequences of a given drug history are influenced by factors other than the pattern and number of previous drug administrations. Wikler (1968) has proposed a theory in which two factors are said to operate in addiction. The first is the classical conditioning of physical dependence through temporal contiguity between specific situations and the occurrence of drug abstinence phenomena. The second factor is the reinforcement of instrumental drug-acquisitive behaviour through the repeated reduction, by the drug, of withdrawal distress that develops during the intervals between drug administrations (negative reinforcement). The addition, by Wikler, of classical conditioning mechanisms in the genesis of addictive behaviour serves as the first suggestion that drug addiction is influenced by the environment in which the potential addict exists.

A related conception of addiction is that proposed by Siegel and co-workers (Siegel, 1990; MacRae, Scoles, & Siegel, 1987). The compensatoryresponse model of drug tolerance proposed by Siegel (1975) has been generalized to account for the development and maintenance of physical dependence on drugs. Briefly, cues previously paired with

drug administration are said to elicit compensatory conditional responses (CCRs) which are opposite in form to the unconditional drug effects. In the absence of the drug, the elicitation of such CCRs results in a homeostatic imbalance which manifests itself as withdrawal distress. This distress can then be alleviated only through further drug intake. It is assumed that this distress is aversive, leading to a craving for the drug. The organism is compelled to seek out further pharmacological stimulation which, in turn, may serve to strengthen the CCRs thought to mediate dependence. Once the organism has entered this "vicious circle", it is said to be addicted.

This model is similar to Wikler's with the exception that the cues thought to elicit withdrawal distress are different. Wikler maintains that such cues are those which have previously been paired with withdrawal distress, while the present formulation maintains that they are those cues which have previously been paired with drug administration per se. Both of these formulations emphasize the importance of the ability of a drug to alleviate such distress (negative reinforcement) in the maintenance of addictive behaviour.

It is apparent, however, that such negative reinforcement cannot be operative in drug-naive organisms. In attempting to deal with the initial use of drugs, a

number of investigators (as previously noted) have invoked the notion that intrinsic reinforcing properties exist as a common feature among drugs of abuse. It seems clear that such drugs are taken, at least in part, for the pleasurable effects they produce. After prolonged use, the development of physical dependence certainly opens the possibility of negative reinforcement being operative in the maintenance of addictive behaviour.

It has repeatedly been demonstrated that the incidence of withdrawal distress can be influenced by the occurrence of stimuli previously paired with drug administration (see Siegel, 1990 for a review). In interviews with both opiate addicts (O'Brien, Testa, O'Brien, & Greenstein, 1976) and alcoholics (Ludwig & Stark, 1974), it has been reported that withdrawal distress is especially pronounced in the presence of cues which have previously been paired with drug self-administration. The suggestion that the CCRs which mediate tolerance may also mediate withdrawal distress is consistent with the observation of a high correlation between tolerance and physical dependence, and the fact that many withdrawal symptoms are opposite in form to acute drug effects (Kalant, 1973).

One of the predictions of the above analysis is that, when confronted with cues previously paired with drug

self-administration, ex-addicts will experience conditional withdrawal distress. The homeostatic imbalance created by the elicitation of drug-preparatory CCRs may be an important determinant of craving. Ludwig and Wikler (1974) have suggested that craving represents "the psychological or cognitive correlate of a 'subclinical conditioned withdrawal syndrome'" (p. 114).

In summary, associative theorists of addiction emphasize the importance of previous drug-related learning in explaining the addictive process. Such learning can influence the development and expression of both tolerance to the drug's various effects and physical dependence upon the drug. The tolerance that develops is considered in terms of it's effects on the reinforcing efficacy of the drug. The physical dependence that develops results in the emergence of negative reinforcement mechanisms in the maintenance of drug self-administration behaviour. One of the defining assumptions of associative theorists is that tolerance and dependence result, in part, from the environmental and/or behavioural contingencies surrounding experience with drugs. The specific conditions of such exposure, and the specific history of the experimental subjects are emphasized.

Two classes of associations are thought to be possible. The first are the stimulus-stimulus associations

emphasized by researchers concerned primarily with Pavlovian conditioning mechanisms. These associations are made possible through the reliable pairing of drug administrations with discrete environmental (or internal) stimuli. The second are the stimulus-response associations emphasized by researchers concerned primarily with operant conditioning mechanisms. These associations are made possible through the presence of behavioural contingencies in which the administration of the drug depends, in part, on the behaviour of the experimental subject.

Investigators interested in the Pavlovian mechanisms underlying the addictive process invariably administer drugs to their experimental subjects under a strict, pre-defined schedule. Investigators interested in the operant mechanisms underlying the addictive process generally employ experimental protocols in which the behaviour of the experimental subjects influences the administration of the drug. A procedure common within such investigations consists of pre-exposing experimental subjects to the drug. This is generally done for one of two reasons. The first is as an attempt to "shape" the appropriate operant behaviour. The second stems from the recognition that physically-dependent organisms are more likely to respond for the drug than are non-dependent organisms.

Adaptive Theories

The physiological and associative views of addiction both emphasize various aspects of experience with the drug in attempting to explain the addictive process. The question remains, however, as to the existence of pre-disposing factors in addiction. The adaptive view of addiction addresses this issue in attempting to explain why certain individuals become addicted, while others do not. The adaptive theories of addiction emphasize the genesis of addiction as either a response to some personality disorder or, alternately, as an attempt to cope with pre-existing distress.

Studies which examine the correlations between addiction and either personality disorders or specific personality sub-types have identified a number of factors which differentiate drug addicts from the normal population (Pittel, 1971; Salmon & Salmon, 1977; Nerviano & Gross, 1983; Miller, 1990). While the possibility exists that such factors as low self-esteem, poor familial identification, and high levels of depression and anxiety may contribute to the development of addiction, the correlational nature of such studies does not justify such conclusions. It is just as possible that the observed differences between addicted and normal populations are a result (and not a cause) of the

addiction. As early as 1920, warnings against assuming that an addictive personality "type" exists were voiced:

> "There are drug addicts constitutionally inferior, and superior; feeble-minded, and strong-minded; physically below, and above par; morally inferior, and superior. No one class of society seems, in our experience, to enjoy a monopoly in this practice." (Hubbard, 1920 quoted in Terry & Pellens, 1928, p. 34).

The other adaptive view of addiction is that which suggests that drug use is initiated in an attempt to reduce pre-existing distress. Alexander and Hadaway (1982) championed this idea, and suggest that the physiological and associative views of addiction traditionally emphasized in the literature fail in their attempts to incorporate "new data" in the field.

The portrayal by Alexander and Hadaway (1982) of adaptive and non-adaptive theories of addiction as mutually-exclusive entities is invalid. It is true that non-adaptive theorists do not, as a rule, invoke pre-existing distress as a factor in the etiology of addiction. Such theorists do, however, make note of the importance of the drug in alleviating withdrawal distress. The common practice of inducing physical dependence in experimental subjects prior to any opportunity for self-administration demonstrates that the importance of pre-existing distress (withdrawal distress) is recognized. The role of the drug in alleviating other forms of negative hedonic states is not

generally emphasized by non-adaptive theorists. Such a role is, however, consistent with the role played by the drug in the alleviation of the negative hedonic state embodied by withdrawal distress.

Alexander and Hadaway list a number of types of adaptive theories dealing with drug use. These are:

1) theories which portray drug use as an attempt to cope with problems stemming from inadequate personality characteristics

2) theories which portray drug use as an attempt to deal with physical or psychological pain--not necessarily originating in personality disorders

3) theories which portray addiction as a normal adaptive response to a hostile society

4) theories which emphasize the use of drugs to alleviate intra-family distress.

In an elaboration on this theme, Alexander (1982) argues that addiction entails an attempt to evade crucial difficulties or conflicts. This attempt consists of "addictive involvements" that are comprised of a number of coping-based behaviours in which no particular behaviour is essential. The notion of an "addictive complex" which may well entail non-drug related behaviours (see also Peele, 1985) is an admirable contribution to the field of addiction studies.

In a sense, adaptive theorists of addiction point out that drug addiction is only a specialized case of an organism's tendency to attempt to cope with negative (or stressful) situations. As with any attempt to cope, the results may be beneficial, neutral, or harmful. Drug use as an attempt to cope is generally viewed as embodying the latter of these possibilities.

Chronic Drug Effects

> "The term addiction is not easily defined, but in all cases it manifests itself in the phenomena of tolerance and physical dependence." (Snyder , 1977, p.11)

Addiction, in this thesis, is defined in terms of the behavioural components of the state. Given this emphasis, the addictive potential of a drug will be defined in terms of its ability to serve as an operant reinforcer. While such a behavioural emphasis favours an associative view of addiction, neither the physiological nor adaptive views summarized above are excluded. Under a view of addiction which stresses the behavioural components of the phenomenon, the effects of chronic exposure to the drug are crucial. Both tolerance and physical dependence influence those aspects of drug administration which are thought to be reinforcing.

Tolerance

The importance of tolerance to the addictive process is commonly viewed as stemming from a desire on the part of the drug user to attain at least the same level of euphoria following a given drug administration as has previously been experienced. As tolerance to the euphoric effects of the drug develops, larger and larger doses are required to attain levels of euphoria comparable to those previously experienced.

Another manner in which tolerance can affect the addictive process is through a decrease in the intensity of hedonically negative components of the drug's effects. Assume, for example, that tolerance to the nausea-inducing effects of opiates develops more rapidly and more completely than does tolerance to their euphoric effects. It would follow that continued exposure to the drug would result in a shift in the hedonic balance (the overall hedonic profile, consisting of the positive less the negative effects of the drug; see Ettenberg, Sgro, & White, 1982 for a discussion of the apparent summation of the affective properties of "rewarding" and "aversive" stimulation). In such a situation, subsequent doses of the drug would, on average, be hedonically "better" (more positive or reinforcing) than previous doses.

Physical Dependence

The importance of physical dependence to the addictive process is commonly viewed as stemming from a desire on the part of the drug user to avoid or escape the discomfort associated with withdrawal. The most reliable way to avoid this discomfort is to administer sufficiently large doses of the drug at a frequency that does not allow withdrawal distress to emerge. Should withdrawal distress be experienced, the quickest and most reliable way to end it is to administer another dose of the drug.

The withdrawal symptoms associated with physical dependence upon the opiates include "restlessness, yawning, chills, pilomotor activity (cold-turkey), excessive nasal secretions, drowsiness, then lacrimation, sneezing, dilation of the pupils, cramps, vomiting, diarrhea, weight loss, excessive perspiration, muscular twitchings, seminal emissions in the male, orgasms in the female" (Light & Torrance, 1929a, p.206) and peak approximately 48-72 hours following the last drug dose. This withdrawal syndrome is described as being unpleasant and uncomfortable. A dose of the drug rapidly terminates these symptoms.

In a sense, those ascribing primary importance to physical dependence in the maintenance of addictive behaviour are formulating addiction in a framework analogous to that of behaviour motivated by electric shock (Miller,

1948). Withdrawal distress, as an unpleasant state is something that is expected to lead to both avoidance and escape-motivated behaviours. The addict can avoid withdrawal behaviours by responding (through drug self-administration) in a timely fashion (before the effects of the last dose have worn off sufficiently). Should the addict experience withdrawal distress, it can readily be escaped through the administration of an additional dose.

This raises the issue of what actually maintains the behaviour in an organism that, as a matter of routine, successfully avoids the unpleasant experience of withdrawal distress. Miller (1948) has suggested that cues reliably paired with shock come, through Pavlovian conditioning, to elicit an unpleasant state (fear). Hence, an avoidance response can be viewed as a response which allows the organism to escape this unpleasant state. As previously mentioned, Light and Torrance (1929a) noted that an emotional component of withdrawal ("mental suffering") reliably preceded any observable physiological withdrawal symptoms in humans. Perhaps drug-taking behaviour in the physically-dependent individual is motivated (and reinforced) in part through the escape afforded from such mental suffering.

Tolerance and Dependence: Similar underlying process?

Both tolerance and dependence reliably develop in organisms repeatedly exposed to physiologically significant levels of opiate. A number of variables have been shown to affect the rate of development of tolerance and dependence. The most important variables identified to date include:

-the dose of drug delivered per administration

-the frequency of administrations

-the overall length of experience with the drug

-the environmental conditions associated with administration.

In general, studies examining tolerance and/or dependence have shown that these two phenomena develop more rapidly with larger drug doses per administration and with greater frequency of administration (e.g., Schmidt & Livingston, 1933). The levels of tolerance and dependence displayed appear to be a function primarily of these variables, coupled with the overall length of experience with the drug. A useful heuristic is that both tolerance and dependence increase with increasing total exposure to the drug. As an individual continues to administer the drug, and as tolerance and dependence continue to increase in intensity, a feedback loop is initiated which results in a spiralling escallation of "need" for the drug.

That tolerance and physical dependence reliably develop in organisms repeatedly exposed to the opiates is well known (Jaffe & Martin, 1990). Traditionally, tolerance and physical dependence have been assumed to be "inextricably linked" both to each other and to the problem of compulsive drug use (Jaffe, 1990, p.524). As Jaffe points out, neither of these assumptions is valid. Examples are common of drugs which do not (on the surface) induce physical dependence, but which are self-administered (e.g., marijuana). Drugs exist in which the development of both tolerance and physical dependence does not lead to self-administration (imipramine). Jaffe also points out that, given an appropriate choice of drug, either tolerance or physical dependence can be produced in isolation.

These points are valid criticisms regarding the necessity and sufficiency of tolerance and physical dependence in the genesis of addiction. The statement that these two phenomena can (and do) contribute to the self-administration of numerous drugs is equally valid. That they do so in the case of the opiates is, after weighing the evidence, a clear statement of fact.

Modelling Addiction

The historical definition of addiction as being physically dependent upon a drug stems, in large part, from the use of opiates as the prototypical addictive drug class.

Repeated exposure to the opiates invariably leads to physical dependence. This, coupled with the clinical importance placed on managing the withdrawal syndrome in the treatment of opiate addicts, has led to what is, perhaps, an undue emphasis on withdrawal distress. The fact that it is so obvious (and, occasionally, dramatic) does not necessitate that withdrawal distress be the fundamental property of addiction.

Nevertheless, the view that physical dependence is central in addiction has heavily influenced the experimental literature attempting to model addiction over the past half century. Early attempts to model opiate addiction generally viewed the presence of physical dependence as the endpoint that defined the state of addiction (e.g., Seevers, 1936). With the shift in definitional emphasis to one concerned with drug-seeking behaviour, physical dependence has come to be used more as a tool to enhance levels of drug self-administration.

A common procedural component of animal research on the mechanisms underlying addiction is the practice of inducing some level of physical dependence in the subjects. This is true both of studies directly examining the tolerance and dependence seen following chronic drug exposure and of studies examining the initiation and maintenance of drug self-administration.

It is clear that making an animal physically dependent upon an opiate increases the likelihood that the animal will, if given the opportunity, administer the drug (e.g., Wikler, Martin, Pescor, & Eades, 1963). Studies that allow just such opportunity, will now be reviewed. It will be shown that these studies typically incorporate a procedure in which experimental subjects are made physically dependent prior to any opportunity for self-administration.

Self-Administration Studies

The emphasis in animal drug self-administration studies is on the behavioural act of drug-seeking. Whether this be defined as the preference for a location previously "associated" with the drug (Beach, 1957), as a preference for the ingestion of the one of two fluids that contains the drug (Nichols, Headlee & Coppock, 1956), or as the performance of some behaviour which leads to the receipt of the drug (Headlee, Coppock & Nichols, 1955), drug-seeking behaviour is interpreted within an operant conditioning framework. By way of review, this framework predicts increases in any behaviour which either leads to the onset of a hedonically positive event (positive reinforcement) or leads to the offset of a hedonically negative event (negative reinforcement).

Spragg's pioneering study (1940), summarized earlier, was the first to demonstrate an apparent preference

for drug-related stimuli. Chimpanzees made physically dependent on morphine ultimately displayed an apparent desire to be taken to the room in which injections occurred when sufficient time had elapsed since the last dose. When given the choice between a box containing food and one containing a morphine-filled syringe, these animals would reliably choose the syringe when morphine-deprived (and only when morphine-deprived).

These results suggest that the relief of withdrawal distress by the administration of morphine is a sufficient condition for the occurrence of drug-seek ng behaviour. This emphasis on the importance of the relief of withdrawal is significant, inasmuch as this paper set the stage for the animal drug self-administration field. For the first time, the importance of drug-seeking behaviour in addiction, as a theoretical stance, was presented in conjunction with the compelling observation that animal models could be employed in its study.

Headlee, Coppock & Nichols (1955) concurred with Spragg's emphasis regarding the importance of physical dependence in pharmacological reinforcement. They demonstrated that rats would learn to prefer a particular head position if morphine delivery was made contingent upon that head position. It was further shown that these animals had to be experiencing withdrawal distress at the time of

drug delivery in order for the head position to increase in frequency. The animals employed in this study had been made physically dependent upon morphine prior to being allowed to operantly respond for drug reinforcement. The reinforcement provided by morphine to these physically-dependent animals was conceptualized in terms of drive reduction (Hull, 1943). The state of physical dependence was thought to reflect a homeostatic imbalance (physiological "need") which was motivating in nature (a "drive"). The delivery of morphine to an organism experiencing abstinence symptoms would reduce this drive, in much the same way that food would reduce the drive of hunger.

One year later these same investigators (Nichols, Headlee & Coppock, 1956) reported the results of two experiments in which rats were allowed to self-administer morphine. This was done through oral intake procedures. The taste of morphine, in solution, is bitter and is quite aversive in nature. In order to enhance the drinking of such aversive solutions, animals were water-deprived for twenty four hours prior to access to the morphine solution. In the second experiment, animals were given access to either morphine solution or an equally aversive solution of quinine. In both experiments, animals were run on a repeating 3-day schedule. On the first day of each such schedule, no fluids were available for consumption. On the

second day, either morphine (in the first experiment) or morphine/quinine (depending on group assignment, in the second experiment) was available for consumption. On the third day, water was available for consumption.

In both experiments, animals previously made physically dependent upon morphine through repeated injections were shown to develop a marked increase in the consumption of morphine solution. This increased consumption of morphine was taken to reflect an instance of escape learning, in which the animals had learned to escape the negative effects of withdrawal through the ingestion of morphine solution. The authors noted that the acquired preference for the morphine solution was still evident weeks after objective signs of withdrawal distress had ended.

The following year, Beach (1957) defined addiction as "purposive drug-seeking behaviour" and indicated that "the important questions for the psychologist are those concerning the nature of the learning and motivational processes involved in drug addiction" (both quotes on p.104). He emphasized that the primary difficulties in demonstrating purposive drug-seeking behaviour in animals are the aversiveness of oral (bitter taste) or injected (irritation & pain) drug administrations and the latency (10-15 minutes) between the administration of the drug via these routes and the onset of drug effects. Beach addressed

both of these difficulties by conducting a place-conditioning (preference) study with rats. Animals were injected with morphine in one environment and then, after a short time, were moved to a second. Beach felt that the discomfort associated with injection would have dissipated, and the pharmacological effects of morphine would have become noticeable and would, thus, be more readily associated with the second environment. In the first of three reported experiments, Beach used rats made physically dependent by daily injections spanning a period of either 4 or 8 weeks. These animals displayed a reliable preference for the arm of a Y-Maze in which they had previously been confined while experiencing the withdrawal-alleviating effects of morphine. In the second reported experiment, this finding was extended to non-dependent rats. The delay between injections of morphine and placement in the conditioning apparatus was extended to minimize the possibility of the reduction of any withdrawal that did develop being associated with the apparatus. This demonstration of place conditioning in non-dependent rats indicated that "the drug's euphoric effects constitute a sufficient condition for the learning of morphine-seeking behaviour" (p.109). This represents the first experimental demonstration that the reinforcing effects of morphine are not based solely on it's withdrawal-alleviating

capabilities. This observation concerned Beach, and he raised the issue that it was inconsistent with drive-reduction theories of reinforcement. The third study in this series served to evaluate the relative retention of place conditioning which had been established by the drive-reduction and the euphoric effects of morphine. Beach concluded that, of the two types of reinforcement that morphine is capable of providing, the drive-reduction effects are more durable than are the euphoric effects.

In 1961, James Weeks published the first report employing a preparation which allowed rats to self-administer morphine through the intravenous (IV) route. Rats were first made physically dependent by repeated hourly IV infusions of morphine through chronic surgically implanted cannulae. Weeks initially infused the rats with 2 mg/kg of morphine and increased the dose logarithmically until a dose of 40 mg/kg per infusion was reached. Following five days of such passive dosing with morphine, these "free" infusions were discontinued. The rats were then presented with a bar which, when pressed, led to the infusion of an additional dose of morphine. The rats rapidly learned to press the bar for morphine. Once bar-pressing for morphine was established, disconnecting the infusion apparatus led to short-lived increases in the rate of bar-pressing. This observation is consistent with the

pattern of responding seen in standard food-reinforced operant conditioning procedures.

The following year, Weeks (1962) elaborated his discussion of what appears to be the same study reported in 1961. In addition to the observations published in 1961, Weeks noted numerous additional details. When the infusion pumps were turned off (the initiation of operant extinction), the response rate of the affected animals increased prior to the display of any overt withdrawal symptoms. Increasing the number of responses required for each infusion (from continuous reinforcement to an FR-5 or an FR-10 schedule of reinforcement) resulted in an increase in overal response rate, but a decrease in net morphine intake. Animals injected with the morphine antagonist, nalorphine, displayed a rapid increase in responding. Weeks noted that, following a period with ratio-schedules of reinforcement, a pattern of responding developed in which prolonged periods of non-responding were followed by a sudden rapid burst of responses which terminated immediately following the resulting infusion. These observations suggest not only that IV morphine is acting in a manner consistent with the traditional operant reinforcers, but that the reinforcement it provides is one of "almost immediate satiation" (p. 144).

In 1962, Davis and Nichols examined the effect of varying the level of physical dependence in rats upon the preference displayed for morphine solution on oral choice tests. Animals made physically dependent upon morphine through bi-daily injections spanning a 3-week period displayed a greater preference for morphine solution following choice training than did rats receiving no morphine pre-treatment.

The importance of physical dependence is clear in the work summarized above. Nichols went on to summarize the experimental findings noted above in two theoretical papers (Nichols, 1963; Nichols, 1965). In these works, the importance of a subject-initiated response in the genesis of addiction is stressed. Nichols argues in favour of the use of the term "sustained opiate-directed behavior" over the use of the term addiction. He suggests that subject-initiated responses appear crucial in the development of sustained opiate-directed behavior.

Many experiments have been conducted in which animals are allowed to self-administer drugs (see Weeks & Collins, 1964; Schuster & Thompson, 1969; Pickens & Thompson, 1972; Spealman & Goldberg, 1978; and van Ree, Slangen & de Wied, 1978 for reviews). Many different drugs have been employed in such studies, as have many different species of animals. One fact that emerges from this work is

that the self-administration of certain drug classes seems a rather general phenomenon. Another is that the patterns of drug-seeking behaviour observed appear consistent with operant behaviours maintained by non-pharmacological reinforcers.

Not all self-administered drugs have been shown to cause tolerance and physical dependence. In the case of the opiates, however, these two effects of chronic drug exposure develop reliably following repeated drug exposures. The state of physical dependence is given such weight in the genesis of drug-seeking behaviour, that it is commonly induced in experimental subjects prior to their first opportunity to self-administer the drug (e.g., Jones & Prada, 1973; Thompson & Ostlund, 1965; Thompson & Schuster, 1964; Weeks & Collins, 1964; Wikler & Pescor, 1970; Young & Khazan, 1987).

The reliance on the use of physically-dependent animals in studies examining the self-administration of drugs is common enough to be called a standard practice. This standard practice stems, in part, from the assumption (voiced by Nichols, 1965, p. 80) that the physiological effects of exposure to opiates (e.g., tolerance and physical dependence) are independent of whether the drugs are self-administered or received passively. This standard practice also stems from the convenience offered by the fact that

physically-dependent organisms more readily, reliably, and rapidly acquire drug self-administration behaviours. Such induction of physical dependence in experimental subjects allows for some degree of experimental control over the levels of physical dependence displayed. Finally, one cannot help but speculate that the practice has become standard, in large part, because the pioneering investigators in the field all employed the technique.

Investigators using animals made physically dependent in an attempt to model human drug addiction have apparently ignored the fact that human drug addicts generally become addicted through active self-administration of the drug, and not through a protracted schedule of passive drug receipt (Smith, Werner & Davis, 1976). Should the assumption of equality between the effects of passively and actively acquired drug doses be invalid, the generality of findings from studies employing the passive induction of physical dependence to the condition of human addiction would be weakened.

Control and Drug Effects

Animal studies

A number of investigators have reported phenomena indicating differential effects of active and passive drug administration. Smith, Lane and co-workers (Smith & Lane,

1983; Smith, Co, Freeman, & Lane, 1982; Smith, Co, Freeman, Sands & Lane, 1980; Smith, Co, & Lane, 1984a; Smith, Co, & Lane, 1984b) have outlined a number of differences in neurotransmitter turnover rates and receptor densities between littermates self-administering morphine intravenously and those passively receiving identical patterns of drug infusions (yoked controls). These investigators suggest that the observed neural differences reflect the "rewarding effects of the drug-taking milieu" (Smith, Co, Freeman, & Lane, 1982, p. 509) in the self-administering animals.

Trusk and Stein (1988), using an [1-14C]octanoate labeled autoradiography technique, have delineated a number of differences in localized cerebral metabolic activity in rats with a history of active self-administration of intravenous morphine when compared to yoked controls. These investigators ascribe the observed functional differences to greater secondaryreinforcing capacity of a drug-associated signal in animals with a history of self-administration.

It is possible that the differences observed by Smith et al. and Trusk and Stein reflect (and/or mediate) an aspect of drug experience other than reinforcement. They could, for example, mediate tolerance and/or sensitization to any of a number of drug effects. They could also mediate physical dependence in the organism. No observations are

reported by the above investigators regarding the presence of behavioural differences in their animals which might reflect differential tolerance to, or dependence upon, morphine. The Smith group did not, apparently, observe any differences in tolerance or dependence between their self-administering and yoked-morphine animals (Smith, personal communication, Nov. 17, 1988). It is quite possible, given the experimental designs and intent of these studies, that the ongoing behaviour of the animals in them was not systematically monitored.

It has been demonstrated that rats self-administering cocaine are less sensitive to the toxic effects of that drug than are rats passively receiving identical drug administrations (Dworkin, Volkmer, & Dworkin, 1988). These investigators observed differential mortality in rats self-administering cocaine intravenously and yoked littermate controls. While 41% of the animals passively receiving cocaine died, only 28% of self-administering rats died. This suggests that the development of tolerance may be augmented in rats self-administering drugs, compared to those passively receiving them.

Moolten and Kornetsky (1990) observed differences in the ability of ethanol to reduce the threshold of electrical brain stimulation in rats dependent upon the level of control over drug administration. While rats allowed to

self-administer ethanol displayed significant lowering of threshold levels, such effects were not observed in yoked animals receiving the drug noncontingently . Recently, Kiyatkin, Wise & Gratton (1993) reported differences in neuro-electrical signals in the nucleus accumbens between rats self-administering heroin intravenously and yoked controls receiving passive infusions. This finding suggests differences in dopaminergic function between these two groups of rats. It is clear from such work that active and passive drug administrations differentially affect their recipients. It is possible that the neurological differences observed by these investigators might be mirrored in observeable behavioural differences. The obvious choices for investigation in the case of the opiates are the reliably evoked observeable responses associated with the development of tolerance and physical dependence.

Human study

Mello and Mendelson (1970) have observed differences in the effects of alcohol in eight chronic alcoholic males depending upon the level of control enjoyed over drug delivery. Control was manipulated by allowing subjects to either ingest alcohol when they wished (spontaneous condition) or only during experimenter-determined intervals (programmed condition). Both tolerance to the effects of high doses of ethanol and withdrawal distress experienced

following removal of access to the drug were greater in the same individuals following spontaneous self-administration than following programmed drug availability. These investigators concluded that "the traditional time-programmed dosage research paradigm may give a very different picture of the alcoholic's response to alcohol than a paradigm which allows spontaneous alcohol consumption" (p. 114).

The mechanism underlying the difference in chronic drug effects displayed by individuals allowed to self-administer drugs, as compared to those not allowed to control their pattern of administrations is unclear. Mello and Mendelson note the consistent ability of their subjects to maintain higher average blood-alcohol concentrations while in the spontaneous drinking condition. They suggest that an individual's pattern of drinking is the fundamental determinant of the extent to which chronic drug effects are evidenced.

Experimental Hypotheses

These observations indicate that the effects of a drug when an organism passively receives it are <u>not</u> the same as when that drug is actively taken. It is one of the primary goals of this thesis to persuade investigators in the field of addiction research that the assumption that the

effects of a drug are independent of the degree of control over its administration is both wrong and misleading.

Experiment 1 was designed to evaluate the effect of the level of control involved in opiate exposure on the development of physical dependence and/or tolerance. The goal was to extend the observations of Smith, Lane and co-workers to encompass behavioural measures of drug effect. Experiment 2 sought to address the hypothesis that the mechanism underlying the differences observed in Expt. 1 was reducable to one of differential signalling of drug doses between self-administering animals and their yoked partners. Experiment 3 addressed the question of whether the imposition of a passive schedule of drug administration following a history of self-administration would in any way affect established levels of physical dependence and/or tolerance. Finally, Experiment 4 sought to further address the hypotheses set forward for Experiments 1, 2, and 3. In addition, the effect of a prior history of passive drug exposure on the subsequent acquisition of self-administration behaviour was evaluated, as was the potential role of signalling in any such observed effects.

CHAPTER 3: YOKED STUDY (EXPT. 1)

"The physiological effect is the same whether an organism passively receives morphine or actively takes it." (Nichols, 1965, p. 80)

Introduction

Active vs. Passive Drug Administrations

The research summarized in the previous chapter indicates that the effects of passivelyreceived drugs are not necessarily equivalent to those of actively self-administered drugs. Two behavioural effects frequently observed in organisms with a history of opiate experience are a decreased responsiveness to various drug effects (tolerance to those effects) and an increase in the number of withdrawal-correlated behaviours (withdrawal signs) observed when access to the drug is terminated (physical dependence upon the drug).

The experiments of Smith and co-workers demonstrate that numerous neurochemical responses to the delivery of morphine can be altered depending on whether that morphine is actively self-administered or passively received. The experiment presented in this chapter will evaluate whether the levels of tolerance and/or physical dependence displayed by morphine-experienced rats are, similarly, sensitive to the degree of control enjoyed over drug administrations.

Yoked Triadic Design

One of the strongest experimental designs intended to allow the separation of the effects of stimulation per se from the level of control over such stimulation is the yoked triadic design (Maier & Seligman, 1976). In this design, one group (the "executive" group) is allowed to respond operantly to produce an environmental change. This change is generally either the delivery of a reinforcing stimulus (e.g., food) or the termination of an aversive stimulus (e.g., shock). A second group (the "yoked" group) receives a pattern of stimuli identical to that delivered to the executive group. The behaviour of the yoked group does not, however, have any effect upon the stimulation. A third group (the "yoked/non-stimulated" group) is treated identically to the yoked group with the exception that it does not receive the relevant reinforcing or aversive stimulation.

Using this design, the effects of the stimulation per se can be evaluated by comparing the yoked and yoked/non-stimulated groups. The effects of operant control over the stimulation (or, alternatively, the effects of the lack of such control) are evaluated by comparing the executive and yoked groups. While the yoked triadic design is generally considered a powerful experimental tool, it has received some criticism.

The primary criticism of yoked control designs was initially raised by Church (1964), and subsequently elaborated by Black (1967). This criticism applies specifically to the use of yoked control designs to determine whether the probability of the occurrence of an operant response is a function of making stimulus delivery contingent upon that response. Church's argument that an executive subject may receive the stimulus at more opportune times than the yoked subject is valid. This difference is said to contribute to a bias for greater rates of operant responding in executive subjects.

The yoked triadic design, as employed in the experiment presented in this chapter, does not employ operant response rate as a dependent measure. Instead, measures of tolerance and physical dependence resulting from exposure to morphine are the dependent variables of interest. The yoked triadic design is employed here as a tool to evaluate the contribution of operant control over drug administration to the development of tolerance and physical dependence. Church's criticism does not speak directly to the existence of a bias in the yoked triadic design that would be expected to affect observed levels of tolerance and/or physical dependence.

Hypotheses

Control can influence behavioural effects of drug experience

The present experiment examines the possibility that rats having control over their experience with morphine will react differently to the effects of repeated drug administrations than will rats receiving identical drug experience independent of their behaviour. Specifically, the levels of tolerance and physical dependence displayed by animals self-administering morphine intravenously will be compared to those displayed by yoked controls that receive identical patterns of drug administrations independent of their behaviour.

A yoked triadic experimental design, using intravenously delivered morphine as the reinforcing stimulation was employed in this investigation. The intravenous route of drug delivery was chosen because of a number of advantages it possesses over alternate routes. The primary advantage is the speed of onset of drug effects following drug delivery. Close temporal proximity both between behaviour and its consequences (in operant conditioning) and between CSs and UCSs (in classical conditioning) is generally held to facilitate the acquisition of learned associations (Mackintosh, 1983, pp.86-89). This route of administration also negates

difficulties presented by the bitter taste of morphine in studies employing oral self-administration of the drug.

Method

Subjects

Eighteen male Long-Evans hooded rats, weighing between 385-620 g at the time of surgery, were used. All were housed in a climate-controlled colony room an a 16-hour/8-hour light/dark cycle. Food and water were available ad libitum. Throughout the study, subjects were housed in clear plastic cages (W 30 cm X D 35 cm X H 16.5 cm) with wood chip bedding. All experimental sessions were run during the light phase of the animals' light/dark cycle.

Surgical Preparation

Chronic indwelling intravenous catheters were implanted in the right jugular vein under sodium pentobarbital anaesthesia (55 mg/kg), using a modified version of the technique of Brown and Breckenridge (1975). The catheters were constructed around the Plastic Products C-313 cannula equipment system (Plastic Products--Roanoke, Virginia) with the catheter itself consisting of silastic tubing (.03-cm inner diameter X .06-cm outer diameter) with a 2.0-mm anchoring sleeve of larger silastic (.06-cm inner diameter X .11-cm outer diameter) cemented midway (7.0 cm) down its length. The silastic was inserted into the vein a

distance estimated to place its end within 1 cm of the heart. The catheter was anchored to the jugular, and it was passed subcutaneously (SC) to a point at the top of the skull where the screw-type cannula guide was anchored in place with dental acrylic cement (see Appendix A for a detailed description of catheter construction and surgical procedure). The catheter was flushed with a solution of heparin and antibiotic (65 micrograms sodium heparin and 1.25 mg ampicillin per cc, dissolved in sterile saline) and sealed with a screw-on cannula cap. Following surgery, animals were housed in large clear plastic cages until the completion of the experiment. Catheters were flushed with heparin/antibiotic solution on the third, fifth, and seventh days following surgery. The experimental procedure commenced between eight and fourteen days following surgery. On the day prior to initiation of experimental manipulations, all animals were infused with heparin/antibiotic solution to ensure both patency and integrity of the catheter.

Apparatus

Three operant chambers (30.4 X 20.5 X 19.0 cm, Lehigh Valley Electronics) containing food cups and water bottles were used. All chambers were equipped with one response lever. A small stimulus light was mounted above the lever. The chambers were located in sound-attenuating

cubicles equipped with ventilation fans which served as background noise generators. Each sound-attenuating cubicle was fitted with a hydraulically-sealed swivel which allowed the rats relatively unrestrained movement while attached to the infusion apparatus. These swivels were made in-house following a modified version of the assembly instructions presented by Brown, Amit, & Weeks (1976). Subjects were connected to the swivels by a spring-protected cannula connector at all times while in the chambers. Polyethylene tubing (PE-90) connected the swivel to a 20-cc syringe (with an in-line 0.22-micrometer micropore filter unit: Millex-GS) held in a Sage Instruments Model 341A syringe pump.

Experimental control and operant data collection were programmed in Commodore Basic (V. 2), running on a Commodore PET 2001 Series computer, interfaced to an electromechanical relay rack through a MIC-02 microcomputer interface controller (Grin City Instruments, Iowa). The house light of each box was programmed to turn off while the lever in that box was depressed. In addition, lever presses in one of the three boxes (that dedicated to the self-administering rats) led to infusions in all boxes. Illumination of the stimulus lights above each lever occurred during the period coincident with each infusion. A solution of 10.0 mg/ml morphine sulfate (Allan & Hanbury, Toronto) dissolved in lactated Ringer's solution was used.

The solution was infused at a rate of .023 ml/sec for a period of 3 seconds. Each infusion, therefore, consisted of a constant dose (not conditional on the subject's body weight) of approximately .69 mg of morphine sulfate delivered in .069 ml of vehicle. Vehicle-alone infusions followed these rate and time parameters.

Analgesia assessments were conducted using the hot-plate procedure (Fennessy & Lee, 1975). This procedure employed an apparatus consisting of a copper plate (30 X 16 X 0.6 cm), completely submerged in a constant-temperature water bath (Narco, Model 210) maintained at 52 ^{o}C (+/- 0.2 ^{o}C). A clear Plexiglas cylinder (12.5 cm inner diameter) was affixed to the centre of the copper plate with a watertight seal, thereby isolating a dry circular surface on which to confine the rat and assess its sensitivity to heat. Analgesia assessment consisted of placing the rat on the surface of the plate and measuring, with a stopwatch, the latency of the first response to thermal stimulation. A response was defined as either a paw lick or a jump. Subjects were confined on the hot-plate surface until either a response had occurred or 30 seconds had elapsed. A subject that did not respond during this interval was assigned a latency of 30 seconds for that determination.

Design

All animals were run in squads of three. Each of these triplets consisted of one rat each in groups SELF-ADMINISTRATION (group S-A), YOKED-MORPHINE (group Y-M), and YOKED-RINGER'S (group Y-R). Each triplet was run through three successive weekly cycles consisting of 6 days of infusion sessions followed by one day of withdrawal testing. During infusion sessions, lever presses by the S-A animal led to morphine infusions both for itself and for the Y-M animal and an equal-volume infusion of Ringer's solution for the Y-R animal. Coincident with each infusion, the signal light above the lever in each chamber was illuminated. Lever-presses by any animal led to the offset of the house light in its chamber for the duration of the lever press. During withdrawal test sessions, these programmed stimulus events remained in effect, but the infusion pumps were turned off. Hence, no infusions occurred during withdrawal testing.

This experiment was initially designed to test the effects of the yoking procedure on the levels of withdrawal distress evidenced. Observation of the first two triplets run suggested that tolerance to the effects of morphine might be developing differentially in groups S-A and Y-M. Hence, for the final four triplets run, measures were taken of morphine's sedating effects following each infusion

session and analgesic effects following completion of the third withdrawal test session.

Procedure

The rats were divided into six triplets matched individually on the basis of body weight. Each matched triplet of animals was then randomly divided between the three groups. Each triplet was subjected to three successive week-long infusion/withdrawal series. The first day of each series consisted of one, 3-hour infusion session in the operant chamber. Lever presses by the S-A rat during an infusion were recorded, but had no consequences other than the offset of the house light in its box for the duration of the lever press. Lever presses by either the Y-M rat or the Y-R rat were recorded, but had no consequences other than the offset of the house light in their respective boxes. The second through sixth days of each series consisted of two, 3-hour infusion sessions in the operant chamber, separated by 3-hour intervals. These sessions were identical to that given on the first day of the series.

The seventh day of each series consisted of one, 2-hour withdrawal test which was identical to drug sessions with the exception that the doors to the sound-attenuating chambers were left open (to facilitate observation) and the infusion pumps were turned off. Hence, lever presses during

withdrawal tests led to stimulus events similar to those occurring in response to lever presses during infusion sessions with the exception that no infusions occurred.

Subjects' weights were recorded prior to the first session of each day, and withdrawal-correlated behaviours (withdrawal signs) were scored during withdrawal tests. In addition, for the last four triplets run, the righting reflex was assessed following each infusion session and tolerance to the analgesic effect of morphine was assessed following the third withdrawal test.

Evaluation of Withdrawal Distress

During each withdrawal test, the rats were placed into the operant chambers and withdrawal scoring commenced immediately with each successive 5-minute period devoted to the observation of a given rat. Each rat received eight observation periods during each test. The order of observation was counter balanced across the three test days (on test day 1 {day T-1}, the order was S-A---Y-M---Y-R---repeat; on test day 2 {day T-2}, the order was Y-M---Y-R---S-A---repeat; on test day 3 {day T-3}, the order was Y-R---S-A---Y-M---repeat). An observer trained in scoring withdrawal signs, but ignorant of the design and intent of the study, was employed on four of the eighteen withdrawal tests to assess the reliability of the experimenter's scoring.

The seven categories of withdrawal signs scored were derived from the withdrawal evaluation procedures previously employed by a number of investigators (Badawy, Evans, & Evans, 1982; Collier, Francis, Henderson, & Schneider, 1974; Laschka, Herz, & Blasig, 1976; Leung, Ogle, & Dai, 1986). These withdrawal signs are described below:

BODY SHAKE--this is a movement analogous to that displayed by a dog shaking water off it's body. The movement tends to crawl up a good portion of the back and usually includes the head and shoulders.

MOUTHING--a rat scored one mouthing behaviour when a burst of non-directed jaw movements occurred. Both movements with and without concomitant tongue protrusions were scored.

REARING--a rat scored one rear for each instance that it stood up on it's hind legs and extended it's upper body upward (note that grooming periods where the forepaws were off the floor were not counted as rears).

HEAD SHAKE--this is basically a wet-dog shake restricted to the head and shoulders area. This behaviour tended to occur either when a rat was lying down, or immediately following a period of grooming.

EAR WIPING--a rat scored one ear wipe each time he was grooming and pulled both paws simultaneously over the ears from the back to front.

PAW TREMOR--a rat scored one paw tremor each time one or both of the forepaws vibrated quickly with no grooming-related function apparent.

TEETH CHATTERING--a rat scored one teeth chatter if rapid jaw movements coincided with a chattering sound (the distinctive feature of this behaviour is the sound of the teeth chattering).

Evaluation of Drug Effect

Righting Reflex

For the final four triplets run, morphine's sedating effect was assessed following each infusion session. Immediately following each session, rats were placed in the supine position in the experimenter's hand to see if they would rig[illegible]hemselves. A failure to right within 5 seconds was counted as a loss of the righting reflex.

Analgesia Assessment

In addition, for these four triplets, the third withdrawal test session was followed immediately by subcutaneous injections of 7.0 mg/kg morphine sulfate. Analgesia assessment occurred on a hot-plate 45 minutes following this injection. The latency to either jump or to lick a hind paw was recorded.

Results

Subject Attrition

Two animals from group Y-R died in the last week of the experiment. No data for these subjects are included in any analyses relating to the third week.

Inter-rater Reliability

The data from the four withdrawal test sessions during which two observers scored withdrawal signs were subjected to a Spearman Rank-Order Correlation test. For each of these four test sessions, the total count of all withdrawal signs for each of the three animals scored was defined as the animal's overall withdrawal score for that session. Each such score was treated as an independent datum for the purpose of this analysis. The resulting Rho (N=12) was equal to .98. This was significantly different than zero ($t[10]=14.55$, $p<.001$), indicating good reliability in scoring between the two observers.

Weight Change

Figure 1 summarizes the mean daily weight recorded for each group throughout the experiment. All animals tended to lose weight across days, but animals receiving morphine tended to lose more than did those receiving Ringer's solution. No significant group differences were observed with this measure during the first or second weeks.

FIGURE 1: Mean daily weights.

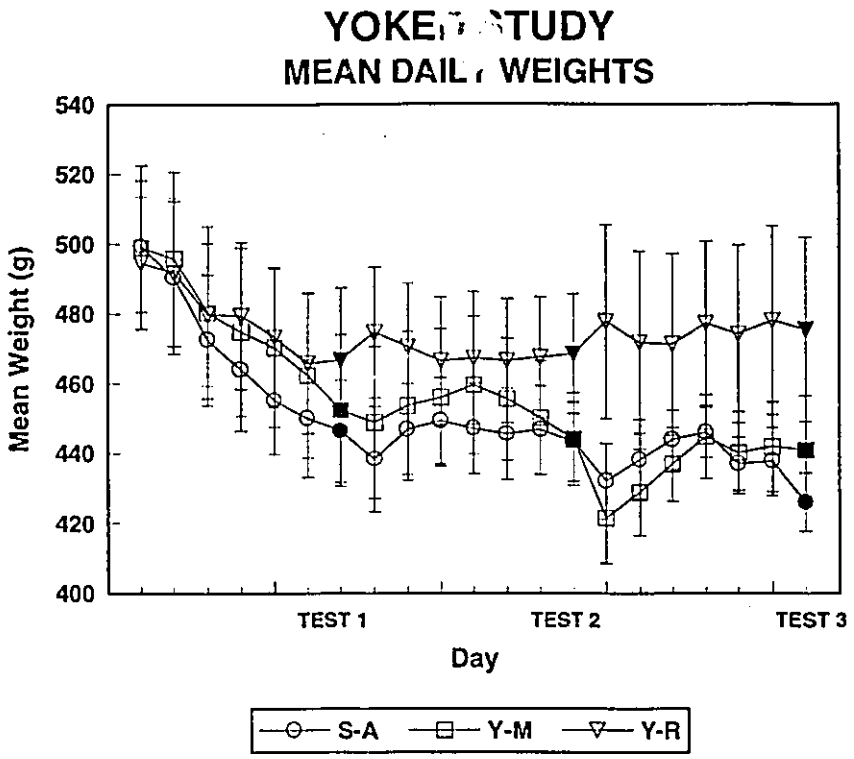
YOKE STUDY
MEAN DAILY WEIGHTS
Mean Weight (g)
540
520
500
480
460
440
420
400
TEST 1
TEST 2
TEST 3
Day
S-A
Y-M
Y-R

FIG. 1

During the third week, a mixed-design ANOVA revealed a significant interaction between GROUP and DAY ($F[12,78]=2.04$, $p<.05$). Post-hoc analyses revealed differences on all seven days of week three between each of the morphine groups and group Y-R (all ps $<.001$). Groups S-A and Y-M differed significantly only on the final test day of week three ($p<.01$).

The effects of drug withdrawal on weight change were assessed by comparing the weights on each of test days 1 and 2 (days T-1 and T-2, respectively) with the weights on the following days (thereby revealing any weight change occurring following a period of approximately 40 hours without infusions). A mixed-design ANOVA conducted on the data related to the first test day (days T-1 vs. T-1+1) revealed no significant effects. A mixed-design ANOVA conducted on the data related to the second test day (days T-2 vs. T-2+1) revealed a significant effect of DAY ($F[1,13]=25.09$, $p<.01$) and a significant interaction between GROUP and DAY ($F[2,13]=13.39$, $p<.01$). Post-hoc analyses revealed significant cross-day effects for both groups S-A and Y-M (both ps$<.01$), but not for group Y-R. Hence, both morphine-experienced groups displayed a significant decrease in weight following the second withdrawal test session, while the morphine-naive group did not.

Lever-press Responses

Figure 2 summarizes the mean number of lever-presses occuring per session for each group throughout the experiment. A mixed-design ANOVA conducted on data from the three weekly series of infusion sessions revealed a significant GROUP effect ($F[2,11]=7.09$, $p<.05$). Post-hoc analyses revealed that each of the morphine groups displayed significantly higher levels of lever-pressing than did group Y-R. No differences were observed between groups S-A and Y-M. The increase in mean lever-presses observed for group SáA during the last three infusion sessions of week three was attributable to a high rate of operant responding by one animal. Hence, the apparent increase in lever-pressing failed to reach statistical significance.

A mixed-design ANOVA conducted on data from the three withdrawal test sessions revealed a significant GROUP effect ($F[2,13]=5.43$, $p<.05$). Post-hoc analyses revealed that group S-A displayed significantly higher levels of lever-pressing than did either group Y-M ($p<.05$) or group Y-R ($p<.01$).

Morphine Delivered

Figure 3 summarizes the mean dose of morphine delivered per session for each day of the experiment. A repeated-measures ANOVA revealed significant WEEK ($F[2,10]=9.19$, $p<.01$) and SESSION ($F[10,50]=2.75$, $p<.01$)

FIGURE 2: Mean lever-presses per session.

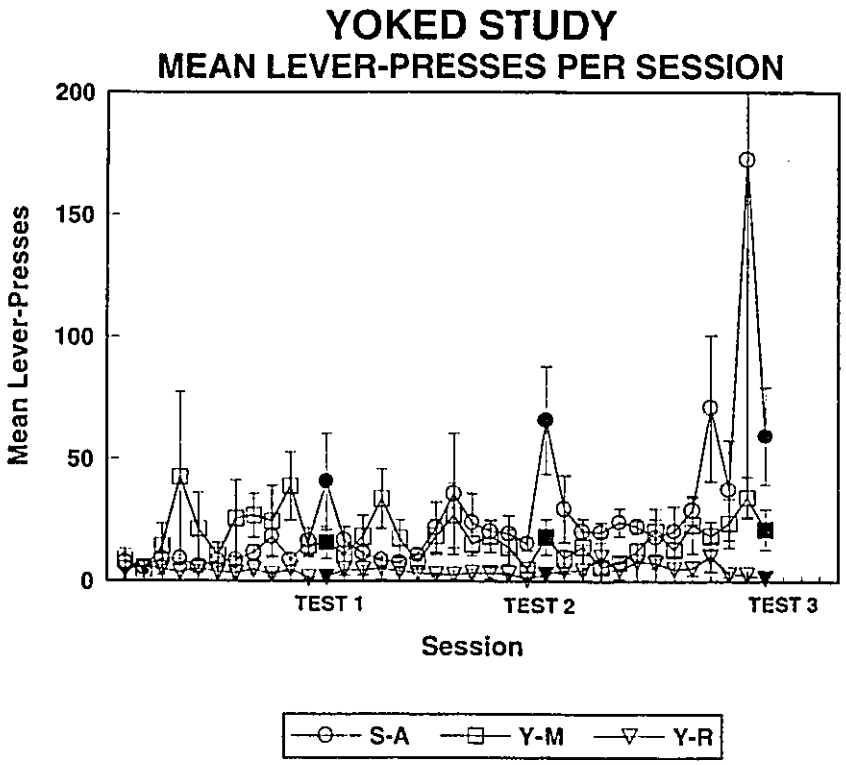
YOKED STUDY
MEAN LEVER-PRESSES PER SESSION
Mean Lever-Presses
200
150
100
50
0
TEST 1
TEST 2
TEST 3
Session
S-A
Y-M
Y-R

FIG. 2

FIGURE 3: Mean morphine per session.

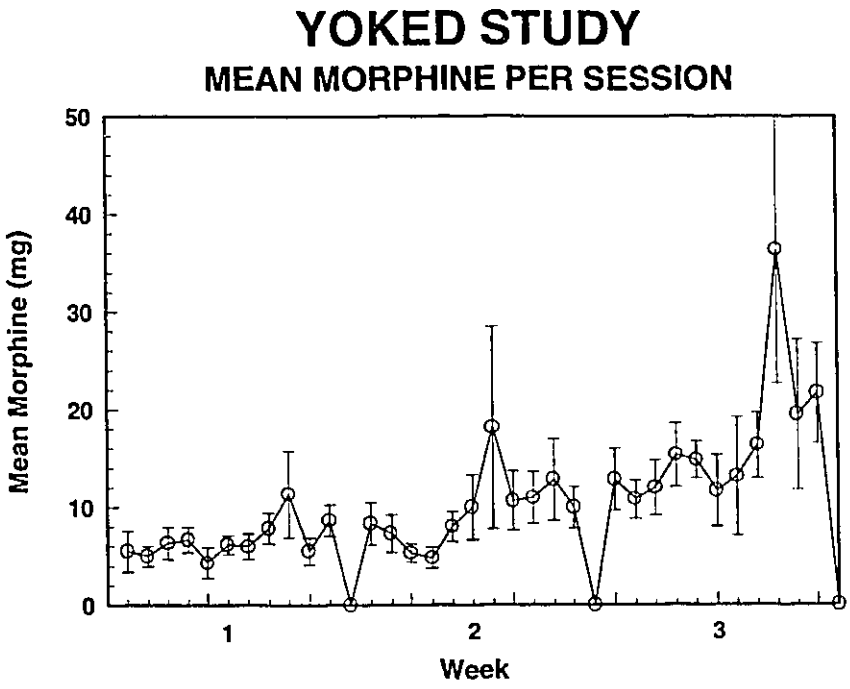
YOKED STUDY
MEAN MORPHINE PER SESSION
Mean Morphine (mg)
50
40
30
20
10
0
1
2
3
Week

FIG. 3

effects. This indicates that the mean dose of morphine delivered per session increased as the experiment progressed.

Withdrawal Distress

The sharp weight loss observed in the morphine animals following the second withdrawal test suggests that physical dependence had developed in these animals. Total counts of the various categories of withdrawal signs are shown per group in Figure 4 (totalled across all animals over all three test days). Clearly, for all seven categories of withdrawal signs, subjects in group S-A evidenced greater levels than did subjects in group Y-M (who, in turn, evidenced greater levels than did subjects in group Y-R). This pattern is consistent with the suggestion that having control over the administration of morphine leads to enhanced levels of physical dependence relative to animals receiving identical drug exposure independent of their behaviour. In order to analyze this suggestion statistically, the total incidence of withdrawal signs was broken down by group for each triplet of rats. This breakdown is summarized in Figure 5 and demonstrates the relative withdrawal distress evidenced by morphine-experienced animals with identical drug histories. Wilcoxon Signed-Ranks tests conducted on each of the pairwise group

FIGURE 4: Total withdrawal signs by category.

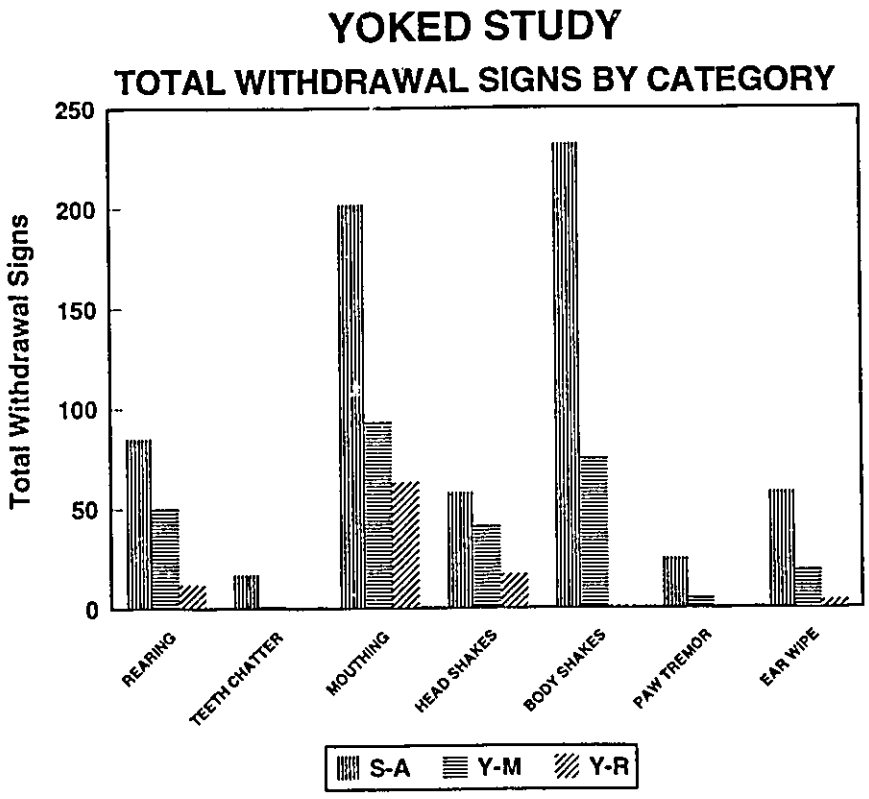
YOKED STUDY
TOTAL WITHDRAWAL SIGNS BY CATEGORY
Total Withdrawal Signs
250
200
150
100
50
0
REARING
TEETH CHATTER
MOUTHING
HEAD SHAKES
BODY SHAKES
PAW TREMOR
EAR WIPE
S-A
Y-M
Y-R

FIG. 4

FIGURE 5: Total withdrawal signs by triplet.

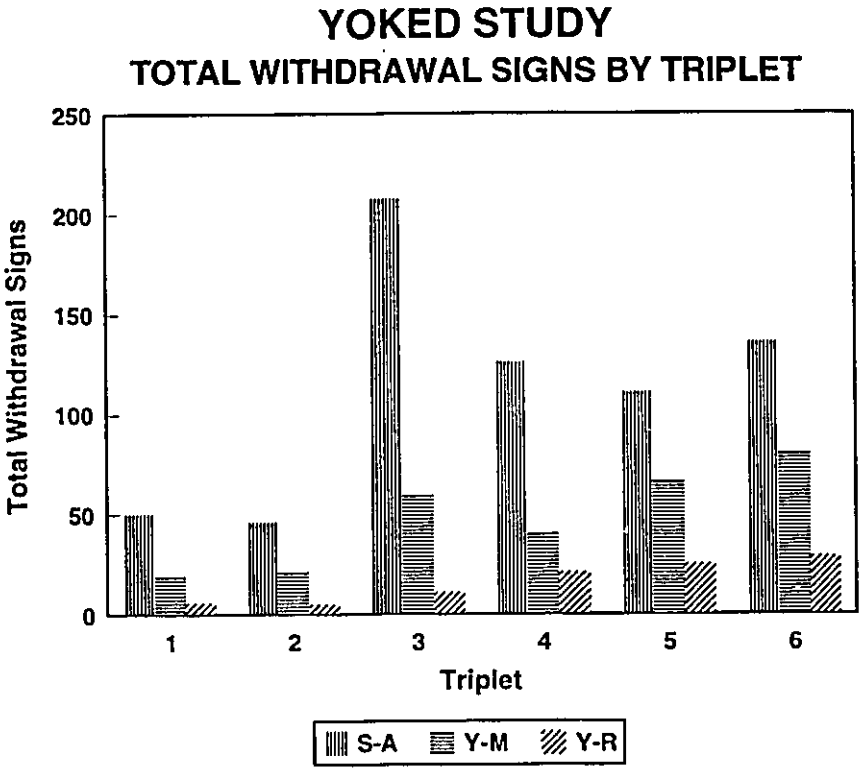
YOKED STUDY
TOTAL WITHDRAWAL SIGNS BY TRIPLET
Total Withdrawal Signs
250
200
150
100
50
0
1
2
3
4
5
6
Triplet
S-A
Y-M
Y-R

FIG. 5

comparisons revealed significant differences between groups S-A and Y-R, groups S-A and Y-M and groups Y-M and Y-R (all Ts[6]=0, $p<.05$). This supports the suggestion that the self-administration of morphine leads to greater levels of physical dependence than does the passive receipt of the drug. In addition, the relatively low number of withdrawal signs observed in animals receiving no morphine reinforces the validity of the withdrawal signs employed in the assessment of physical dependence.

The mean incidence of withdrawal signs observed across the three test days did not change systematically in any of the three groups (see Figure 6). This suggests that the mechanism underlying the observed differential withdrawal distress is "fully" functional within a period of six days of exposure to morphine under the current procedural conditions.

Drug Effects

Righting Reflex

On thirteen different occasions, rats failed to right themselves. Ten failures to right were attributable to group Y-M rats and three were attributable to group S-A rats (note that on each occasion in which an animal in group S-A failed to right, its yoked partner in group Y-M also failed to right). A sign test applied to these data showed

FIGURE 6: Mean withdrawal signs per test.

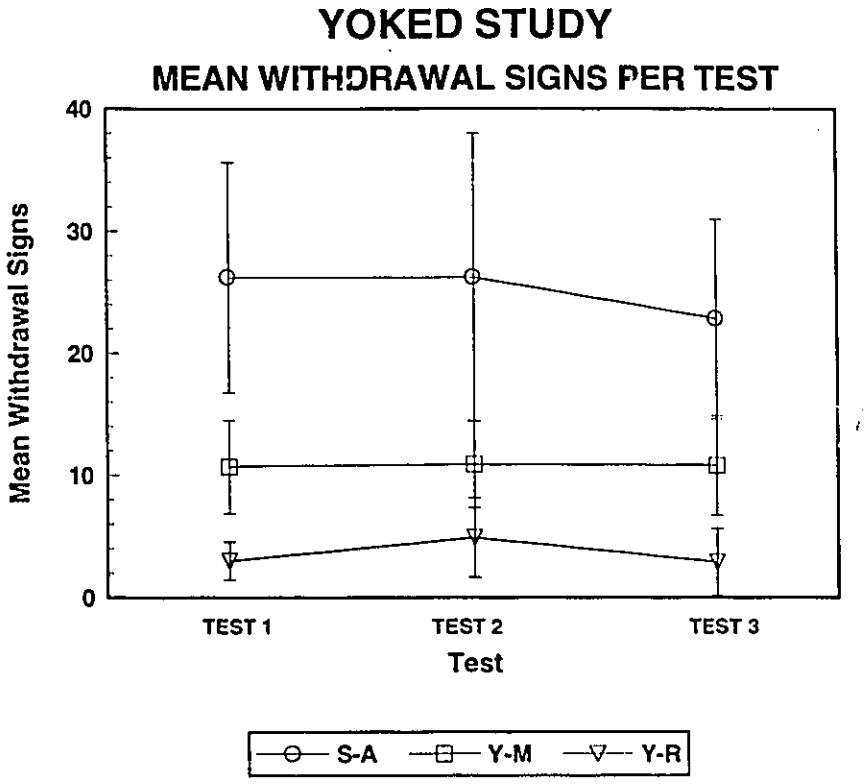
YOKED STUDY
MEAN WITHDRAWAL SIGNS PER TEST
Mean Withdrawal Signs
40
30
20
10
0
TEST 1
TEST 2
TEST 3
Test
S-A
Y-M
Y-R

FIG. 6

FIGURE 7: Analgesia assessment: Mean paw-lick latency.

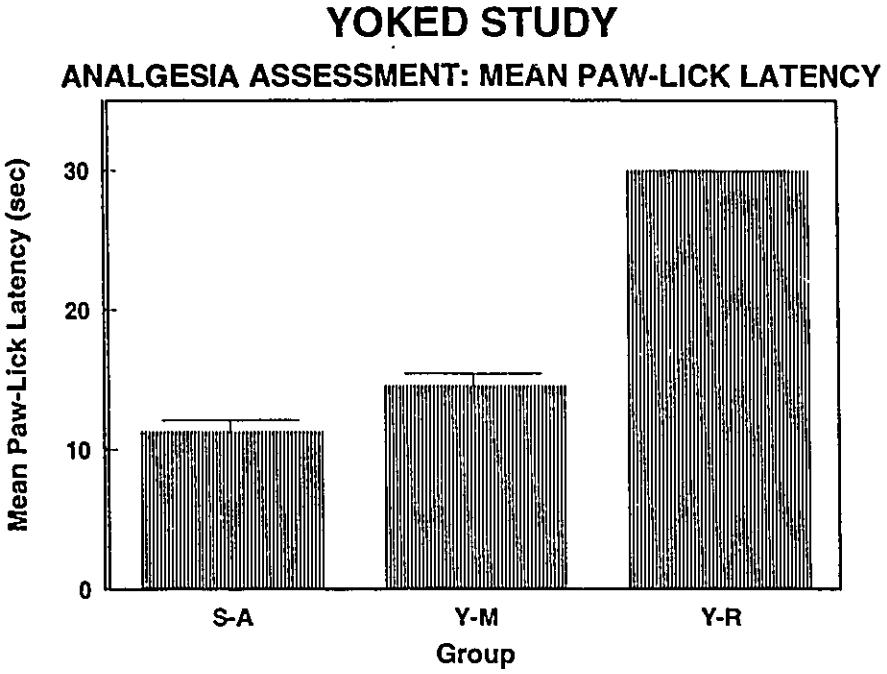
YOKED STUDY
ANALGESIA ASSESSMENT: MEAN PAW-LICK LATENCY
Mean Paw-Lick Latency (sec)
30
20
10
0
S-A
Y-M
Y-R
Group

FIG. 7

a significant difference in the number of righting failures evidenced by the two groups ($p<.05$, 1-tailed).

Analgesic Tolerance

The mean paw-lick latencies and standard errors of the mean for the three groups are summarized in Figure 7. The difference between the means of groups S-A and Y-M was found to be statistically significant (dependent samples $t[3] = 2.6$, $p<.05$, 1-tailed), as was the difference between the means of each of the morphine groups and group Y-R (S-A vs. Y-R, $t[2] = 20.8$, $p<.01$, 1-tailed; Y-M vs. Y-R, $t[2] = 13.1$, $p<.01$, 1-tailed). While tolerance to the analgesic effect of morphine had clearly developed in both groups S-A and Y-M, these results suggest that the self-administration of morphine leads to greater levels of tolerance than does passive receipt of the drug.

Discussion

Physical Dependence Differences

The current study serves as the first demonstration that rats self-administering intravenous morphine display enhanced withdrawal distress, in the absence of drug, relative to yoked partners having identical drug histories. This finding argues strongly that some aspect of self-administration can contribute to the development of physical dependence. It is also contrary to a common assumption in

work on physical dependence and self-administration namely that: "the physiological effect is the same whether an organism passively receives morphine or actively takes it." (Nichols, 1965, p. 80).

Tolerance Differences

In addition to the observed differences in withdrawal distress, the current results suggest that animals self-administering morphine display greater levels of tolerance to the analgesic and sedating effects of the drug than do rats passively receiving equivalent drug administrations. This finding indicates that some aspect of self-administration can contribute to the development of drug tolerance.

Rapidity of Development of Physical Dependence

Both the levels of physical dependence observed and the group differences in those levels were fully evident following the first six-day cycle of drug administration sessions. Given the procedures traditionally employed to induce physical dependence through passive drug administrations, this observation was somewhat unexpected. During this first six-day cycle, morphine animals received, on average, less than 75 mg. of the drug. Experiments inducing physical dependence through passive drug

administrations typically employ much greater exposure to morphine (Risner & Khavari, 1973).

Physical dependence has been observed following acute administrations of relatively small doses of opiates (Ritzmann, 1981; Krystal & Redmond, 1983). In such studies, withdrawal distress is evidenced only following challenge with an opiate antagonist such as naloxone. Non-precipitated withdrawal (such as that observed in the present experiment) is generally not reported in such "low-dose" studies. It would appear that physical dependence can develop more rapidly (and with less exposure to morphine) than is expected on the basis of experiments inducing physical dependence through passive drug administrations.

Mechanism of Effect

The mechanism underlying the accelerated development of tolerance and physical dependence displayed by rats self-administering morphine in the current study is unclear. The primary difference between these two groups was the level of control they had over their respective drug administrations. A number of psychological and physiological mechanisms through which differential control might influence the development and/or expression of tolerance and physical dependence will be presented in the general discussion section.

One possibility is that the control over drug administrations experienced by animals in group S-A led to greater predictability of upcoming drug administrations than was the case for animals in group Y-M. An animal that is in control of the occurrence of an event has greater information regarding the impending occurrence of that event than does an animal lacking such control. An animal having control over drug administrations is in a better position to expect upcoming drug administrations than is its yoked partner.

The work of Siegel and co-workers has demonstrated that the explicit signalling of drug administrations can influence the levels of tolerance and physical dependence displayed by animals exposed to morphine (e.g., Siegel, 1990; MacRae, Scoles, & Siegel, 1987). The reliable pairing of an explicit signal with drug administration lends a degree of predictive value to that signal. Under Siegel's formulation, animals expecting drug administrations will, if the drug is administered, display an attenuated response to that drug (i.e., greater tolerance). Similarly, in the absence of the drug, such expectations will lead to the unmasked display of CCRs (i.e., greater withdrawal distress).

If it is the case that upcoming drug administrations were signalled more effectively for the S-A rats than for

the Y-M rats, the current pattern of results are exactly what Siegel's model would predict. The "better" signal(s) of upcoming drug administration in the S-A animals would have resulted in a stronger compensatory conditional response being elicited in these animals than in the Y-M rats. During drug-delivery sessions, this would be manifest as increased levels of tolerance. During drug-withdrawal tests, this would result in increased levels of withdrawal distress. The next chapter describes a study which examines the ability of explicit exteroceptive signals to mimic the effects of self-administration in rats passively receiving morphine.

CHAPTER 4: SIGNALLED/NON-SIGNALLED STUDY (EXPT. 2)

Introduction

The current study is intended to examine the mechanism underlying the differential withdrawal distress and tolerance observed in the previous chapter. While the consistency of those data with Siegel's model of tolerance and dependence is appealing, it is difficult to specify what the source of the required differential cuing might have been.

Three possible sources of differential cuing between the S-A and Y-M animals in the previous study have been considered. Proprioceptive stimulation attendant on pressing the lever would, of necessity, be reliably paired with drug infusions in group S-A. There is no such necessity in group Y-M. Beck and O'Brien (1980) have provided evidence that mild electrical stimulation of a foreleg can serve as a very powerful cue in rats self-administering morphine intravenously. To the extent that such stimulation activates systems involved in proprioception, this observation suggests that proprioceptive feedback might be an effective cue in pharmacological conditioning. Alternatively, the "internal state" of the self-administering animal might act as a cue for upcoming drug infusions. This "state" would likely take

the form of a central nervous system condition reliably preceding operant responding for drug reinforcement. However, this "state" is neither well specified at present, nor is it likely to be amenable to well-controlled scientific investigation. A third possible source of differential cuing in the previous study results from the stimulus consequences programmed for the lever-pressing behaviour of individual rats. Whereas the signal light above the lever was illuminated during infusions for both the S-A and Y-M animals, the S-A animals were required to be in contact with that lever at the time that an infusion (with its attendant light signal) was initiated. There was no such restriction placed on the Y-M animals. Hence, the signal light may have been a relatively more effective cue for the S-A animals than for the Y-M animals.

Hypotheses

The present experiment examines the possibility that the effects observed in the previous chapter can be reproduced through the differential signalling of passively-received morphine. Specifically, the levels of tolerance and physical dependence displayed by animals receiving externally signalled passive infusions of morphine will be compared to those displayed by animals receiving an identical pattern of passive morphine infusions but lacking the predictive signal. Should increased cuing of drug

administrations lead to enhancements in the observed levels of tolerance and physical dependence, a mechanism of drug-compensatory conditional responses would be suggested.

A design was employed in which two groups of rats both received a predetermined schedule of passive morphine administrations. Each group received a 3-second light signal coincident with each infusion, as did the Y-M animals of the previous study. In addition, one group was presented with a 30-second light signal immediately prior to each infusion. The ability of this exteroceptive signal to mimic the effects of self-administration observed in the previous study was evaluated.

Method

Subjects

Sixteen male Long-Evans hooded rats, weighing between 464-522 g at the time of surgery, were used. They were housed identically to the animals in Expt. 1. All experimental sessions were run during the light phase of the animals' light/dark cycle.

Surgical Preparation

The surgical preparation and post-operative care were similar to that of Expt. 1, with the exception that the experimental procedure commenced between six and eleven days following surgery.

Apparatus

The apparatus employed was identical to that of Expt. 1 with the following exceptions. Four operant chambers were employed in the current study. Experimental control and operant data collection were programmed in ASYST (V. 1.56, Macmillan Software Company), a Forth-based laboratory automation language. Appendix B provides an example of source code written in this language for experimental control and data collection purposes. This system was run on a Tandy Model 3000 microcomputer, interfaced to an electromechanical relay rack through a Tecmar LabTender interface board and an optically-isolated digital interface.

Lever-presses in each box led to the offset of the house light in that box for the period of time that the lever remained depressed. Infusion parameters were identical to those of Expt. 1, with a 3-second signal light coincident with each infusion in each box. In addition, two of the four operant chambers were programmed to deliver an additional 30-second onset of the signal light immediately prior to each infusion. The intensity of the signal light was substantially less than that of the overhead house light.

A "tiltometer" was used to assess the narcotizing effect of morphine following each session. This device

consisted of a clear Plexiglass chamber (width 18cm X depth 60cm X height 30cm) which could be tilted from the horizontal plane. The degree of tilt at which a rat placed in the chamber initially slid down the tilted plane could be recorded directly from the device.

Design

Two groups of rats were each run through three successive weekly cycles consisting of 6 days of infusion sessions followed by one day of withdrawal testing. Lever presses by any rat were recorded, but resulted only in the offset of the house light in that animal's box for the duration of the lever-press. All animals received an identical pattern of morphine infusions, independent of lever-pressing activity. This infusion pattern was based on the responding of an "average" S-A animal from Expt. 1 that delivered an intermediate amount of morphine to itself (ranked 4th out of the 6 on total morphine delivered), and displayed intermediate numbers of withdrawal signs (ranked 3rd out of 6 on total number of withdrawal signs evidenced) relative to its group.

The two groups consisted of NON-SIGNALLED-MORPHINE (group N-M) and SIGNALLED-MORPHINE (group S-M). Group N-M animals experienced their infusions in a manner identical to that of the Yoked-Morphine animals in Expt. 1. Group S-M animals differed in that each infusion was immediately

preceded by a 30-second illumination of the signal light above their levers. The 30-second duration was chosen in order to ensure discriminability between this signal and the 3-second light that occurred during infusions.

Levels of withdrawal were scored during each withdrawal test. Following every infusion session, each animal was assessed for loss of the righting reflex and for angle of slippage on the tiltometer. Hot-plate analgesic assessments were conducted following the third withdrawal test, as in Expt. 1.

Procedure

The rats were divided into two groups of eight, matched individually on the basis of body weight. Each matched pair of animals was then randomly divided between the two groups. Two animals each from groups S-M and N-M were run as one squad. Each rat was subjected to three successive week-long infusion/ withdrawal series identical to those of Expt. 1, with the following exception. All infusions were delivered on a programmed schedule derived from the response pattern of one of the S-A animals from Expt. 1.

Evaluation of Withdrawal Distress

An observer trained in scoring withdrawal signs, but ignorant of the design and intent of the study, was employed

on all twelve of the withdrawal tests. The behaviours scored during withdrawal tests were identical to those of Expt. 1. The format of each two hour session was a 3-minute time sampling observation period alternating between the four rats, with a fifth period in each series during which each observer did not score any animals. This resulted in each rat being scored for a total of 24 minutes during each withdrawal test. Each observer alternated across withdrawal tests between an order of observation consisting of S-M---N-M---S-M---N-M---rest---repeat or rest---N-M---S-M---N-M---S-M---repeat. On any given withdrawal test, one observer followed one of the above orders of observation, while the other observer followed the alternate one. This resulted in independent scoring for three of the four animals in each squad and simultaneous scoring for one of the animals in each squad. All withdrawal data reported consist of average totals observed by the two observers.

Evaluation of Drug Effects

All of the animals were evaluated for loss of righting reflex immediately following each session, as described in Expt. 1. A tiltometer was used to assess the narcotizing effect of morphine following each session. It was assumed that the narcotizing effects of morphine would be reflected in a decrease in the angle of first slippage recorded on this device. In addition, analgesic tolerance

was assessed using the hot-plate following the third withdrawal test in a manner identical to that of Expt. 1.

Results

Subject Attrition

Two animals (one each from groups S-M and N-M) died over the course of the experiment. No data for these subjects are included in any analyses.

Inter-rater Reliability

The data from the 12 withdrawal tests during which two observers scored withdrawal signs were subjected to a Spearman Rank-Order Correlation test. The total overall withdrawal score for each animal on each test was treated as an independent datum for the purpose of this analysis. The resulting Rho (N=12) was equal to .99. This was significantly different than zero ($t[10] = 23.74$, $p<.001$), indicating good reliability in scoring between the two observers.

Weight Change

Figure 8 summarizes the mean daily weight recorded for each group throughout the experiment. All animals tended to lose weight across days. A mixed-design ANOVA conducted on data from all three weeks revealed significant WEEK ($F[2,24]=17.29$, $p<.001$), DAY ($F[6,72]=6.39$, $p<.001$) and

FIGURE 8: Mean daily weights.

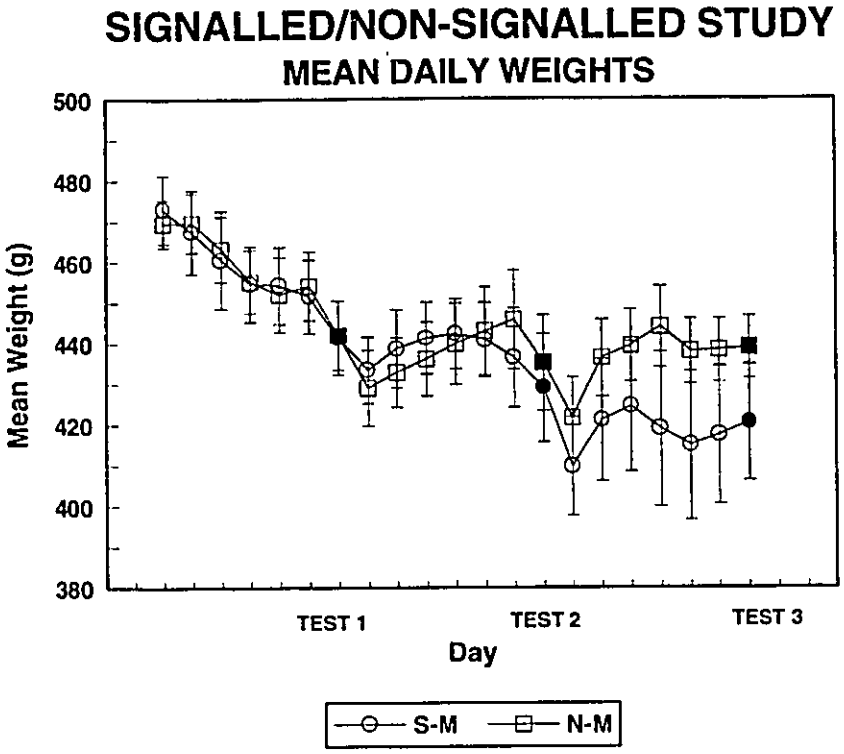
SIGNALLED/NON-SIGNALLED STUDY
MEAN DAILY WEIGHTS
Mean Weight (g)
500
480
460
440
420
400
380
TEST 1
TEST 2
TEST 3
Day
S-M
N-M

FIG. 8

WEEKxDAY ($F[12,144]=10.24$, $p<.001$) effects. No effects involving GROUP were statistically significant

The effects of drug withdrawal on weight change were assessed by comparing the weights on each of test days 1 and 2 with the weights on the following days (thereby revealing any weight change occurring following a period of approximately 40 hours without infusions). Significant effects of DAY were revealed by mixed-design ANOVAs conducted on the data related to both the first ($F[1,12]=49.79$, $p<.001$) and second ($F[1,12]=63.56$, $p<.001$) test days. These analyses failed to reveal any significant GROUP or GROUPxDAY effects.

Lever-press Responses

Figure 9 summarizes the mean number of lever-presses occuring per session for each group throughout the experiment. A mixed-design ANOVA conducted on data from the three weekly series of infusion sessions revealed a significant effect of WEEK ($F[2,18]=6.71$, $p<.01$) and a significant WEEKxSESSION interaction ($F[20,180]=2.48$, $p<.001$). This analysis failed to reveal any significant GROUP effects. A mixed-design ANOVA conducted on data from the three withdrawal test sessions failed to reveal any significant GROUP, TEST, or GROUPxTEST effects.

FIGURE 9: Mean lever-presses per session.

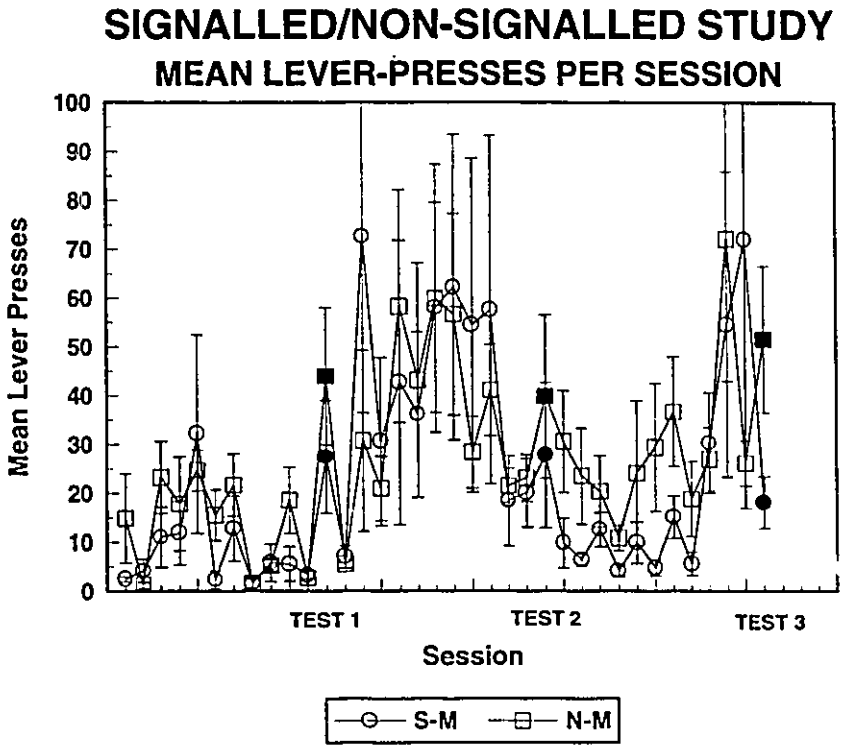
SIGNALLED/NON-SIGNALLED STUDY
MEAN LEVER-PRESSES PER SESSION
Mean Lever Presses
100
90
80
70
60
50
40
30
20
10
0
TEST 1
TEST 2
TEST 3
Session
S-M
N-M

FIG. 9

Morphine Delivered

Figure 10 summarizes the mean dose of morphine delivered per session across weeks. This dose increased across weeks, in a manner consistent with the increasing doses observed in the YOKED experiment reported in Chapter 2. No analyses were conducted on the dose of morphine delivered, as the dosage was determined by the response pattern of a subject in group S-A from the YOKED experiment and was fixed and identical for all subjects.

Withdrawal Distress

The sharp weight loss observed in the morphine animals following each of the first two withdrawal tests suggests that physical dependence had developed in these animals. Total counts of the various categories of withdrawal signs are shown per group in Figure 11 (totalled across all animals over the three test days). Clearly, no systematic difference is apparent across the seven categories of withdrawal signs between subjects in group S-M and Y-M.

The mean incidence of withdrawal signs for each group on each test day is shown in Figure 12. Mann-Whitney U tests conducted on data from each of the three test days failed to reveal any significant differences in the number of withdrawal signs evidenced by the groups.

FIGURE 10: Mean morphine per session.

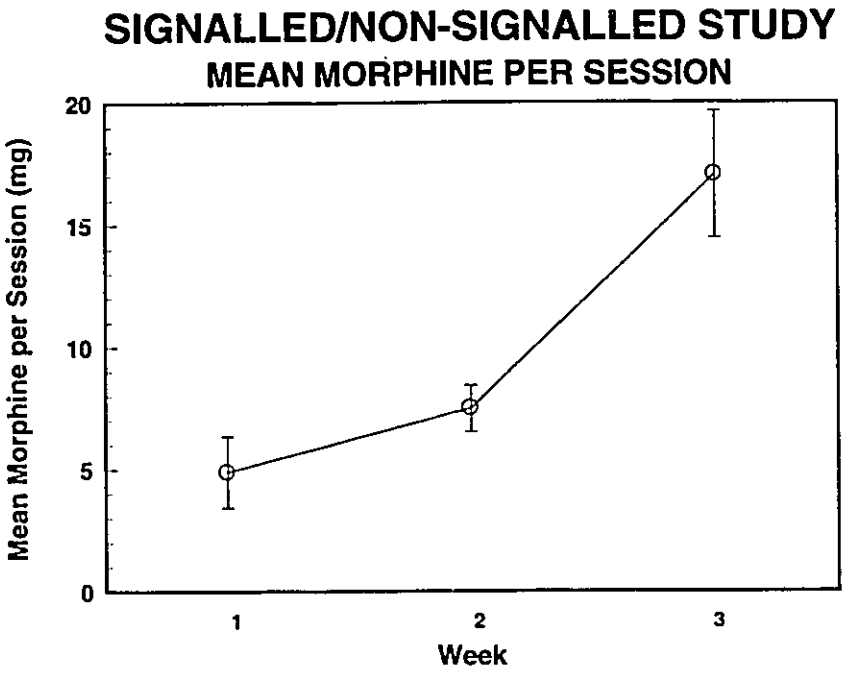
SIGNALLED/NON-SIGNALLED STUDY
MEAN MORPHINE PER SESSION
Mean Morphine per Session (mg)
20
15
10
5
0
1
2
3
Week

FIG. 10

FIGURE 11: Total withdrawal signs by category.

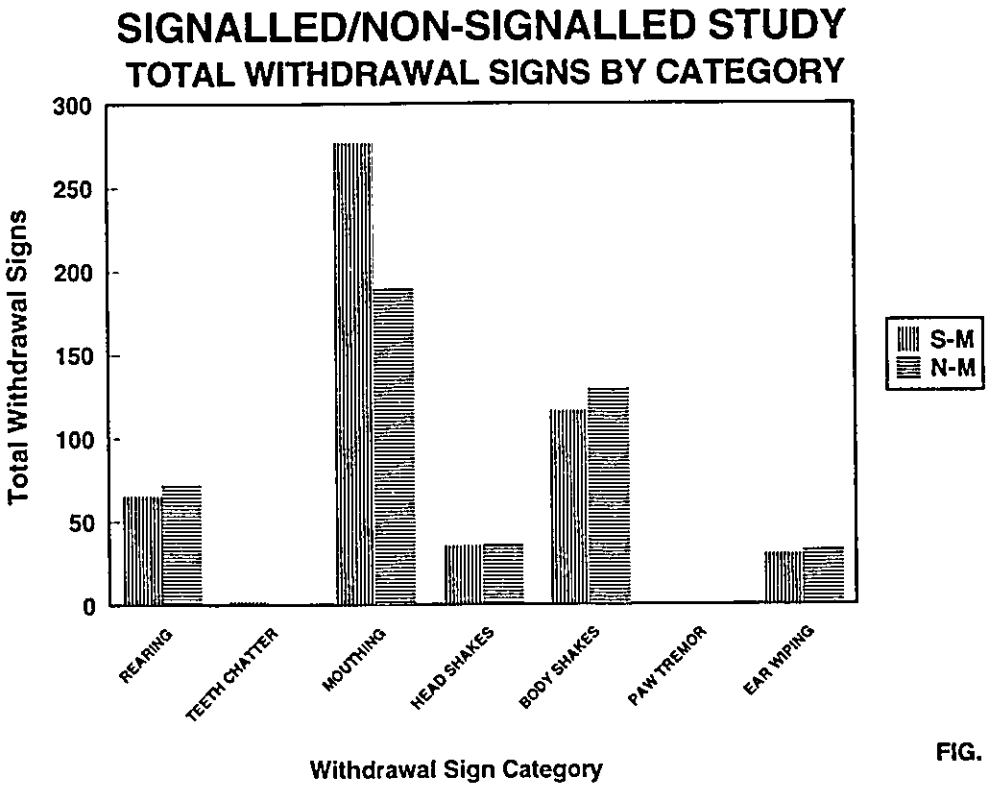
SIGNALLED/NON-SIGNALLED STUDY
TOTAL WITHDRAWAL SIGNS BY CATEGORY
Total Withdrawal Signs
300
250
200
150
100
50
0
REARING
TEETH CHATTER
MOUTHING
HEAD SHAKES
BODY SHAKES
PAW TREMOR
EAR WIPING
S-M
N-M
Withdrawal Sign Category

FIG. 11

FIGURE 12: Mean withdrawal signs per test.

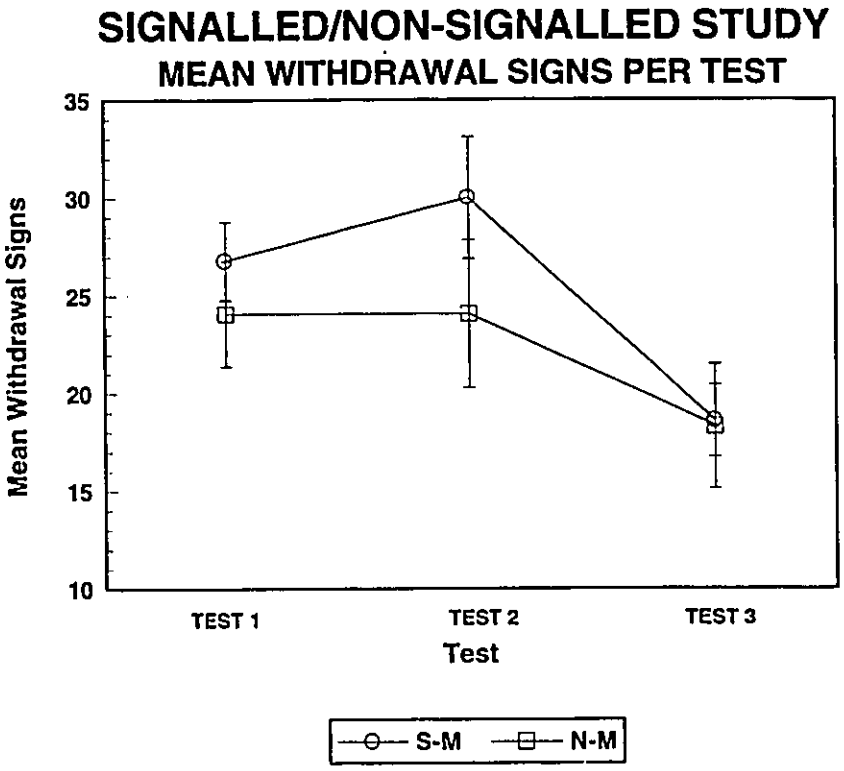
SIGNALLED/NON-SIGNALLED STUDY
MEAN WITHDRAWAL SIGNS PER TEST
Mean Withdrawal Signs
35
30
25
20
15
10
TEST 1
TEST 2
TEST 3
Test
S-M
N-M

FIG. 12

Comparison with S-A Animal from Expt. 1

The total number of withdrawal signs observed in each of the animals in this experiment was compared against the total number of withdrawal signs observed in the S-A animal which determined their drug delivery schedule. All fourteen animals in the current experiment displayed fewer total withdrawal signs than did the S-A animal from the YOKED experiment reported in Chapter 2 ($p<.001$, 1-tailed sign test). This is consistent with the difference observed in the total incidence of withdrawal signs between groups S-A and Y-M in the YOKED experiment.

Drug Effects

Righting Reflex

On thirty-six different occasions, rats failed to right themselves. Of these thirty-six, 22 were attributable to animals in group N-M and 14 were attributable to animals in group S-M. A Chi-squared test applied to these data failed to show a significant difference in the number of righting failures evidenced by the two groups.

Tiltometer

The mean angle of slippage for the two groups is summarized in Figure 13. The tiltometer data also failed to show any reliable difference between the groups. A mixed-

FIGURE 13: Tiltometer: Mean angle of slippage.

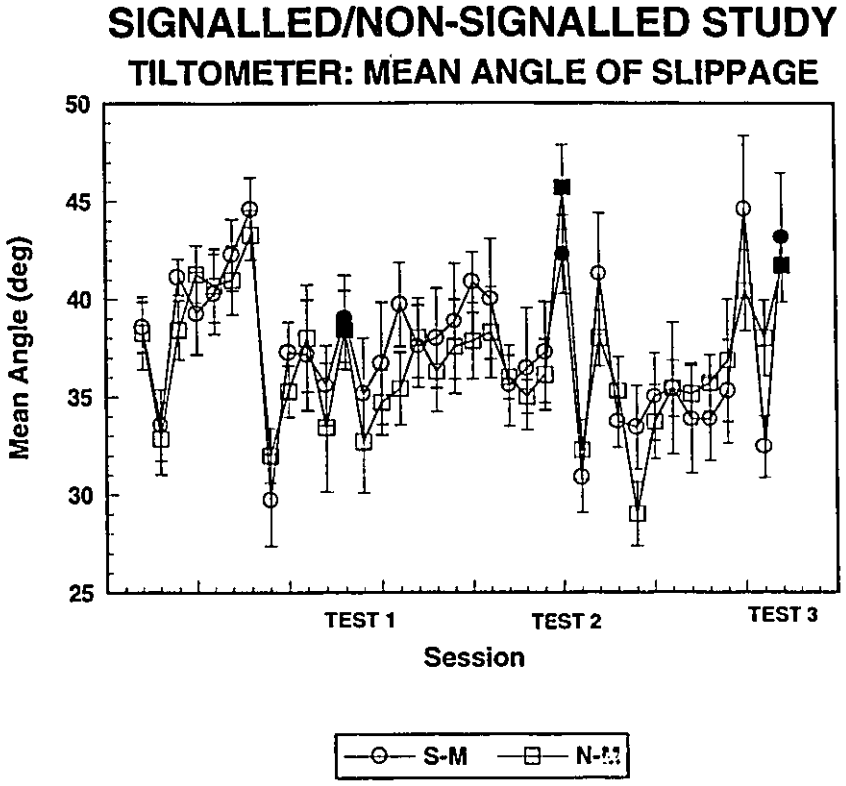
SIGNALLED/NON-SIGNALLED STUDY
TILTOMETER: MEAN ANGLE OF SLIPPAGE
Mean Angle (deg)
50
45
40
35
30
25
TEST 1
TEST 2
TEST 3
Session
S-M
N-M

FIG. 13

design ANOVA conducted on these data failed to indicate any significant GROUP, GROUPxWEEK or GROUPxSESSION effects.

Inspection of Figure 14 reveals a tendency for the mean angle of slippage to decrease on sessions where the dose of morphine administered increases. A regression analysis conducted on the mean angle of slippage (across all animals) against the dose of morphine administered per session revealed a significant negative correlation between these two variables ($r=-.72$, $p<.001$). While the dose of morphine received is a function of session (and, by extension, prior drug experience), this negative correlation reinforces the validity of the tiltometer as a measure of morphine's narcotizing effect.

Analgesic Tolerance

The analgesic tolerance test also failed to show any reliable difference between the groups. The mean paw-lick latencies and standard errors of the mean for the two groups were: S-M (21.4 +/- 2.82 s) and N-M (19.06 +/- 2.24 s). The difference in these means was not found to be statistically significant (independent samples $t[12] = 0.65$, $p>.25$, 1-tailed).

Comparison with S-A Animal from Expt. 1

The paw-lick latencies observed in each of the animals in this experiment were compared with the paw-lick

FIGURE 14: Tiltometer: Mean angle of slippage vs. morphine dose per session.

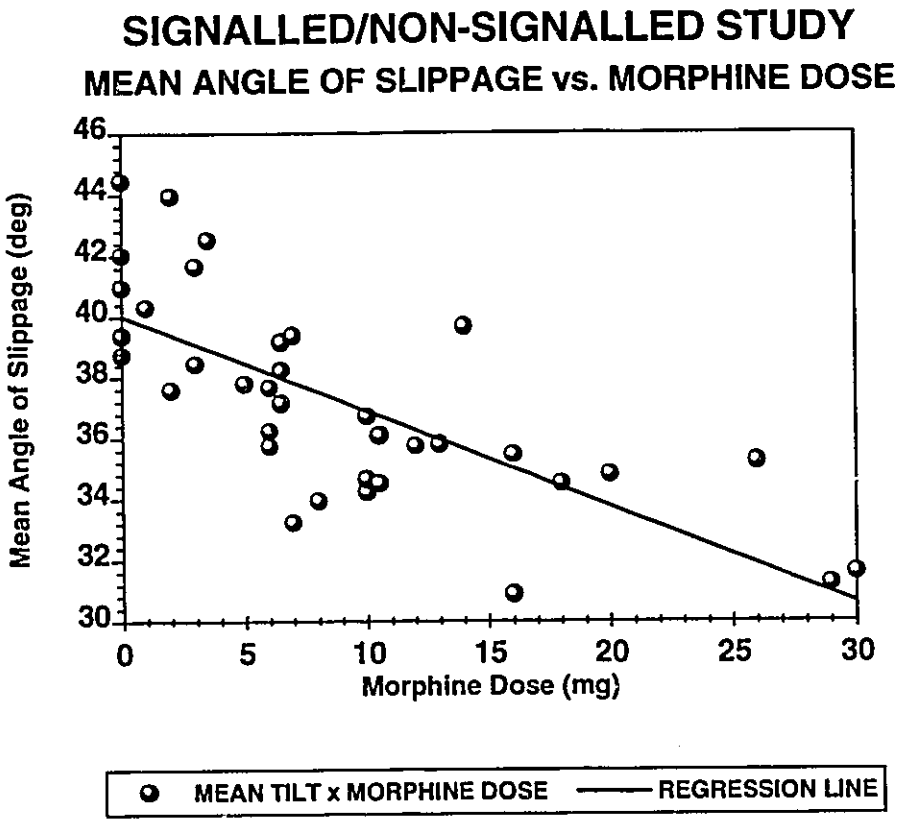
SIGNALLED/NON-SIGNALLED STUDY
MEAN ANGLE OF SLIPPAGE vs. MORPHINE DOSE
Mean Angle of Slippage (deg)
46
44
42
40
38
36
34
32
30
0
5
10
15
20
25
30
Morphine Dose (mg)
MEAN TILT x MORPHINE DOSE
REGRESSION LINE

FIG. 14

latency observed in the S-A animal which determined their drug delivery schedule. Eleven of the fourteen animals in the current experiment displayed a greater paw-lick latency than did the S-A animal from the YOKED experiment reported in Chapter 2 ($p<.05$, 1-tailed sign test). This is consistent with the difference observed in mean paw-lick latency between groups S-A and Y-M in the YOKED experiment.

Discussion

There were no statistically significant differences between the two groups in the current study on either measures of physical dependence or tolerance. It is clear from these data that the exteroceptive signal, as presented, did not mimic the effects of self-administration seen in the previous chapter.

Such failure to reject the null hypothesis does not allow any definitive statement to be made regarding the set of hypotheses specified. The failure to reject any of the null hypotheses regarding tolerance or physical dependence specified in the current experiment, therefore, lends no support to the suggestion that signalling (through the mechanism of compensatory conditional responses) might mediate the differences in tolerance and physical dependence observed in the previous study.

Signalling Did Not Mimic YOKED Effects

The failure to observe any effect of differential signalling of passive drug administrations in this study is consistent with a number of explanations. While the negative results observed do not in any way allow for comparative evaluation of these alternatives, they are listed for the purpose of guiding further discussion.

It may be the case that the effects observed in the YOKED study are not in any way mediated by differential signalling of self-administered vs. passively-received drugs. Alternatively, it is possible that, while such mediation does exist, the explicit signals of impending drug delivery presented to animals in group S-M were inadequate to elicit observeable levels of drug-compensatory conditional responding. Should this second alternative be true, the inadequacy of the signals employed could take on a number of forms. They could be inadequate in terms of their intensity (e.g., overshadowing by the house light) or temporal correlation (e.g., the extended 30-second duration may have decreased the signal value of the light) with drug administration. Alternatively, they could be inadequate in terms of their explicity exteroceptive nature. These issues will be discussed further in the conclusion section in terms of suggestions for future research which might be able to better distinguish between the above alternatives.

CHAPTER 5: (S-A)-TO-Y STUDY (EXPT. 3)

Introduction

The failure in the previous experiment to reject any of the hypotheses regarding the ability of differential signalling to mimic the effects observed in Expt. 1 led to the decision to terminate that line of research. It is felt that the variables relevant to the signalling hypothesis, and the resulting combinations of those variables, are too numerous to allow adequate examination within the constraints of this dissertation. Hence, it was decided to further examine the conditions under which differences in tolerance and physical dependence such as those observed in Expt. 1 would occur.

The results of Expt. 1 indicate that tolerance and physical dependence develop differentially in animals having control over drug administrations compared to animals lacking such control. In Expt. 1, both groups of animals received their total experience with morphine under the conditions of differential controllability outlined. The question remains as to whether or not varying the level of control over drug administrations can in any way affect established levels of tolerance and physical dependence.

At least two strategies are possible in the evaluation of this question. In the first, animals made

physically dependent upon morphine through passive administrations would subsequently be allowed to either self-administer morphine or receive equivalent doses in a passive, yoked fashion. In the second, animals allowed to achieve a state of physical dependence through morphine self-administration would be exposed to further drug administrations that were either self-administered or passively received.

It has been noted that drug-taking behaviour in humans does not generally stem from a baseline of established physical dependence (Smith, Werner, & Davis, 1976). In the interest of maximizing the generality of this experiment to the human condition, it was decided to follow the latter of the above strategies. Hence, animals were initially allowed to achieve a state of physical dependence through self-administration (as do most humans that ultimately display compulsive drug-taking behaviour).

Hypotheses

The present experiment examines the possibility that the imposition of a passive schedule of drug administration following a history of self-administration will alter established levels of physical dependence and tolerance.

A design was employed in which all rats were initially allowed to self-administer morphine for a period sufficient to yield observable physical dependence. Animals

were then matched individually on the basis of their level of drug administration and randomly assigned to either a group allowed to continue drug self-administration or a yoked group in which drug delivery was dependent upon the behaviour of the paired self-administering animal. Following a cycle of such sessions, where the level of control over otherwise identical drug administrations differed between the groups, an additional cycle occured during which all animals were allowed to self-administer morphine. Levels of tolerance and physical dependence were assessed throughout the experiment in a manner similar to that of Expt. 2.

Method

Subjects

Sixteen male Long-Evans hooded rats, weighing between 402-603 g at the time of surgery, were used. They were housed identically to the animals in Expt. 1. All experimental sessions were run during the light phase of the animals' light/dark cycle.

Surgical Preparation

The surgical preparation and post-operative care were similar to that of Expt. 1, with the exception that the experimental procedure commenced between four and sixteen days following surgery.

Apparatus

The apparatus employed in this study was identical to that of Expt. 2 with the exception that the operant chambers were not programmed to deliver any signals prior to infusions. Initially, all boxes were programmed for self-administration, as in Expt. 1 for the S-A animals. During the second phase of this study, two of the boxes were programmed for self-administration and two were programmed for yoked infusions. During the third phase of this study, all boxes were once again programmed for self-administration.

Design

All animals were run in squads of four. During the first week, animals were allowed to self-administer morphine solution for six days. On the seventh day, a withdrawal test was conducted. Prior to the second week, animals were divided into two groups, matched individually on the basis of total drug dose administered during the first week. During this second week, animals assigned to the SELF-ADMINISTRATION group (group S-A) continued to self-administer morphine for six days. Animals assigned to the SELF-ADMINISTRATION-TO-YOKED group (group (S-A)-TO-Y) received a pattern of morphine infusions determined by (and identical to) that of their paired group S-A partners. On the fourteenth day, a withdrawal test was conducted. In the

third week, all animals were allowed to self-administer morphine solution for five days, followed by a final withdrawal test session. Hence, group S-A animals received three cycles of morphine self-administration followed by withdrawal testing. Group (S-A)-TO-Y animals received one self-administration/withdrawal test cycle, followed by one yoked-infusion/withdrawal test cycle, followed by a final self-administration/withdrawal test cycle.

Levels of withdrawal were scored during each withdrawal test. Following every session, each animal was assessed for loss of the righting reflex and for angle of slippage on the tiltometer.

Procedure

Phase 1

All rats were allowed to self-administer morphine during the first six days. One, 3-hour session was run on the first day, followed by two, 3-hour sessions on each of the following 5 days. Infusion parameters were identical to those used in Experiments 1 and 2. Lever presses occurring during an ongoing infusion resulted in the resetting of the infusion timer to provide 3 seconds of morphine delivery timed from the onset of the response. As in Experiments 1 and 2, lever presses resulted in the offset of the house light for that box for the duration of the lever press.

During each infusion, the signal light above the lever in the affected box was illuminated. On the seventh day of the self-administration cycle, rats were placed in the operant chamber with the infusion pumps turned off. Withdrawal signs were then scored.

Phase 2

Following phase 1, animals were matched individually on the basis of total dose of drug administered. Following rank-ordering on this measure, one member of each pair was then randomly assigned to group S-A, which was allowed to self-administer morphine for an additional week exactly as in phase 1. The other member of each matched pair was assigned to group (S-A)-TO-Y, whose pattern of drug infusions during the second week was determined by and identical to that of its matched S-A partner. During this second week, lever-presses in a (S-A)-TO-Y animal's box resulted only in offset of that box's house light. Infusions initiated by an S-A animal were accompanied by onset of the signal light in each of the two affected boxes. Following this 6-day cycle of drug availability, a withdrawal test was conducted.

Phase 3

A third cycle of self-administration was then initiated for all animals. Each lever press led to stimulus

changes and morphine infusions identical to those described for Phase 1. This cycle consisted of one, 3-hour session on the first day, followed by two, 3-hour sessions on each of the following 3 days, followed by one, 3-hour session on the fifth day. A final withdrawal test was conducted on the sixth day. This test differed from previous withdrawal tests in that all levers were removed from the operant chambers.

Evaluation of Withdrawal Distress

The behaviours scored during withdrawal tests were identical to those of Experiments 1 and 2. During each withdrawal test, behaviours were scored in 4 animals for 96 minutes, with each animal being scored sequentially for eight, 3-minute bins. This resulted in each rat being scored for a total of 24 minutes during each withdrawal test.

Scoring was conducted by the experimenter and, for half of the animals run, by an observer trained in scoring withdrawal signs, but ignorant of the design and intent of the study (this was the same observer employed in Experiment 1). During tests in which two observers scored the animals, each observer scored different animals in any given time bin. All withdrawal data collected during such tests are presented as the average scores obtained by the two observers.

Evaluation of Drug Effect

Immediately following each session, righting reflex and angle of slippage on the tiltometer were assessed as before.

Results

Subject Attrition

In the first week of the experiment, one animal developed a leak in its catheter. Data from this animal were excluded from the following analyses.

Matching the Groups

Of the remaining 15 animals, 8 (S-A)-TO-Y animals were yoked to 7 S-A animals. Animals were assigned to groups by matching individually on the basis of total dose of morphine administered during the first week. While the average dose self-administered by 14 of the animals during this week was 56 mg, one animal self-administered 1053 mg of morphine in the same period. This "superrat" was assigned to group S-A and two animals were yoked to it. This was done due to the expectation that such high levels of morphine administered passively might lead to mortality. It was felt that yoking two animals to this "superrat" improved the chances that at least one of the (S-A)-TO-Y animals so yoked might survive. Neither of these two (S-A)-TO-Y animals died over the course of the experiment. Due to the

extreme levels of morphine administered by the S-A rat in this triplet, the data for these three animals have been excluded from the following analyses. The resulting sample size was six per group.

Weight Change

Figure 15 summarizes the mean daily weight recorded for each group throughout the experiment. Mixed-design ANOVAs conducted for each of the three weeks revealed no significant GROUP or GROUPxDAY effects. Significant effects of DAY were seen during weeks one ($F[6,60]=35.00$, $p<.001$) and three ($F[5,50]=16.27$, $p<.001$), indicating a significant decrease in body weight for both groups during week one, and a significant increase in body weight for both groups during week three.

The effects of drug withdrawal on weight change were assessed by comparing the weights on each of test days 1 and 2 with the weights on the following days (thereby revealing any weight change occurring following a period of approximately 40 hours without infusions). A mixed-design ANOVA conducted on the data related to the first test day (days T-1 vs. T-1+1) revealed a significant effect of DAY ($F[1,14]=8.55$, $p<.05$). A mixed-design ANOVA conducted on the data related to the second test day (days T-2 vs. T-2+1) revealed no significant effects.

FIGURE 15: Mean daily weights.

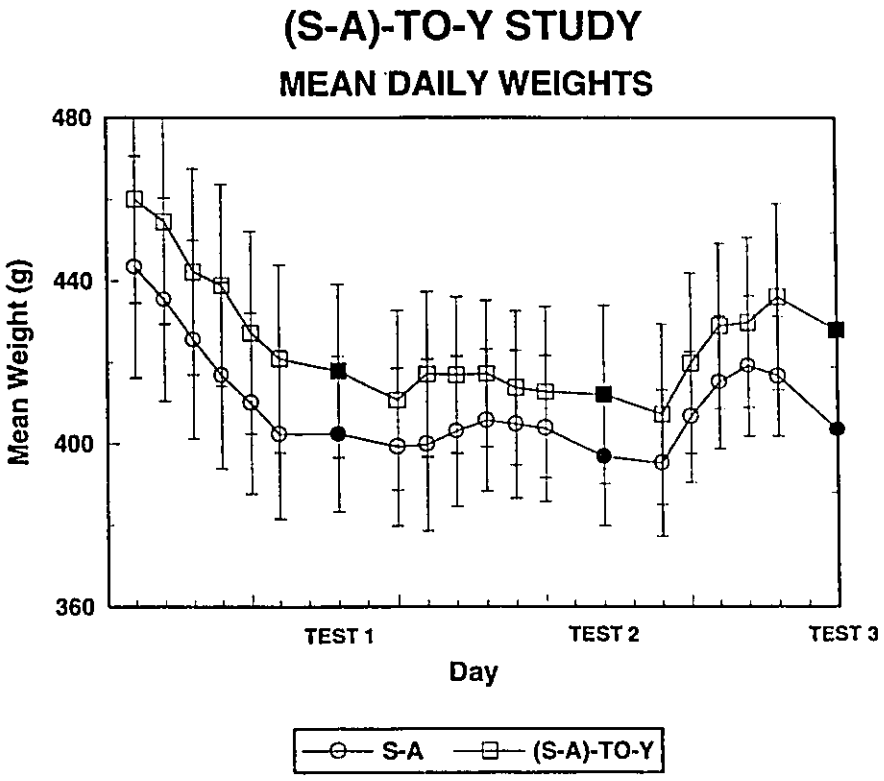
(S-A)-TO-Y STUDY
MEAN DAILY WEIGHTS
480
440
400
360
Mean Weight (g)
TEST 1
TEST 2
TEST 3
Day
S-A
(S-A)-TO-Y

FIG. 15

Lever-press Responses

Figure 16 summarizes the mean number of lever-presses occurring per session for each group throughout the experiment. Mixed-design ANOVAs conducted on data from each of the three weeks revealed no significant GROUP, SESSION or GROUPxSESSION effects.

Morphine Delivered

Figure 17 summarizes the weekly mean dose of morphine received per session by each of the groups. The average doses of morphine administered per session during the first week were 4.7 mg for group S-A and 4.6 mg for group (S-A)-TO-Y. This indicates an adequate matching of animals in terms of the total drug dose administered during week one.

Mixed-design ANOVAs conducted on data from each of the first and third weeks failed to reveal any significant GROUP effects. This suggests that the interpolation of one week of passive drug administration for the (S-A)-TO-Y animals in week two does not disrupt the subsequent "re"-acquisition of intravenous morphine self-administration during week three.

Withdrawal Distress

The mean incidence of withdrawal signs for each group during each test day is presented in Figure 18.

FIGURE 16: Mean lever-presses per session.

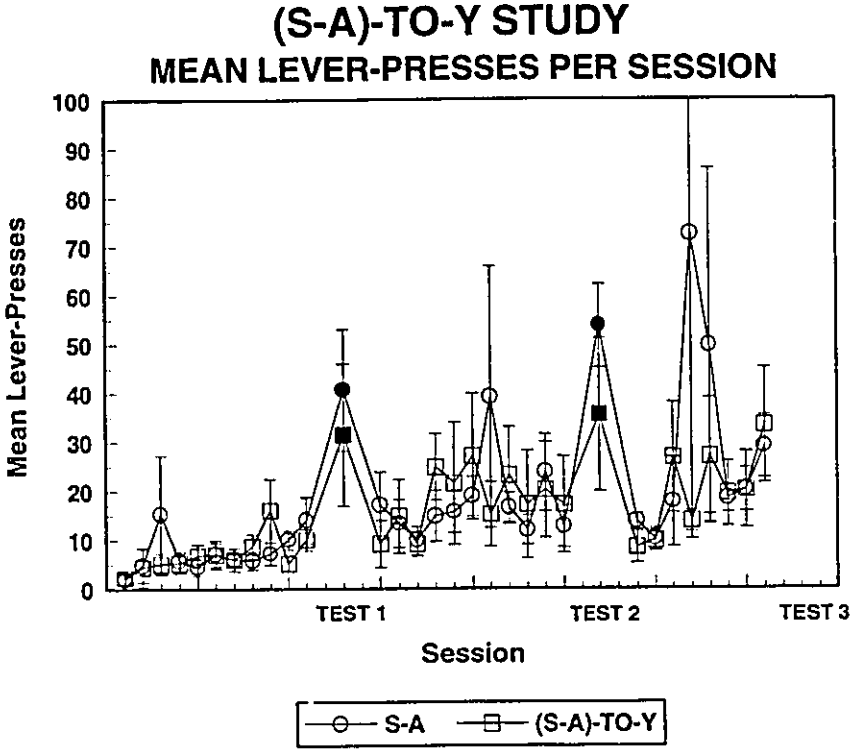
(S-A)-TO-Y STUDY
MEAN LEVER-PRESSES PER SESSION
Mean Lever-Presses
100
90
80
70
60
50
40
30
20
10
0
TEST 1
TEST 2
TEST 3
Session
S-A
(S-A)-TO-Y

FIG. 16

FIGURE 17: Mean morphine per session.

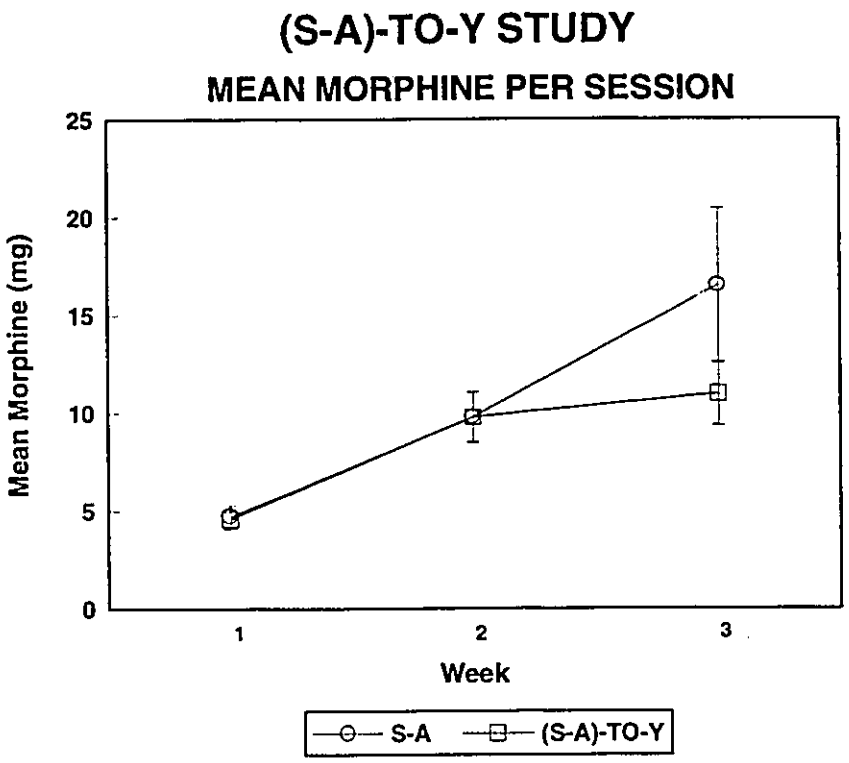
(S-A)-TO-Y STUDY
MEAN MORPHINE PER SESSION
Mean Morphine (mg)
25
20
15
10
5
0
1
2
3
Week
S-A
(S-A)-TO-Y

FIG. 17

FIGURE 18: Mean withdrawal signs per test.

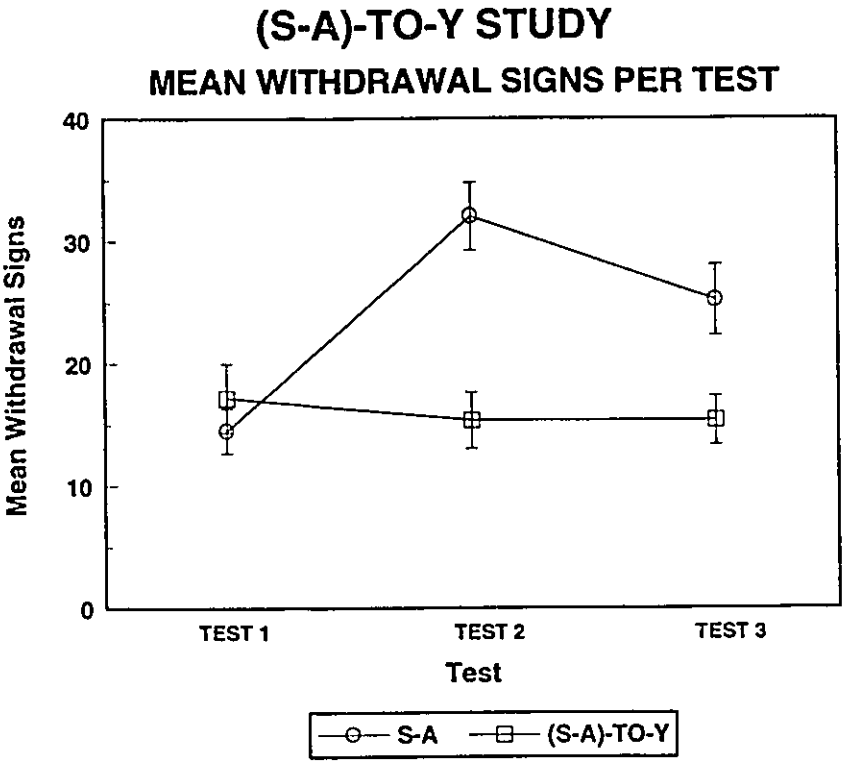
(S-A)-TO-Y STUDY
MEAN WITHDRAWAL SIGNS PER TEST
Mean Withdrawal Signs
40
30
20
10
0
TEST 1
TEST 2
TEST 3
Test
S-A
(S-A)-TO-Y

FIG. 18

Test 1

There was no significant difference between the number of withdrawal signs displayed by the two groups on the first test day, following a week in which both groups were allowed to self-administer morphine (for independent samples: $U = 14$, $p>.10$, 2-tailed).

Test 2

Following week two, during which the S-A animals were allowed to self-administer morphine, and the (S-A)-TO-Y animals were yoked to passively receive the drug, the S-A animals displayed significantly greater numbers of withdrawal signs than did the (S-A)-TO-Y animals (for dependent samples: Wilcoxon $T[6] = 0$, $p<.05$).

Test 3

The S-A animals displayed significantly greater numbers of withdrawal signs than did the (S-A)-TO-Y animals again during the third test day, following a week in which both groups were once again allowed to self-administer morphine (for independent samples: $U = 4$, $p<.05$, 2-tailed).

These results suggest that the initiation of a passive schedule of administration in the (S-A)-TO-Y animals resulted in a decrement in their level of physical dependence relative to the S-A animals. Further, this

difference persisted through a subsequent week in which all animals were allowed to self-administer the drug.

Within-Group Comparisons

Within group comparisons across test days revealed that the S-A animals displayed a consistent increase in withdrawal signs from the first to the second test day (Wilcoxon $T[6] = 0$, $p<.05$). The level of withdrawal did not change significantly from the second to the third test day in the S-A animals (Wilcoxon $T[6] = 2$, $.05<p<.10$).

The level of withdrawal did not change significantly across test days in the (S-A)-TO-Y animals. While the difference between the first and second test days did not reach statistical significance (Wilcoxon $T[6] = 8$, $p>.10$), 4 of the 6 (S-A)-TO-Y animals displayed a decrease in the level of withdrawal across these tests. This can be contrasted with 6 of the 6 S-A animals displaying an increase in the level of withdrawal across the same two tests.

Drug Effects

Righting Reflex

The total number of failures to right displayed by each group for each of the 3 cycles are presented in Figure 19. Meaningful comparisons between groups can only be made on the data from week 2, as drug doses among pairs of

FIGURE 19: Failures to right.

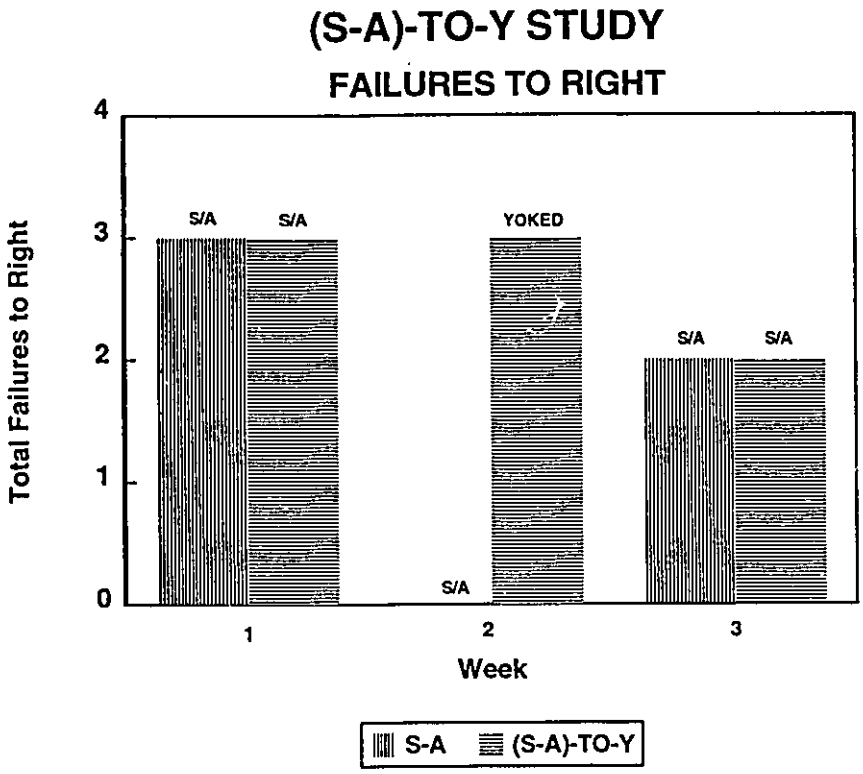
(S-A)-TO-Y STUDY
FAILURES TO RIGHT
Total Failures to Right
4
3
2
1
0
S/A
S/A
S/A
YOKED
S/A
S/A
1
2
3
Week
S-A
(S-A)-TO-Y

FIG. 19

animals were equivalent throughout this cycle. As can be seen in Figure 19, each of the 3 failures to right during week 2 occurred in the (S-A)-TO-Y animals. Given the small number of observed failures to right during this week, there is inadequate power to justify conducting any statistical analysis.

The equivalence in failures to right between the two groups seen during both weeks one and three, coupled with the complete lack of failures to right in animals self-administering morphine during week two, is consistent with the observation in the YOKED experiment that, under conditions of passive receipt of morphine, animals are less tolerant to its narcotizing effects than are those self-administering equivalent doses.

Tiltometer

The mean angle of slippage displayed by both groups after each session is presented in Figure 20. While no systematic differences are apparent between the groups during the first and third weeks, the S-A animals appear, on average, to resist slippage better than do the (S-A)-TO-Y animals during the second week.

Statistical comparison of the angle of slippage of the groups is meaningful only during the second week, as the dose of morphine delivered per session was identical for each matched pair of animals. During the first and third

FIGURE 20: Tiltometer: Mean angle of slippage per session.

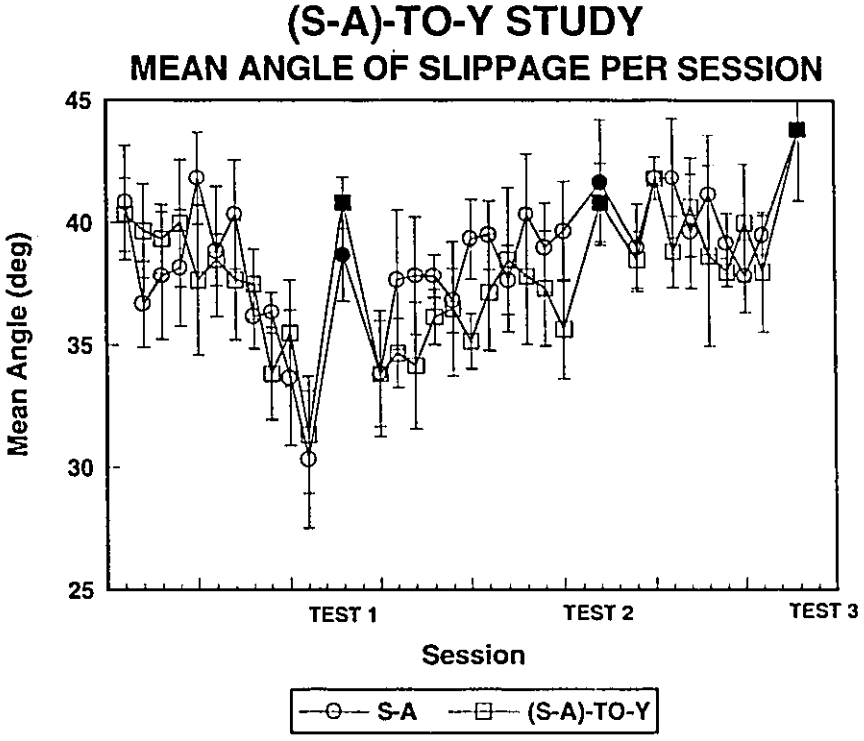
(S-A)-TO-Y STUDY
MEAN ANGLE OF SLIPPAGE PER SESSION
Mean Angle (deg)
45
40
35
30
25
TEST 1
TEST 2
TEST 3
Session
S-A
(S-A)-TO-Y

FIG. 20

weeks, the dose of morphine received by each animal was independently determined by the lever-press responding of the individual animals.

Figure 21 displays the proportion of trials for each session on which the S-A animal of a pair displayed a greater angle of slippage than did its matched (S-A)-TO-Y animal. If all 6 S-A animals were to display greater angles of slippage than their respective (S-A)-TO-Y partners on a given session, this proportion would be 1.0. Figure 21 also indicates the proportion (0.5) that would be expected if the mode of drug delivery (active vs. passive receipt) had no effect on angle of slippage (essentially, under Ho each trial can be viewed as being analogous to the flip of a fair coin: it is equally likely that the (S-A)-TO-Y animal or the S-A animal would have the greater angle of slippage). Data from weeks one and three are included in Figure 21 for comparison, but it should be emphasized that the pairing of animals during these two weeks is artificial in terms of both dose of morphine delivered and the active/passive nature of such delivery.

Week 2

In order to test the equivalence of morphine's effects on angle of slippage between the groups, a sign test was applied to the individual paired session data of week two. For the purposes of this analysis, the data from each

FIGURE 21: Tiltometer: Proportion of trials per session where the angle of slippage for group S-A was greater than the angle of slippage for group (S-A)-TO-Y.

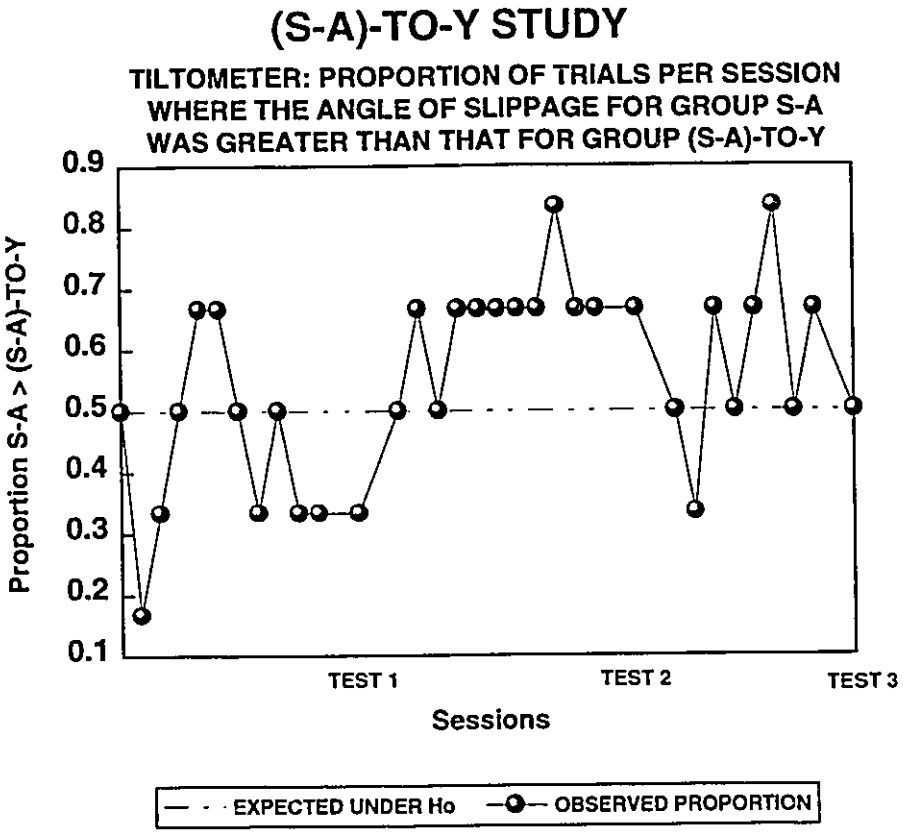
(S-A)-TO-Y STUDY
TILTOMETER: PROPORTION OF TRIALS PER SESSION
WHERE THE ANGLE OF SLIPPAGE FOR GROUP S-A
WAS GREATER THAN THAT FOR GROUP (S-A)-TO-Y
Proportion S-A > (S-A)-TO-Y
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
TEST 1
TEST 2
TEST 3
Sessions
EXPECTED UNDER Ho
OBSERVED PROPORTION

FIG. 21

animal on different sessions were treated as statistically independent. Trials on which the two animals of a pair displayed an equivalent angle of slippage were excluded from these calculations. Of the 66 pairs of tiltometer angles occurring during week two, the S-A animal displayed a larger angle of slippage than did the respective (S-A)-TO-Y animal 43 times (with 2 tied pairs of angles, $p < .01$). This suggests that, during the week in which they received passive drug infusions, the (S-A)-TO-Y animals were less tolerant to the narcotizing effects of a given dose of morphine than were the S-A animals.

Discussion

As was the case in Expt. 1, the current study demonstrates that the level of control over drug administrations influences observed levels of physical dependence and tolerance. The current study extends the results of Expt. 1 by revealing that level of control over drug adminstrations can influence the display of tolerance and physical dependence in animals whose prior history of drug experience is one of active self-administration.

In addition, the difference in levels of withdrawal distress observed during the third withdrawal test suggests that a history of uncontrolled drug experience can have lasting effects. This suggestion of the presence of an "innoculation" against future self-administration-induced

physical dependence by a history of passive drug experience is, however, just that: a suggestion. Other differences between weeks 2 and 3 could also have contributed to the difference in levels of physical dependence observed during the third test. The removal of the response levers from the operant chambers during the third test may have differentially affected the two groups. In addition, the average dose of morphine administered by the S-A animals was greater than that administered by the (S-A)-TO-Y animals during the third week. If nothing else were relevant, the attenuated levels of self-administration displayed by the (S-A)-TO-Y animals during week 3 would alone be sufficient to account for this difference.

While statistically insignificant, the decrement in levels of withdrawal distress observed in the (S-A)-TO-Y animals from the first to the second test day is worth note. Should the trend observed turn out to be reliable, it would imply that established levels of physical dependence can be attenuated by the institution of a schedule of passive drug administrations. The management of withdrawal symptomatology is one of the primary emphases in the early treatment of physically-dependent individuals (Jaffe, 1990). The addition of a new effective tool (and particulary one that would be expected to result in high levels of patient compliance) would be of aid to physicians faced with the

treatment of such individuals. Clearly, further research is required to address the statistical reliability of this effect.

The study described in the next chapter further examines the possibility that the initiation of a passive schedule of drug delivery following a history of active self-administration has the capacity to attenuate established levels of physical dependence and/or tolerance. In addition, hypotheses concerning both the contribution of differential signalling to the effects of controllability and the effects of a history of passive drug exposure to the subsequent development of self-administration will be addressed.

CHAPTER 6: QUAD STUDY (EXPT. 4)

Non-completion of Experiment

Due to the occurrence of a severe allergic response to rats in the experimenter, only two quadruplets were run to completion in this experiment. The data from these animals are presented and discussed, as they suggest an interesting series of trends. With such small sample sizes, statistical inference is innappropriate due to inadequate power and is, therefore, not attempted.

Introduction

The current study examined a number of issues surrounding the influence of differential signalling on the effects of chronic exposure to morphine. In the previous three studies, depression of the operant lever led to offset of the house-light in the affected chamber for the duration of lever depression. This led to a difference in signalling of infusions between self-administering and yoked animals in Expts. 1 and 3 (for self-administering animals in these two studies, the light signal accompanying infusions was, of necessity, initiated against the background of a darkened experimental chamber; the same was not necessarily the case for yoked animals). The current study equated these signals across self-administering, yoked-morphine, and yoked-

Ringer's animals throughout the first phase. During this phase, the depression of the operant lever by the self-administering animal resulted in the offset of the houselight in all three affected chambers. Hence, the purpose of the current study was to replicate the basic self-administering vs. yoked differences observed in Expt. 1 while holding external stimulation equal across yoked triplets. The first phase of the study for these three groups was, then, similar to the design of Expt. 1, with the exception that external signals of drug delivery were equated across the groups.

Signalling effects were further evaluated by the inclusion of a fourth control group. During the first phase, this group received passive morphine infusions identical to those of the yoked-morphine group. However, these infusions were delivered in a distinctive environment in which external stimulation was held constant. Comparison of this group with that receiving passive signalled morphine administrations allowed evaluation of the possibility that the lower levels of tolerance and physical dependence observed in yoked animals result, in part, from differential signalling.

In addition, the current study further examined the possibility that a schedule of passive drug receipt, following a history of self-administration, can attenuate

established levels of tolerance and physical dependence. This was evaluated in animals that had self-administered morphine during the three cycles of Phase 1. During the two cycles of Phase 2, these animals were passively administered a series of morphine infusions identical to those they had self-administered during the last cycle of Phase 1. This allowed evaluation of changes in the levels of both physical dependence and tolerance across three cycles comprising identical patterns of drug infusions, but with the last two being passively received.

Also during Phase 2, the three yoked groups (yoked-morphine, yoked-Ringer's, and yoked-morphine-distinctive) were allowed to self-administer morphine for the first time. This allowed evaluation of the contribution of a prior history of morphine experience to the development of drug self-administration (through evaluation of differences in the acquisition of drug self-administration in morphine-experienced vs. morphine-naive animals). In addition, yoked-morphine-distinctive animals were run in operant chambers for the first time in Phase 2. Thus, comparison of the acquisition of self-administration in the yoked-morphine and yoked-morphine-distinctive groups allowed for the evaluation of the environmental specificity of the influence of prior morphine exposure on the development of self-administration.

Method

Subjects

Sixteen male Long-Evans hooded rats, weighing between 505-663 g at the time of surgery were used. They were housed identically to the animals in Expt. 1. All experimental sessions were run during the light phase of the animals' light/dark cycle.

Surgical Preparation

The surgical preparation and post-operative care were similar to that of Expt. 1, with the exception that the experimental procedure commenced between four and seven days following surgery.

Apparatus

The apparatus employed in this study was similar to that of Expt. 3 with the exception that the levers were initially retracted from all but one of the operant chambers. In addition, a new chamber (the distinctive chamber) in which morphine could be passively administered intravenously was employed. The distinctive chamber consisted of a circular metal trash can (40-cm height by 22-cm diameter at the base and 30-cm diameter at the top) with a removable wire-mesh base placed above standard wood-chip bedding material. A hole was drilled in one wall to allow a drinking tube to be attached. A bracket on the top of the

distinctive chamber secured the swivel in place, and chicken wire secured over the opening ensured that the rat remained in the chamber during sessions. A small light was mounted just outside of the chicken wire above one wall of the distinctive chamber. This light remained on throughout all sessions. No exteroceptive stimulus events were programmed to occur in the distinctive chamber.

Design

All animals were run in squads of four. Each of these quadruplets consisted of one rat each in groups SELF-ADMINISTRATION (group S-A), YOKED-MORPHINE (group Y-M), YOKED-RINGER'S (group Y-R) and YOKED-MORPHINE-DISTINCTIVE (group Y-M-D). Animals in group Y-M-D were run in the distinctive chamber described above, while the other three groups were run in the operant chambers. Each quadruplet was run through a series of five, 6-day infusion/withdrawal test cycles. The first three of these cycles constituted Phase 1 of the study, and the last two constituted Phase 2.

Phase 1

The first three cycles consisted of 5 daily 5-hour infusion sessions, followed by a withdrawal test on the sixth day. Subsequent cycles began later in the same day following the withdrawal test that terminated the previous cycle. During this initial phase, lever-presses by the S-A

animal resulted in both infusions of morphine solution for itself and its Y-M and Y-M-D partners and infusions of Ringer's solution for its Y-R partner. Infusions for groups S-A, Y-M and Y-R were accompanied by the illumination of the signal light in their respective chambers. In addition, lever-presses by the S-A animal led to the offset of the house light in its box and that of its Y-M and Y-R partners.

From the third withdrawal test on, the Y-M-D animal was moved from the distinctive chamber and all subsequent sessions were conducted in an operant chamber.

Phase 2

The final two cycles consisted of 5 daily 5-hour infusion sessions, followed by a withdrawal test on the sixth day. During this final phase of the study, the lever was retracted from the S-A animal's operant chamber and the levers were extended in the other three chambers. Throughout this phase, animals in groups Y-M, Y-R, and Y-M-D were allowed to self-administer morphine in a manner identical to that group S-A experienced during Phase 1. Animals in group S-A were now administered infusions passively in a pattern identical to that they had received during the final cycle of Phase 1. During each passive infusion, the house lights were turned off and the signal light above the now-retracted lever was illuminated.

Procedure

Subjects were grouped into quadruplets, matched individually on the basis of body weight. Each member of a quadruplet was randomly assigned to one of the four groups.

Phase 1

During this phase, the S-A rat was placed in the operant chamber with the lever present, the Y-M and Y-R animals were placed in the operant chambers with the levers removed, and the Y-M-D rat was placed in the distinctive chamber. A cycle consisted of 5 days of drug availability, with a withdrawal test occurring early on the sixth day. The next cycle was initiated on the same day as the withdrawal test which had terminated the previous cycle. During this phase of the experiment, lever-presses by the S-A rat initiated infusions of morphine to itself and to its paired Y-M and Y-M-D animals. Similarly, Ringer's solution was infused to its paired Y-R animal. While no external stimulus events were programmed for the Y-M-D animals in this phase, the external stimulus events for groups S-A, Y-M and Y-R were identical. Every time the S-A animal depressed its lever, the house lights in the three operant chambers went off. Similarly, coincident with each infusion, a 3-second signal light was illuminated in the three operant chambers.

Phase 2

Following the three cycles of Phase 1, two additional cycles conducted under the same temporal parameters were immediately run. These Phase-2 cycles differed from those of Phase 1 in the following ways. The S-A animals had the levers removed from their chambers throughout this phase, and all of their infusions were administered passively in a pattern identical to that they had received during the third cycle of Phase 1. The Y-M and Y-R animals now had levers inserted in their chambers and were allowed to self-administer morphine for the first time. The Y-M-D animals were placed into an operant chamber with a lever present and also allowed to self-administer morphine for the first time. Withdrawal tests were conducted following each of the two cycles of Phase 2.

Evaluation of Withdrawal Distress

The behaviours scored during withdrawal tests were identical to those of Experiments 1, 2 and 3. During each withdrawal test, behaviours were scored for a quadruplet for 64 minutes, with each animal being scored sequentially for eight 2-minute bins. This resulted in each rat being scored for a total of 16 minutes during each withdrawal test. Scoring was conducted by the experimenter and an observer trained in scoring withdrawal signs, but ignorant of the design and intent of the experiment (this was the same

observer employed in Expts. 1 and 3). During each test, each observer scored different animals in any given time bin. All withdrawal data collected during these sessions are presented as the average scores obtained by the two observers.

For the third withdrawal test, animals in group Y-M-D were moved for the first time to the operant chambers, in an attempt to evaluate any environmentally-specific component to their established physical dependence. Otherwise, all animals received withdrawal tests under conditions identical to those of the preceding 5-day cycle, with the exception that all infusion pumps were turned off.

Evaluation of Drug Effect

Immediately following each session, righting reflex and angle of slippage on the "tiltometer" were assessed as before.

Results & Discussion

Weight Change

Figure 22 summarizes the mean daily weight per week for each group throughout the experiment.

FIGURE 22: Mean daily weights.

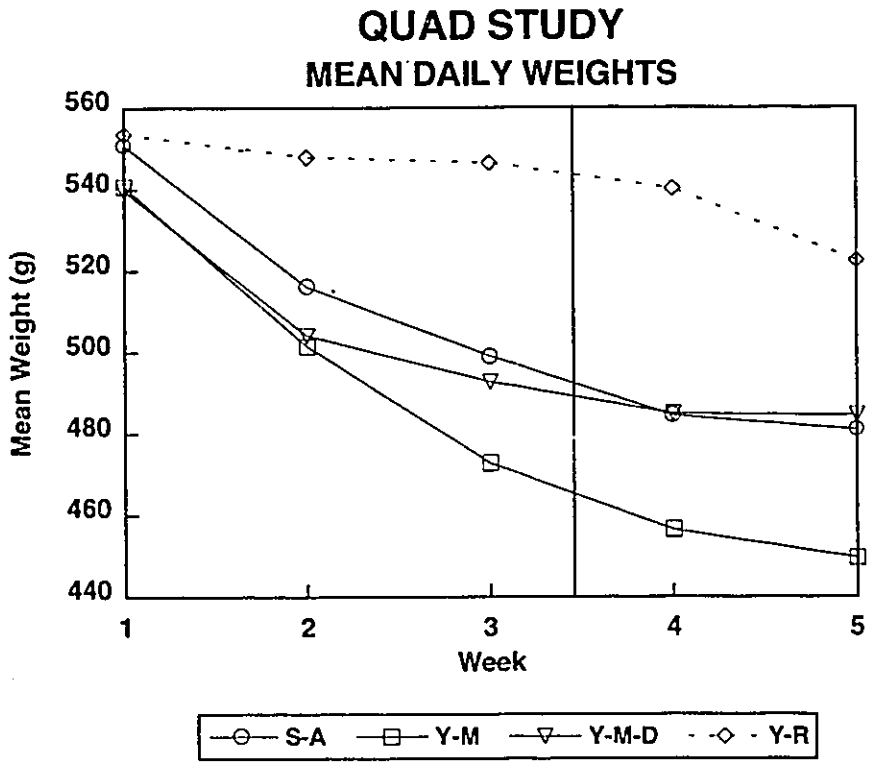
QUAD STUDY
MEAN DAILY WEIGHTS
Mean Weight (g)
560
540
520
500
480
460
440
1
2
3
4
5
Week
S-A
Y-M
Y-M-D
Y-R

FIG. 22

Phase 1

The differential weight loss observed over the first three weeks between the three morphine groups and group Y-R is consistent with that observed in the YOKED experiment.

Phase 2

After being allowed to self-administer morphine, animals in group Y-R displayed greater weight loss than they did over the three weeks during which they were infused with Ringer's solution only. It appears that the intravenous delivery of morphine leads to increased weight loss relative to the intravenous delivery of Ringer's solution.

Lever-press Responses

Figure 23 summarizes, by week, the mean number of lever-press responses occurring per session for each group throughout the experiment.

Phase 1

During the first three weeks, when only animals in group S-A were able to lever-press for morphine, lever-presses consistently increased across weeks.

Phase 2

During the last two weeks, when animals in groups Y-M, Y-M-D and Y-R were all able to lever-press for morphine, lever pressing increased in all three groups. The

FIGURE 23: Mean lever-presses per session.

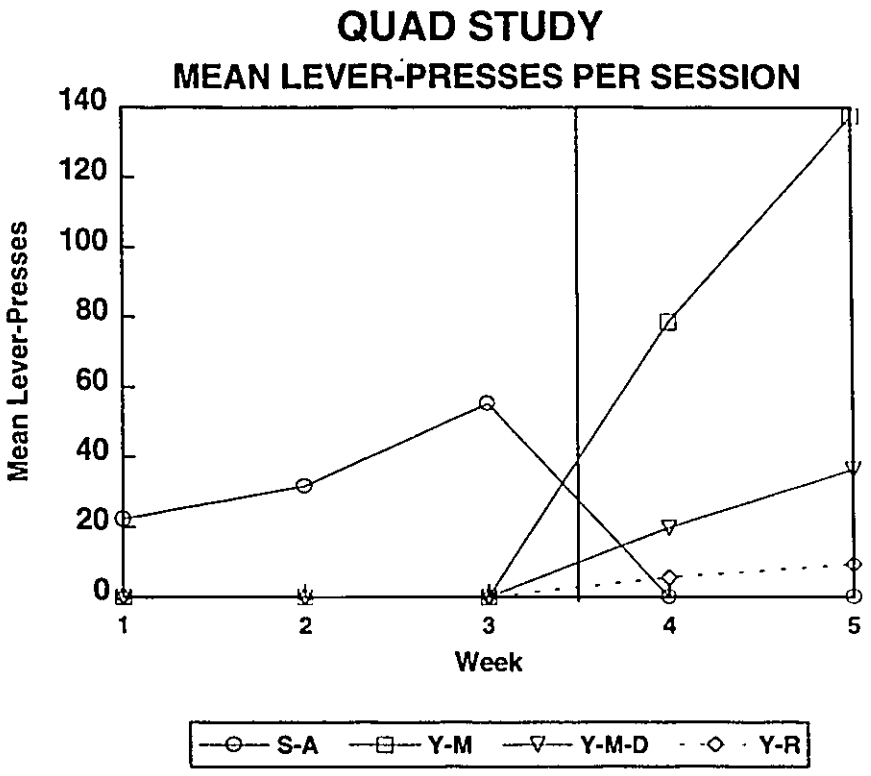
QUAD STUDY
MEAN LEVER-PRESSES PER SESSION
Mean Lever-Presses
140
120
100
80
60
40
20
0
1
2
3
4
5
Week
S-A
Y-M
Y-M-D
Y-R

FIG. 23

levels of lever-pressing were highest in group Y-M, lowest in group Y-R and intermediate in group Y-M-D. This suggests that the prior passive receipt of morphine leads to increased levels of drug-reinforced behaviour relative to that displayed by animals lacking such morphine experience (compare groups Y-M and Y-M-D with group Y-R). In addition, the increased level of drug-reinforced behaviour in group Y-M compared to group Y-M-D suggests that such behaviour may be sensitive to signals previously paired with drug delivery.

Morphine Delivered

Figure 24 summarizes, by week, the mean dose of morphine administered per session in each group.

Phase 1

The dose of drug administered by the S-A animals consistently increased across the first three weeks of the experiment.

Phase 2

It appears that a prior history of passive morphine predisposes rats to self-administer morphine when it is made available (compare groups Y-M and Y-M-D with group Y-R). In addition, it appears that there is an environmentally-specific component to this effect (compare group Y-M with group Y-M-D).

FIGURE 24: Mean morphine per session.

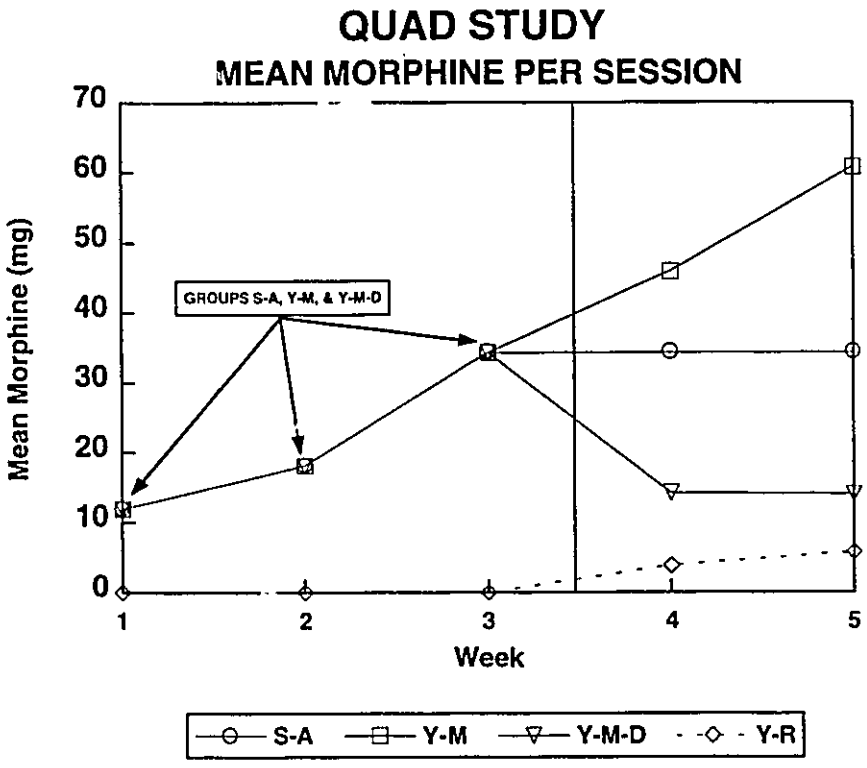
QUAD STUDY
MEAN MORPHINE PER SESSION
Mean Morphine (mg)
70
60
50
40
30
20
10
0
GROUPS S-A, Y-M, & Y-M-D
1
2
3
4
5
Week
S-A
Y-M
Y-M-D
Y-R

FIG. 24

Withdrawal Distress

The mean total number of withdrawal signs evidenced by the four groups on each withdrawal test are presented in Figure 25.

Phase 1

The higher level of withdrawal signs observed in group S-A compared to group Y-M is consistent with the results of the YOKED study. In addition, the similar levels of withdrawal signs observed in groups Y-M and Y-M-D suggest that the difference in physical dependence between self-administering animals and those passively receiving the drug is not dependent on the presence of external signals of drug delivery.

The third withdrawal test for animals in group Y-M-D was conducted (for the first time) in an operant chamber. A comparison of the levels of withdrawal signs evidenced by group Y-M-D from test 2 to test 3 does not appear to support the suggestion that the physical dependence displayed by these animals is environmentally-specific. This observation is further supported by the lower levels of withdrawal signs evidenced by group Y-M on test 3 when compared to those evidenced by group Y-M-D on test 3.

FIGURE 25: Mean withdrawal signs per test.

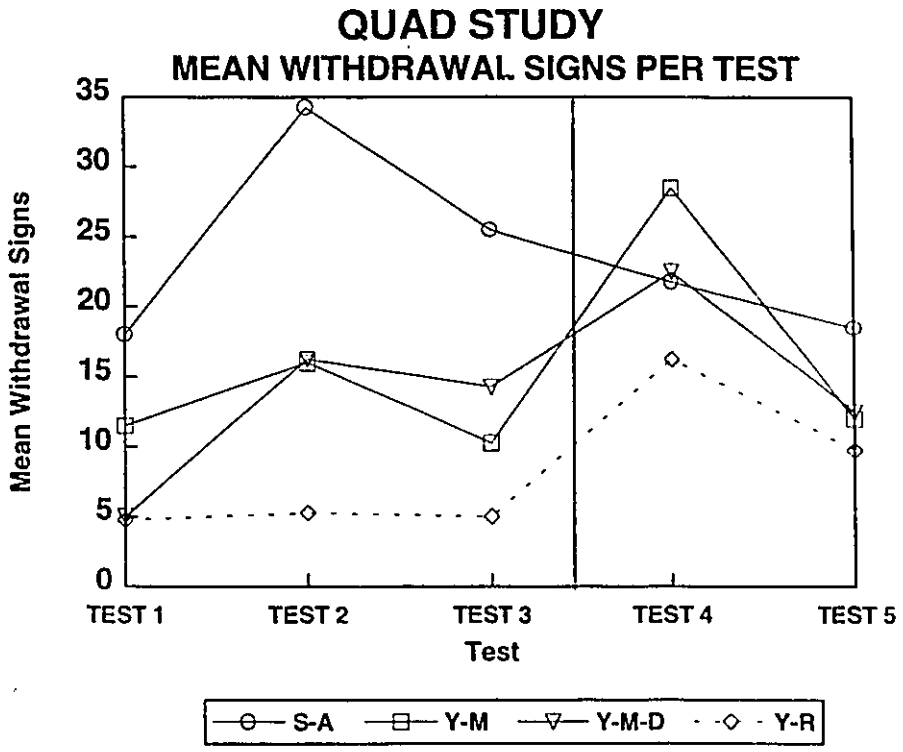
QUAD STUDY
MEAN WITHDRAWAL SIGNS PER TEST
Mean Withdrawal Signs
35
30
25
20
15
10
5
0
TEST 1
TEST 2
TEST 3
TEST 4
TEST 5
Test
S-A
Y-M
Y-M-D
Y-R

FIG. 25

Phase 2

The increase in withdrawal distress seen in groups Y-M and Y-M-D from the third test to the fourth test does not support the existence of an "innoculation" against subsequent self-administration-induced physical dependence by a prior history of passive drug receipt.

The level of withdrawal distress evidenced by animals in group S-A suggests that, with drug dose held constant, the level of physical dependence displayed decreases with a switch from self-administration to passive drug delivery.

Drug Effects

Righting Reflex

The total number of failures to right occurring in each group during each week of the experiment are summarized in Figure 26. Missing bars indicate the absence of failures to right in a group during a given week.

Weeks 1 to 3

A comparison of the three morphine groups is consistent with the observation in the YOKED experiment that passive receipt of morphine leads to lower levels of tolerance to the narcotizing effect of morphine than does self-administration of identical doses of the drug.

FIGURE 26: Failures to right.

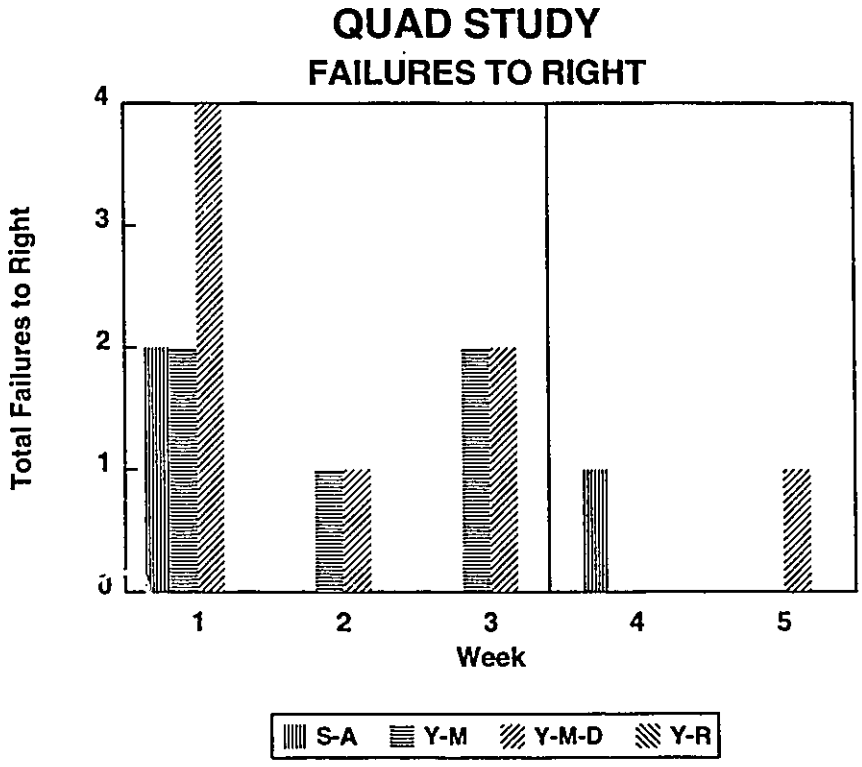
QUAD STUDY
FAILURES TO RIGHT
Total Failures to Right
4
3
2
1
0
1
2
3
4
5
Week
S-A
Y-M
Y-M-D
Y-R

FIG. 26

Weeks 4 and 5

Since no failures to right were observed in group S-A during the third week, the failure to right observed in this group during week 4 suggests a decreased level of tolerance to the narcotizing effect of a given dose of morphine resulting from the switch to passive receipt of the drug. The decreased number of failures to right observed in groups Y-M and Y-M-D over the last two weeks suggests an increased level of tolerance to the narcotizing effect of morphine resulting from the switch to self-administration of the drug.

Tiltometer

Figure 27 summarizes, by week, the mean angle of slippage occurring per session in each group throughout the experiment.

Weeks 1 to 3

No differences are apparent in the angle of slippage displayed by any of the three groups receiving morphine. The consistently greater angles of slippage displayed by the animals receiving Ringer's solution reinforces the validity of the tiltometer as a measure of morphine's narcotizing effect.

FIGURE 27: Tiltometer: Mean angle of slippage per session.

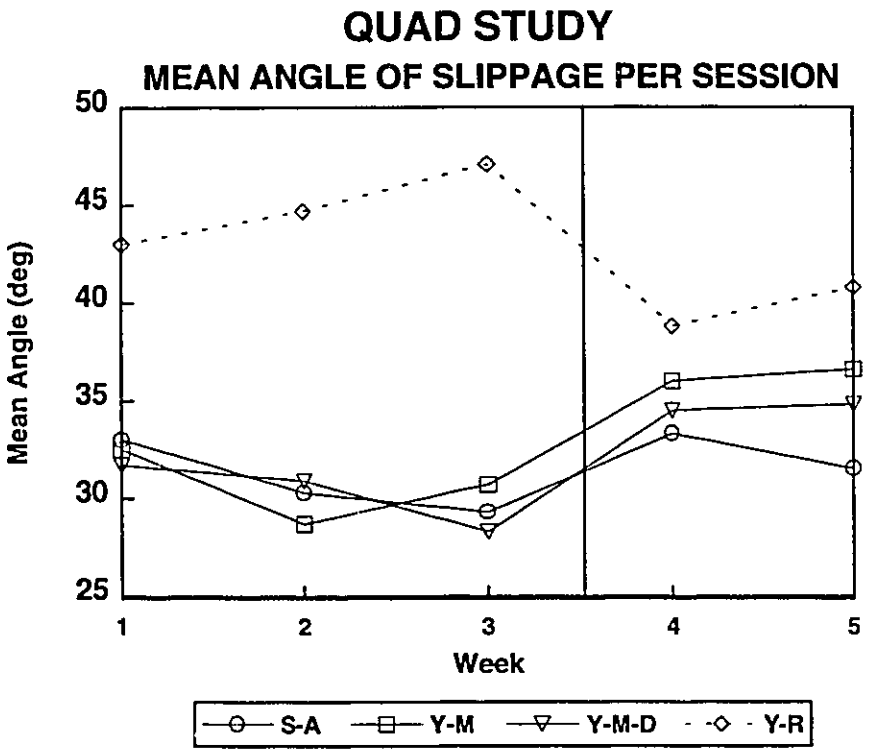
QUAD STUDY
MEAN ANGLE OF SLIPPAGE PER SESSION
Mean Angle (deg)
50
45
40
35
30
25
1
2
3
4
5
Week
S-A
Y-M
Y-M-D
Y-R

FIG. 27

Weeks 4 and 5

Exposure to morphine led to a decrease in the angle of slippage displayed by group Y-R relative to that displayed in week 3. The switch from passive receipt of morphine to self-administration of the drug in groups Y-M and Y-M-D led to an increase in the angle of slippage displayed by these groups, suggesting increased levels of tolerance. Animals in group S-A also displayed an increase in angle of slippage relative to that displayed in week 3. This suggests, given the identical dosing experienced by group S-A across weeks 3, 4, and 5, that a switch from active self-administration to passive receipt of morphine led to increased levels of tolerance.

Conclusion

Interpretation of the results presented in this chapter is, at best, tentative. A number of aspects of the data have been highlighted which suggest mechanisms that may have influenced the effects observed in Experiments 1 and 3. It is hoped that, in the future, other investigators will continue with research on the effects of controllability on the effects of chronic exposure to drugs of abuse. It would be satisfying to see the completion of a study based on the experimental design employed above.

CHAPTER 7: GENERAL DISCUSSION

> I know that most men--not only those considered clever, but even those who really are clever and capable of understanding the most difficult scientific, mathematical or philosophic problems--can seldom discern even the simplest and most obvious truth if it be such as obliges them to admit the falsity of conclusions they have formed, perhaps with much difficulty--conclusions of which they are proud, which they have taught to others, and on which they have built their lives.
> Leo Tolstoy (1896) (cited in Dyson, 1984, p. 213)

Summary of Results

Experiment 1

Differences in the Acquisition of Tolerance & Dependence

The YOKED study demonstrated that rats self-administering intravenous morphine display enhanced levels of withdrawal distress (in the absence of drug) and tolerance (in the presence of drug) relative to yoked partners having identical drug histories. These findings indicate that self-administration can contribute to the development of physical dependence and tolerance. They are also contrary to the common assumption in animal research on addiction that the physiological effect of a drug is the same whether an organism passively receives or actively takes it.

Rapidity of Acquisition

The levels of physical dependence observed and the group differences in those levels were fully evident following a single six-day cycle of drug sessions. This observation was somewhat surprising, given the procedures traditionally employed to induce physical dependence in rats through passive administrations of morphine. The morphine animals in the YOKED study received, on average, less than 75 mg of morphine during the first cycle of drug sessions. As mentioned earlier, studies inducing physical dependence through passive administrations of morphine typically employ much larger doses administered multiple times per day at increasing levels over an extended period of time. For example, Weeks (1962) employed 122 hourly intravenous infusions of morphine on an increasing schedule of doses ranging from 2 to 40 mg/kg per infusion to induce physical dependence. Prior to the initiation of self-administration, these rats were maintained for 2 days on hourly 40 mg/kg infusions of morphine. To induce physical dependence, Martin et al (1963) gave rats intraperitoneal injections of morphine twice daily for 6 weeks. The doses employed increased steadily from an initial daily dose of 10 mg/kg to a final dose, maintained for a full week, of 320 mg/kg/day.

It is apparent that physical dependence can, under the conditions employed in the YOKED study, develop more

rapidly and with much lower doses of morphine than are traditionally employed in efforts to experimentally induce physical dependence.

Experiment 2

Explicit Signalling Did Not Mimic S-A Effect

The SIGNALLED/NON-SIGNALLED study failed to reveal any statistically significant differences between the two groups on measures of either physical dependence or tolerance. The exteroceptive signal, as presented, did not mimic the effects of self-administration seen in the YOKED study. As is always the case with traditional hypothesis-testing logic, a failure to reject the null hypothesis does not allow any definitive statement to be made regarding the set of hypotheses specified. The failure to reject any of the null hypotheses relating to tolerance and physical dependence specified in the SIGNALLED/NON-SIGNALLED study, therefore, lends no support to the suggestion that differential signalling might mediate the differences in tolerance and physical dependence observed in the yoked study.

Analysis of the tiltometer data, however, suggested that the measure is sensitive to morphine dose. The significant negative correlation between the dose of morphine delivered and the mean angle of slippage suggests

that increasing doses of morphine will be associated with lower angles of slippage.

Experiment 3

Effect of Passive Drug Receipt Following Self-Administration

The (S-A)-TO-Y study extends the demonstration of the YOKED study that passive schedules of morphine administration have the ability to attenuate observed levels of tolerance and physical dependence. During the first cycle, when all animals were allowed to self-administer morphine, no significant group differences were observed in the levels of tolerance or physical dependence displayed. During the second cycle, when half of the animals were yoked to animals that continued to self-administer morphine, the passively-dosed animals displayed attenuated levels of withdrawal distress (in the absence of drug) and tolerance (in the presence of drug) relative to animals actively self-administering identical doses of morphine. Each of the six animals allowed to continue self-administering morphine during the second cycle displayed an increase in the number of withdrawal signs from the first to the second withdrawal test. In contrast, four of the six animals switched to passive receipt of morphine during the second cycle displayed a decrease in the number of withdrawal signs from the first to the second withdrawal test. This decrease in

withdrawal distress was not statistically significant. However, that such a decrease occurred in 2/3 of the (S-A)-TO-Y animals in the face of doses of morphine that more than doubled from the first to the second cycle is surprising.

During the third cycle, when all animals were allowed to self-administer morphine, animals that had been allowed to self-administer morphine throughout the experiment once again displayed significantly higher levels of withdrawal distress than did animals that received morphine passively during the second cycle.

Experiment 4

Although the small sample sizes in the QUAD study precluded statistical analysis of the data, a number of the results merit note. This study afforded the first opportunity to compare the angle of slippage displayed by animals exposed to morphine with the angle displayed by drug-naive animals. Comparison of group Y-R with each of the three groups receiving morphine during the first three weeks reinforces the validity of the tiltometer as a measure of morphine's narcotizing effect. This is consistent with the sensitivity of the tiltometer to morphine dose observed in the SIGNALLED/NON-SIGNALLED study. As the dose of morphine increases, the observed angle of slippage decreases.

The data also suggest that self-administration of morphine in animals with a prior history of passive drug receipt may be influenced by environmental cues previously paired with morphine. In contrast, the withdrawal observed in morphine-experienced animals does not appear to be sensitive to external signals of drug availability. External signals paired with drug delivery were equated in groups S-A and Y-M and were minimized in group Y-M-D. The levels of physical dependence displayed by groups Y-M and Y-M-D during weeks 1 to 3 were substantially lower than that displayed by group S-A. There were, however, no apparent differences in the levels of physical dependence displayed by groups Y-M and Y-M-D.

Finally, the fact that the levels of withdrawal distress observed during the first three weeks were higher for group S-A than for group Y-M is consistent with the results of the YOKED study. As such, the reliability of the difference in levels of withdrawal distress observed in the YOKED study is strengthened.

Question of Mechanism

The mechanism underlying the observed effects has not been elucidated in this thesis. Some aspect of self-administration of morphine leads to increased levels of tolerance and physical dependence relative to those produced by equivalent doses of passively-administered morphine. A

putative physiological mechanism implicating stress-induced alterations in the endogenous opioid system is presented. Finally, both behavioural and associative mechanisms which may be relevant to the observed effects are outlined. With this framework, it is hoped that the delineation of the mechanisms underlying the effects observed in this thesis might be facilitated.

Stress-Induced Increases in Endogenous Opioids

One manner in which the level of control over drug administrations might have an impact upon tolerance and physical dependence is through the relative stressfulness of controllable vs. uncontrollable events. Uncontrollable events have been described as more stressful than otherwise comparable (but controllable) events (Overmier, Patterson, & Wielkiewicz, 1980). This suggests a physiological mechanism through which the response to morphine might be altered.

One of the fundamental changes noted by Selye (1975) as characteristic of the physiological response to stress is an increase in the activity of the hypothalamic-pituitary-adrenal axis, resulting in increased excretion of ACTH and cortisol. In summarizing the neurochemical events which lead to the release of ACTH, Kelly (1985) indicates that the precursor of ACTH (proopiomelanocortin) is also the precursor of the peptide beta-lipotropin. Kelly points out that both of these peptides are released in response to

stressful stimulation. Of particular interest is the fact that beta-lipotropin is converted into beta-endorphin. It follows that increases in stress will lead to increases in levels of beta-endorphin. Beta-endorphin is the most potent of the endogenous opioids in terms of its morphine-like activity. It has been estimated that beta-endorphin, administered intraventricularly, is over 40 times more potent than morphine in terms of opiate-like effects (Kelly, 1985, p.339).

If the passive receipt of morphine is, indeed, stressful, it follows that animals receiving drug independent of their behaviour would have elevated levels of endogenous opioids. In this case, the expression of physical dependence during withdrawal testing might be inhibited by the relatively high baseline levels of endogenous opioids. While both self-administering and yoked animals would have been free from exogenously administered morphine for equivalent durations, the relatively high levels of endogenous opioids in the yoked animals would be expected to diminish the expression of withdrawal signs.

Similarly, during drug administration sessions, the observed narcotic effect in each animal would actually be the summation of the effects of the endogenous opioids and the exogenously administered morphine. The higher levels of endogenous opioids in the yoked animals would be expected to

result in relatively greater drug effect than that observed in animals self-administering identical doses of morphine. Such an increased narcotic effect would be interpreted as a relative lack of tolerance in the yoked animals.

The ability of stress to activate endogenous opioid release to levels that are behaviourally relevant is supported by a number of lines of research. Stress has been shown to result in decreased sensitivity to pain that is reversible by the opiate antagonist naloxone (Akil, Madden, Patrick, & Barchas, 1976; Sherman, Strub, & Lewis, 1984). This finding implies that levels of endogenous opioids are elevated in organisms exposed to stressful stimulation. Similarly, exposure to uncontrollable shock leads to increased levels of naloxone-precipitated withdrawal signs in animals exposed to morphine (Williams, Drugan, & Maier, 1984). These authors indicate that their results are consistent with the activation of endogenous opioid systems in response to inescapable shock.

Przewlocka, Sumova, & Lason (1990) observed decreases in the levels of beta-endorphin in the hypothalamus and pituitary gland in rats following conditioned fear-induced stress. In addition, a three-fold increase in plasma levels of beta-endorphin was observed. This stands as direct evidence that stress has the ability to increase peripheral levels of endogenous opioid peptides.

Should this stress-induced mechanism actually account for the results reported in this thesis, a distinction would have to be made between the development and the expression of physical dependence and tolerance. With all other factors being equal, the development of both tolerance and physical dependence is expected to be more rapid in animals possessing higher levels of endogenous opioids. Elevated levels of endogenous opioids would be expected to stimulate the opioid receptors to a greater extent during periods where relatively little morphine is present in the animals' systems. With greater net stimulation of these receptors, the adaptive mechanisms underlying tolerance and physical dependence would be active to a greater degree. Under this analysis, the levels of developed (potential) tolerance and physical dependence would actually be higher in the yoked animals than in their self-administering partners. The expression of such tolerance and physical dependence would, however, be blocked by the very factor that led to their existence: elevated baseline levels of endogenous opioids. A line of experimental investigation designed to test this putative mechanism is presented in the next chapter.

Behavioural Mechanism: Optimization of Drug Delivery

Another manner in which having control over drug administrations might have an impact upon the consequences

of continued use is through what will be called the "optimization" of drug delivery. An animal having control over its drug administrations can, by definition, choose either to administer a dose or to not administer a dose at each moment in time that a dose is available. The factors that contribute to the form of that choice are, no doubt, numerous. Two that may be relevant to the current discussion are the administration of drug in either response to or anticipation of a "need" or a "want".

If it is assumed that there exists individual variation in the pharmacokinetics of morphine across different animals, it follows that each animal's response, across time, to a given dose (or pattern of doses) of the drug will be somewhat different. An animal allowed to self-administer morphine will be able to time the delivery of each dose in such a way as to optimize the drug's efficacy. An animal yoked to such a self-administering partner enjoys no such privilege. It's doses occur in a pattern independent not only of it's behaviour, but also of it's current physiological state. Presumably, as the physiological state changes, only the self-administering animal will be able to titrate the dosing pattern with respect to it's ongoing "needs" and "wants".

It follows that the benefit gained by an animal exposed to morphine (be it the alleviation of either a

"need" or a "want") will, potentially, be greater in an animal self-administering the drug than in an animal receiving an identical pattern of doses without control over their occurrence. Consistent with this suggestion are observations that rats prefer events over which they have control to events over which they have no control (Ettenberg, Laferriere, Milner, & White, 1981).

The specific mechanisms through which differential optimization of drug delivery might be expected to influence observed levels of tolerance and physical dependence are unclear. However, such differential optimization is analogous to a key component of Church's (1964) criticism of the use of yoked-control designs to assess the reinforcing efficacy of stimulus events. As mentioned in Chapter 3, the bias inherent in yoked-control designs highlighted by Church does not speak to the differences in levels of tolerance and physical dependence observed in this thesis.

Associative Mechanism: Compensatory Conditional Responding

A final manner in which having control over drug administrations might have an impact upon tolerance and physical dependence is through differential predictability of upcoming drug administrations. It is necessarily the case that an animal that is in control of the occurrence of an event has greater information concerning the impending occurrence of that event than does an animal lacking such

control. With the initiation of the behaviour that results in the occurrence of an event, the initiating animal is in a position to predict (if not expect) the imminent occurrence of that event. It follows that the manipulation of the level of control over the occurrence of an event is confounded by systematic alterations in the level of predictability of that event. With increased control comes increased predictability.

The increased predictability of upcoming events in organisms having control over those events can stem from a number of sources. The interoceptive state of the organism leading to emission of the response which results in the occurrence of the event (the operant) is reliably paired with the occurrence of that event. Such interoceptive states could, therefore, acquire predictive value regarding the occurrence of the event. In a sense, the interoceptive state preceding event occurrence becomes a signal for the occurrence of that event. In a similar fashion environmental stimuli reliably paired with the performance of the operant (such as the sight of one's paw coming into contact with the response lever) could serve as signals for the occurrence of the event contingent upon performance of the operant. Finally, proprioceptive feedback from the actual performance of the operant could, potentially, serve a similar signalling function.

Siegel's conditioning model of tolerance and dependence (Siegel, 1979; 1990; MacRae, Scoles, & Siegel, 1987) allows for a mechanism through which differential predictability could account for the results presented in this thesis. If upcoming drug administrations were signalled more effectively for the S-A rats than for the Y-M rats, the observed pattern of results is exactly what Siegel's model would predict. The "better" signal(s) of upcoming drug administration in the S-A animals would have resulted in a stronger compensatory conditional response being elicited in these animals than in the Y-M rats. During drug-delivery sessions, the greater strength of this compensatory conditional response would be manifest as increased levels of tolerance. During drug-withdrawal tests, the stronger compensatory conditional response would be observed as increased levels of withdrawal distress. While Expt. 2 failed to support this hypothesis, it is possible that the exteroceptive signalling employed was simply inadequate to mimic the differential signalling inherent between self-administering rats and yoked controls.

Arguments have been presented which suggest possible mechanisms for the observed differences between rats self-administering morphine and those passively receiving the drug. Further experimentation will be required before the validity of these mechanisms is clear.

CHAPTER 8: CONCLUSION

"human drug-taking behavior does not usually originate from a baseline of physical dependence"
Smith, Werner & Davis (1976)

Conclusion

Animal Research on Addiction

The current experiments call into question the interpretation of results in experiments "passively addicting" animals prior to examining the roles of physical dependence and/or tolerance in drug-acquisitory behaviour. Specifically, the amount of opiate experience considered necessary to establish appreciable levels of these two states has, it would appear, been overestimated. In general, such studies may have systematically underestimated the contribution of physical dependence and tolerance to the acquisition, maintenance, and reinstatement of drug self-administration behaviour.

One such influential study (Weeks & Collins, 1968) employing passive addiction techniques concluded that "prior exposure to morphine is only a minor factor in etiology of relapse " (p. 297). Similary, van Ree, Slangen, & de Wied (1978) concluded that tolerance and physical dependence do not play a significant role in heroin self-administration. These investigators based their conclusion on data stemming

from passive, forced infusions of heroin conducted prior to allowing the opportunity for self-administration. While such assertions may well be true, it should be noted that any such conclusion based on the assumption that "passive addiction" leads to physical dependence and tolerance identical to that seen in an "actively-addicted" organism is highly suspect.

The contribution of both tolerance (Smith, Werner, & Davis, 1976) and physical dependence (Thompson & Schuster, 1964; Schuster & Thompson, 1969) to opiate self-administration has been amply demonstrated. The emphasis on physical dependence as a key feature of opiate addiction stems from the theoretical view that reinforcement, by definition, involves the reduction of some form of drive ("need") (Hull, 1943). Opiate administration by a physically-dependent organism appears to reduce just such a need. The rapid alleviation of withdrawal distress following drug administration essentially returns the organism to a "normal" state. The acquired drive of withdrawal distress is reduced by the drug and only by the drug.

The emphasis on reinforcement as drive-reduction has encouraged the practice of making animals physically-dependent on opiates prior to allowing the self-administration of the drugs. The bulk of early research

examining the reinforcing effects of morphine employed animals made physically-dependent upon the drug (Beach, 1957; Headlee, Coppock, & Nichols, 1955; Nichols, Headlee, & Coppock, 1956; Spragg, 1940; Thompson & Schuster, 1964; Weeks, 1962; Weeks & Collins, 1964). As a result, the use of animals made physically-dependent upon opiates became a virtually standard practice in studies investigating opiate self-administration.

Given the results presented in this thesis, this practice is questionable. That some motivation is required to support operant behaviour is clear. However, the assumption that this motivation must in some way reflect the amelioration of a "need" is, perhaps, innappropriate to the case of drug reinforcers. Wise (1988) has argued that, since drugs can activate central reinforcement mechanisms directly, they represent a class of reinforcers much more intense than those represented by environmental stimuli such as food and water.

It is generally accepted that physical dependence can contribute to drug self-administration in terms of the negative reinforcement afforded through the relief of withdrawal distress. However, it has been argued that physical dependence is neither a necessary nor sufficient condition for the initiation or maintenance of drug self-administration (Spealman & Goldberg, 1978; Woods & Schuster,

1968). It should be remembered that the processes leading to the development of physical dependence are initiated following the first experience with opiates. The rapidity with which physical dependence was seen to develop in Expt. 1 underscores this point. Physical dependence was observed within a period of 6 days during which as little as 50 mg (in total) of morphine was administered. Given this, it is reasonable to assume that the negative reinforcement resulting from the amelioration of withdrawal distress by self-administered opiates is active and able to contribute to the development of drug addiction much earlier than is generally believed possible.

Future Directions

The results presented in this thesis raise a number of issues that merit further investigation. These include the identification of both the physiological mechanisms underlying the observed effects and the environmental and/or behavioural variables which control the activation of these mechanisms. In addition, the suggestion in Expt. 4 that differential signalling of passive drug infusions may influence the subsequent acquisition of drug self-administration is worth further study. Together, these issues point to a need for the reformulation of current views concerning the etiology of tolerance, dependence, and addiction.

Physiological Mechanisms Underlying Effects

Blood Sampling of Endorphin Levels

The physiological mechanism proposed in the previous chapter to explain the observed differences was that of higher levels of endogenous opioids (likely beta-endorphin) being present in the yoked animals than in the self-administering animals. This hypothesis could be tested through the regular monitoring of endogenous opioid levels in the blood of animals in an experiment employing the yoked triadic design (as employed in Expt. 1; procedures for assaying blood levels of both morphine and beta-endorphin are summarized in Graves, Arrigo, Foster, Baumann, & Batenhorst, 1985). In addition, following the last withdrawal test session the levels of the endogenous opioids in the cerebrospinal fluid or specific brain regions could be assayed.

Precipitated Withdrawal

A further suggestion of the "elevated endorphin" model is that the developed levels of tolerance and physical dependence should be greater in the YOKED animals than in the self-administering animals. However, the high levels of baseline endogenous opioids would tend to mask the expression of such states. An indirect method by which this hypothesis could be tested is through the use of naloxone to

precipitate an acute state of withdrawal. If, as has been suggested earlier, YOKED animals have relatively high levels of endogenous opioids, they would be expected to display a more intense precipitated withdrawal syndrome than would self-administering animals.

The precipitated abstinence test could be conducted under an experimental design similar to that employed in the YOKED study. During each of the non-precipitated withdrawal tests, it would be expected that the self-administering animals would display more withdrawal signs than would their yoked controls. Following the final withdrawal test, naloxone could be infused through the indwelling catheter. A rapid reversal in the relative levels of withdrawal signs evidenced by the two groups would be supportive of the proposed model.

Behavioural/Environmental Variables Underlying Effects

Control vs. Signalling

The issue of whether it is differential control *per se*, or differential signalling of upcoming drug administrations resulting from such control that results in the observed results remains unanswered. Experiments which vary the degree of control over drug administrations may be confounded by differential signalling (predictability) of

upcoming drug infusions. Animals having greater control necessarily have greater predictability of such infusions.

One avenue of research which might aid in the ultimate specification of the variables important to the observed effects is that concerned with the evaluation of signalling. Operant control can not easily be manipulated independently of differential signalling. However, signalling can be manipulated independently of the degree of operant control. Experiment 2 represents a first attempt at just such a line of research. The negative results of the experiment were disappointing, but cannot be taken as refutation of the signalling hypothesis. Perhaps the signalling employed was inadequate to mimic the differences in tolerance and physical dependence observed in Expt. 1. Such inadequacy might lie in terms of the intensity, duration/timing, or modality of the signals that were paired with drug administrations.

Additional experiments varying the intensity, duration/timing, or modality of signalling paired with drug administrations may be more successful in evaluating the potential contribution of signalling to the observed effects. As was mentioned previously, one means by which self-administering animals could be exposed to increased signalling of upcoming drug administrations is through the proprioceptive feedback inherent in the performance of the

operant response. One manner in which proprioceptive feedback could be mimicked is through the administration of low-level cutaneous shock. One group of animals would have such shock paired reliably with drug infusions. An appropriate control would have an equivalent pattern of infusions and an equivalent number of shocks. For the control animals, however, the shocks would be delivered on a random schedule, uncorrelated with the pattern of delivered infusions.

The primary goal of this line of research would be to demonstrate that effects like those observed in Expt. 1 can be replicated in situations where the only difference between groups of animals receiving identical patterns of drug administrations is the level of signalling of those administrations. It would be advisable for such studies to employ heroin as the drug of choice, as it's latency of onset following intravenous infusion is appreciably shorter than that of morphine.

Effects of Signalling on Subsequent Self-Administration

The differences in self-administration observed in Expt. 4 between animals having identical histories of passive drug receipt, but different signals associated with those administrations is also worth further study. The small sample sizes in Expt. 4 do not allow for statistical validation of the observed differences. However, animals

allowed to self-administer morphine in the same environment and with the same signals as were paired with morphine when delivered passively (group Y-M) displayed average levels of self-administration from four to six times that displayed by animals with identical passive pharmacological histories, but lacking such reliably drug-predictive cues (group Y-M-D).

This suggestion of a strong environmental component in the acquisition of drug self-administration in organisms with a history of passive drug receipt has potential clinical relevance. The low rates of continued opiate use displayed following termination of treatment by patients chronically administered opiate analgesics in a hospital setting may stem, in part, from exactly such changes in environmental drug-associated cues. A clearer demonstration of such "environmental specificity" of drug self-administration would add a great deal to existing knowledge concerning the effects of similar environmental manipulations on the effects of chronic drug exposure (Siegel & MacRae, 1984).

Etiology of Addiction

Exposure Alone is Not Enough

The results presented in this thesis emphasize the inadequacy of any view of tolerance, dependence, or

addiction that focuses solely on pharmacological principles. Previous research which has evaluated the role of prior drug experience in both tolerance and dependence has, in large part, failed to acknowledge a role of self-administration in the development of these phenomena. By so doing, application of the results of such research to the genesis of human addictive behaviour has systematically underestimated the potential contribution of both tolerance and physical dependence to the addictive process.

There can be little doubt that a pre-existing state of physical dependence increases the likelihood that drugs, when made available, will be self-administered. The point to be emphasized is, however, that attempts to model human addictive behaviour should recognize that passive and active schedules of drug administration do not result in equivalent levels of tolerance and physical dependence. To the extent that human drug addiction results primarily from a history of drug self-administration, models employing passive schedules of drug administration have consistently missed an important component of the development of chronic drug effects.

The theoretical view that physical dependence is equivalent to addiction has frequently been questioned (e.g., Nichols, 1965). The primary reason for this debate has resulted from the acknowledgement that addiction

involves a behavioural component. The behavioural component is that of drug-seeking behaviour. The results presented in this thesis extend the role played by drug-seeking behaviour in the definition of addiction to include the influence of drug-seeking behaviour on the development of some of the defining characteristics of the addict. That drug-seeking behaviour can influence the development of tolerance and physical dependence argues strongly that the importance of such behaviour extends beyond that of being a definitional component of the end-state of addiction. Drug-seeking behaviour appears to be an integral component of the processes that influence the development of chronic drug effects. As such, drug-seeking behaviour should be viewed as an integral component of the addictive process. It is simply not adequate to view physical dependence and tolerance as the sole driving forces underlying the development of addictive behaviour.

Final Conclusion

The central question motivating the experiments was that of the role played by the act of <u>self</u>-administration in the effects of chronic exposure to drugs. The common assumption that the effects of drug administrations are the same whether a drug is actively taken or passively received was challenged. Experiments 1, 3, and 4 indicate that this assumption is invalid. The levels of tolerance and physical

dependence displayed in animals self-administering morphine were consistently higher than in animals passively receiving identical doses of the drug.

The experiments highlight a shortcoming of research strategies which employ passive drug administration in attempts to model human drug addiction. If it is assumed that human drug addicts generally develop their addictions without a history of passively receiving such drugs, the results presented herein would suggest that such research may have systematically underestimated the role played by both tolerance and physical dependence in the development of addictive behaviour. That tolerance and physical dependence develop differently through passive and active drug receipt is clear. What role this may play in the development, maintenance, and loss of addictive behaviour remains to be determined.

The inadequacy of employing passive administrations to model situations where active self-administrations are the norm may well generalize beyond the drug paradigm outlined above. It will be interesting to see if further research reveals that similar differences are observed with other passively-delivered reinforcers when compared to actively-obtained reinforcers.

The role of control over the delivery of physiologically meaningful stimuli on the impact of those

stimuli merits further investigation. Pavlovian conditioning experiments actively deny control to their subjects (although CRs can be viewed as a mechanism through which a degree of control is exerted over upcoming events). The ability to exercise some degree of control over the external and internal environments is an important (if not defining) quality of living organisms. The results presented in this thesis suggest that experiments which deny any possibility of such control may, as a result, be inadvertently confounded.

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Appendix A: Catheter Construction & Surgical Procedure

CATHETER CONSTRUCTION

-Cut small (.012 x .025) silastic 15 cm long.

-Dilate one end of the silastic with xylene and fit it over the cannula guide.

-Cut PE-100 (or 90) tubing 1.2 cm long. Slide over the silastic and cannula guide (taking care not to tear the silastic). This provides support and protection for the silastic when bending the cannula for attachment to the skull.

-Cut a piece of large (.025 x .047) silastic about 2 mm long. Dilate with xylene and slide it over small the silastic until it is about 7 cm from the cannula guide. This yields an anchoring collar mid-way along the catheter.

-Bond silastic collar to silastic catheter with small blobs of silastic medical adhesive (type A) at each end of collar, being sure to spread it around the collar and up the cannula about .5 mm on each end.

-Let this dry for at least four days.

-Test the catheter for leaks or blockage by squirting water through it both freely and with the open end blocked.

-Store the finished catheters in alcohol for about two or three days prior to implantation.

SURGICAL PROCEDURE

Preparation

-Check the catheter once again for leaks, and see if its collar is firmly anchored in place.

-Anaesthetize animal.

-Shave head, dorsal right shoulder-neck area, and ventral neck and thoracic area.

-Swab all shaved areas with ethanol.

Surgery

-Make a small (1 cm) incision in the dorsal skin near the shoulder blade.
-Make a 2 cm incision on the ventral surface in the area of the jugular vein. This should only be as large as is required to dissect out the vein.
-Using blunt dissection, create a subcutaneous tunnel from the dorsal incision, just over the shoulder-neck region to meet the ventral incision.
-Pull the catheter through this tunnel and position it such that its collar will fit against the hole to be cut in the vein.
-Blunt dissect out the vein (small straight hemostats and forceps work nicely for this).
-Cut the silastic (with a non-bevelled or straight cut) so that when it is in the vein it reaches just to the entrance of the auricle (be very careful not to make it too long, which occasionally happens if the vein rips a bit upon catheter implantation). To locate the auricle, put your finger on the chest and determine where the heartbeat is the strongest, then cut the silastic to reach just to the edge of your finger--ie about .5 cm rostral of this "strongest" heartbeat.
-Rinse a dilute heparin/ampicillin solution through the catheter and leave it attached to a filled syringe during surgery.
-The catheter will enter the vein just rostral to the collar bone. Dissect <u>all</u> overlying tissue from the vein (or else implantation will be difficult).

-Gently lift the vein and push a large (18 g) needle under it. This provides some support to the vein while you are working, a partial tourniquet effect, and clearance between the vein and surrounding tissues for the upcoming gluing stage.

-With surgical micro-scissors make a small incision in the vein (be careful not to sever the vein through more than 1/3 of its circumference).

-Using curved sharp forceps to lift the edge of the cut vein, use flat blunt forceps to gently insert the end of the silastic into the vein until the collar fits against the hole. Note that this is often the most difficult stage of the surgery.

-Put one suture (gut) around the vein at each end of the collar. To ensure that these are not tied too tightly, repeatedly check for patency by retracting and infusing from the heparin-filled syringe. If blood is seen to pass unimpeded rostral to the suture upon syringe retraction, the suture is not too tight.

-Put a small drop of crazy glue around each suture and the vein. BE CAREFUL TO HOLD THE SKIN, FUR , INSTRUMENTS, AND SURROUNDING TISSUE AWAY FROM THIS GLUE UNTIL SET...APPROX 30 SEC. (a small drop of water or saline placed on the suture prior to gluing facilitates bonding). Watch, in particular, that the 18 g needle does not bond to the vein.

-Sprinkle area with topical antibiotic. Suture tissue over the vein (with silk...be careful not to catch the vein or catheter in these sutures, and make the sutures relatively deep--i.e. into the muscle).

-Sprinkle more topical antibiotic and wound-clip the skin back together (takes about 4 clips).

-Position animal in stereotaxic. Reswab head with ethanol.
-Make scalp incision (1.5 cm) and drill four holes in skull.
-Put four skull screws in.
-Using blunt dissection, make a subcutaneous tunnel between the skull and dorsal shoulder incision and pull the cannula guide through to the skull.
-Bend the cannula guide to an angle of about 80 degrees with needle nose pliers that have had their tips wrapped with electrical tape (to minimize damage to the catheter).
-Position the cannula guide on the skull and apply dental acrylic to the screws being very careful not to leave any sharp edges and, particularly, not to leave anything around the silastic past the PE 100 protector.
-Apply topical antibiotic to shoulder and skull openings.
-Suture the head incision both rostral and caudal to cap, being careful with the caudal suture not to nick or catch the catheter.
-Wound clip the shoulder incision (takes 1 or 2 clips).
-Check catheter patency (by withdrawing until blood is visible, then re-infusing blood and heparin), and then infuse about .1 cc of heparin solution into the animal.
-Inject I.M. penbritin 1 cc (in the thigh).
-Remove heparin lead and screw the cannula cap in place.
-Place the animal under a heat source on a paper towel and observe periodically until it wakes up. Be prepared to aspirate if necessary.

Appendix B: Example Experimental Control Program.

```
\    ASYST PROGRAM SOURCE FILE
\    FOR (S-A)-TO-Y STUDY (Expt. 3)
\
\ This program shall initiate externally-programmed injection
\   sequences for each response performed by each animal.
\
\ The code will produce a single output to initiate the injection
\   sequence in each of the four boxes as appropriate. (Note
\   that each response in a given box will result in the
\   initiation of 3-seconds of infusion for that animal.
\   Responses occurring during an infusion re-cycle the infusion
\   clock
\
\ In addition, the time (from start of session) of each lever
\   press and the total number of lever-presses per box shall
\   be recorded
\
\ The first week of the experiment involves all animals
\   self-administering.  During the second week, half of these
\   will be yoked to the other half and any changes in withdrawal
\   levels or drug effects will be noted.  The yoking will be
done
\   using the external relay rack, and not the program.

\ The program assumes the presence of a Tecmar Lab-Tender
\   Interface Board, although the code is fairly generalizable
\   to any Digital I/O board employing the 8255 controller chip
\   (i.e., the Data Translation Series, the Lab-Master, etc.)
```

```
\ ************************************************************
\                          LIST OF SCALARS
\ ************************************************************

INTEGER
SCALAR #.RESP.1          0 #.RESP.1 :=    \ Response counter for
box 1
SCALAR #.RESP.2          0 #.RESP.2 :=    \ Response counter for
box 2
SCALAR #.RESP.3          0 #.RESP.3 :=    \ Response counter for
box 3
SCALAR #.RESP.4          0 #.RESP.4 :=    \ Response counter for
box 4

DP.INTEGER
SCALAR SESSION.LENGTH  10800000 SESSION.LENGTH := \ 3 hours =
10800000
SCALAR START.TIME                         \ REL.TIME at start of
session
SCALAR RESP.TIME                          \ REL.TIME of each
response

12 STRING FILENAME

\************************************************************
\*********************  LIST OF ARRAYS **************************
\************************************************************

  \ RESP.TIME.1 (n)   Stores array of response times for box 1
DP.INTEGER DIM[ 800 ] ARRAY RESP.TIME.1
```

```
  \ RESP.TIME.2 (n)   Stores array of response times for box 2
DP.INTEGER DIM[ 300 ] ARRAY RESP.TIME.2

  \ RESP.TIME.3 (n)   Stores array of response times for box 3
DP.INTEGER DIM[ 300 ] ARRAY RESP.TIME.3

  \ RESP.TIME.4 (n)   Stores array of response times for box 4
DP.INTEGER DIM[ 300 ] ARRAY RESP.TIME.4

\*****************************************************************
\******************  BEGINNING OF SOURCE CODE ********************
\*****************************************************************
\ Define and initialize digital templates
0 DIGITAL.TEMPLATE INPUT.PORT
     BINARY
     00001111, DIGITAL.MASK
     DECIMAL
     DIGITAL.INIT

5 DIGITAL.TEMPLATE OUTPUT.PORT.1
     BINARY
     00000001, DIGITAL.MASK
     DECIMAL
     DIGITAL.INIT

5 DIGITAL.TEMPLATE OUTPUT.PORT.2
     BINARY
     00000010, DIGITAL.MASK
     DECIMAL
     DIGITAL.INIT
```

```
5 DIGITAL.TEMPLATE OUTPUT.PORT.3
     BINARY
     00000100, DIGITAL.MASK
     DECIMAL
     DIGITAL.INIT

5 DIGITAL.TEMPLATE OUTPUT.PORT.4
     BINARY
     00001000, DIGITAL.MASK
     DECIMAL
     DIGITAL.INIT

: INJECT
     SET.BITS
     5 MSEC.DELAY
     RESET.BITS
;

: STORE.BOX.1
     OUTPUT.PORT.1
     INJECT
     #.RESP.1 1 + #.RESP.1 :=
     #.RESP.1 801 <
IF RESP.TIME RESP.TIME.1 [ #.RESP.1 ] :=  CR ." BOX 1 Rs = "
#.RESP.1 . CR
 ELSE ." TOO MANY RESPONSES IN BOX 1.  NOW AT N = " #.RESP.1 . CR
     RESP.TIME . CR
   THEN
;
```

```
: STORE.BOX.2
   OUTPUT.PORT.2
   INJECT
   #.RESP.2 1 + #.RESP.2 :=
   #.RESP.2 301 <
IF RESP.TIME RESP.TIME.2 [ #.RESP.2 ] :=  CR ." BOX 2 Rs = "
#.RESP.2 . CR
 ELSE ." TOO MANY RESPONSES IN BOX 2.  NOW AT N = " #.RESP.2 . CR
   RESP.TIME . CR
 THEN
;

: STORE.BOX.3
   OUTPUT.PORT.3
   INJECT
   #.RESP.3 1 + #.RESP.3 :=
   #.RESP.3 301 <
IF RESP.TIME RESP.TIME.3 [ #.RESP.3 ] :=  CR ." BOX 3 Rs = "
#.RESP.3 . CR
 ELSE ." TOO MANY RESPONSES IN BOX 3.  NOW AT N = " #.RESP.3 . CR
   RESP.TIME . CR
 THEN
;

: STORE.BOX.4
   OUTPUT.PORT.4
   INJECT
   #.RESP.4 1 + #.RESP.4 :=
   #.RESP.4 301 <
IF RESP.TIME RESP.TIME.4 [ #.RESP.4 ] :=  CR ." BOX 4 Rs = "
#.RESP.4 . CR
```

```
 ELSE ." TOO MANY RESPONSES IN BOX 4.  NOW AT N = " #.RESP.4 . CR
   RESP.TIME . CR
 THEN
;

\SAVE.RESPONSE.DATA
\Expects numerical representation of last response-onset state on
\ top of number stack.  Stores time of response for each box
\ and increments #.RESPONSES.BOXn for each box indicated.
\ CASE..OF..ENDOF..ENDCASE is structured to have most likely
\ events tested first (e.g., 1 box only with a response onset,
\ followed by 2 simultaneous onsets, then 3, and, lastly, 4)
\Leaves number stack clear when exited


: SAVE.RESPONSE.DATA
     REL.TIME RESP.TIME :=
     CASE
     1  OF STORE.BOX.1                                        ENDOF
     2  OF STORE.BOX.2                                        ENDOF
     4  OF STORE.BOX.3                                        ENDOF
     8  OF STORE.BOX.4                                        ENDOF
     3  OF STORE.BOX.1 STORE.BOX.2                            ENDOF
     5  OF STORE.BOX.1 STORE.BOX.3                            ENDOF
     9  OF STORE.BOX.1 STORE.BOX.4                            ENDOF
     6  OF STORE.BOX.2 STORE.BOX.3                            ENDOF
     10 OF STORE.BOX.2 STORE.BOX.4                            ENDOF
     12 OF STORE.BOX.3 STORE.BOX.4                            ENDOF
     7  OF STORE.BOX.1 STORE.BOX.2 STORE.BOX.3                ENDOF
     11 OF STORE.BOX.1 STORE.BOX.2 STORE.BOX.4                ENDOF
     13 OF STORE.BOX.1 STORE.BOX.3 STORE.BOX.4                ENDOF
```

```
     14 OF STORE.BOX.2 STORE.BOX.3 STORE.BOX.4          ENDOF
     15 OF STORE.BOX.1 STORE.BOX.2 STORE.BOX.3 STORE.BOX.4  ENDOF
     ENDCASE
;

\ CHECK.FOR.PRESS
   \ PRIMARY routine
\
\Reads input ports and returns true (bit-mapped) if a response
\  has begun since the last read operation
\If response has begun, the numerical representation is returned
\  to the number stack and the data are saved.  If not, then
\  a test is made for the end of the session and this routine may
\  occur again.
\Note that this expects an initial response state on the symbol
\  stack (which must be initialized to 0, before first execution)
\  and leaves the last response state on the symbol stack after
\  execution.
\
: CHECK.FOR.PRESS
     INPUT.PORT
     READ.BITS
     ?SWAP
     ?OVER
     XOR
     ?OVER
     AND
     ?DUP
     IF MASK>#  SAVE.RESPONSE.DATA ELSE ?DROP THEN
;
```

```
: INJECTION.TIME?
     BEGIN
          CHECK.FOR.PRESS
          SESSION.LENGTH START.TIME + REL.TIME <=
     UNTIL
;

: GET.FILENAME
  CR ." Enter filename for session data storage " CR CR
  "INPUT   FILENAME ":= CR
  ." You have specified the data file as "  FILENAME "TYPE CR CR
;
\ END.ROUTINE

: END.ROUTINE
    FILE.TEMPLATE
        DP.INTEGER DIM[ 800 ] SUBFILE
        DP.INTEGER DIM[ 300 ] SUBFILE
        3 TIMES
    END
CR ." I am saving data files to disk now " CR
FILENAME DEFER> FILE.CREATE
FILENAME DEFER> FILE.OPEN
    1 SUBFILE RESP.TIME.1 ARRAY>FILE  ." SUBFILE 1 SAVED " CR
    2 SUBFILE RESP.TIME.2 ARRAY>FILE  ." SUBFILE 2 SAVED " CR
    3 SUBFILE RESP.TIME.3 ARRAY>FILE  ." SUBFILE 3 SAVED " CR
    4 SUBFILE RESP.TIME.4 ARRAY>FILE  ." SUBFILE 4 SAVED " CR
FILE.CLOSE
     ." # RESPONSES IN BOX 1 = " #.RESP.1 . CR
     ." # RESPONSES IN BOX 2 = " #.RESP.2 . CR
     ." # RESPONSES IN BOX 3 = " #.RESP.3 . CR
```

```
     ." # RESPONSES IN BOX 4 = " #.RESP.4 . CR
     ." TIME OF LAST RESPONSE OCCURRED " RESP.TIME START.TIME -
1000 / .
     ." SECONDS INTO SESSION. " CR CR
;
\*****************************************************************
\ RUN.EXPT-----------MASTER CONTROL WORD
\*****************************************************************

: RUN.EXPT
GET.FILENAME
0 #.RESP.1 :=
0 #.RESP.2 :=
0 #.RESP.3 :=
0 #.RESP.4 :=
0 RESP.TIME.1 :=
0 RESP.TIME.2 :=
0 RESP.TIME.3 :=
0 RESP.TIME.4 :=
10800000 SESSION.LENGTH :=
CR
CR
." HIT ESCAPE KEY TO BEGIN SESSION " CR CR
BEGIN KEY 27 = UNTIL
." SESSION HAS STARTED AT REL.TIME = "
REL.TIME START.TIME :=  START.TIME . CR CR
     0,
     INJECTION.TIME?
     END.ROUTINE
;
```

APPENDIX C: Data Tables (Yoked Study).

YOKED STUDY
DAILY WEIGHT (g)

	GROUP	S-A	S-A	S-A	S-A	S-A	S-A	Y-M	Y-M	Y-M	Y-M	Y-M	Y-M	Y-R	Y-R	Y-R	Y-R	Y-R	Y-R
DAY	TRIPLET	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
D1		492	463	531	574	487	449	510	452	542	582	480	428	486	450	520	571	493	447
D2		486	442	536	570	476	432	506	448	539	588	471	423	478	431	528	574	488	452
D3		454	435	515	540	467	424	476	440	520	577	462	407	456	422	507	562	486	446
D4		440	424	510	522	464	424	466	428	535	557	454	409	458	415	509	563	482	450
D5		441	416	506	489	463	416	460	426	522	553	451	410	441	415	508	552	469	455
D6		435	411	506	490	453	405	450	421	507	555	441	400	444	406	496	546	464	439
T-1		422	411	496	477	458	414	427	413	503	533	438	400	448	400	499	545	474	434
D8		447	410	481	477	426	389	457	411	490	527	427	381	462	414	504	548	465	455
D9		454	417	483	482	436	409	462	408	492	532	432	395	458	415	478	551	464	457
D10		449	409	482	485	446	425	460	408	488	530	445	405	464	414	458	550	456	457
D11		449	413	486	483	434	417	472	407	494	531	437	416	466	419	459	556	446	457
D12		441	413	485	481	442	411	460	409	489	519	440	416	459	414	466	547	456	457
D13		452	409	477	483	445	414	451	411	480	511	441	407	465	422	462	548	451	457
T-2		443	415	471	481	434	416	454	412	484	476	431	407	458	423	457	548	470	455
D15		440	412	451	471	417	401	448	389	449	451	409	383		421		554	470	466
D16		448	422	451	481	426	401	455	392	450	459	423	394		414		541	467	465
D17		449	422	453	477	439	423	453	402	452	469	431	414		408		535	477	465
D18		444	429	464	471	438	430	462	417	470	481	425	414		423		538	477	471
D19		418	426	450	461	449	418	446	407	467	477	434	410		423		545	464	465
D20		401	437	464	454	451	419	447	406	477	479	429	413		419		550	473	470
T-3		391	421	437	449	439	419	448	390	472	493	429	413		412		541	477	472

YOKED STUDY
LEVER-PRESS RESPONSES PER SESSION

GROUP	S-A	S-A	S-A	S-A	S-A	S-A	Y-M	Y-M	Y-M	Y-M	Y-M	Y-M	Y-R	Y-R	Y-R	Y-R	Y-R	Y-R
TRIPLET	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
S1	20	5	0	5	8	7	30	3	2	4	0	12	21	2	0	0	1	4
S2	5	5	2	9	9	5	2	14	0	5	1	7	6	5	0	0	7	18
S3	11	11	17	0	10	5	5	11	6	61	2	1	2	5	0	0	17	7
S4	12	9	13	1	9	10	1	4	216	5	30	0	22	0	0	0	0	0
S5		12	3	0	14	1		1	3	8	15	80		3	0	0	7	16
S6	10	13	5	4	9	9	10	14	0	2	3	34	13	1	0	0	8	1
S7	11	2	7	9	14	8	25	103	1	5	11	9	1	0	1	3	13	2
S8	4	9	17	24	12	5	59	24	46	6	24	3	13	1	1	1	9	5
S9	9	12	57	13	13	6	98	10	15	8	7	10	2	1	2	0	7	6
S10	6	0	19	7	12	9	25	50	102	4	29	24	6	8	4	0	9	5
S11	5	15	43	13	15	8	0	18	9	18	10	33	1	4	2	0	2	0
T-1	18	42	136	31	11	7	10	8	8	4	47	19	5	0	0	0	6	0
S13	12	9	10	10	45	14	4	23	2	8	15	15	7	3	1	1	15	2
S14	5	9	6	7	23	18	25	56	2	0	20	8	2	7	1	12	6	0
S15	3	12	9	3	14	11	49	35	12	19	3	85	1	27	1	0	3	0
S16	6	4	7	6	7	17	45	2	16	6	36	1	4	7	1	2	10	2
S17	4	6	19	8	17	11	3	14	18	10	4	0	0	16	3	1	0	0
S18	6	5	73	9	26	12	36	38	24	4	10	0	4	4	3	2	4	0
S19	12	13	160	9	11	10	9	29	10	90	22	2	0	1	11	2	2	0
S20	5	15	82	9	25	8	4	2	28	11	28	19	2	9	7	0	3	0
S21	6	10	25	24	39	19	45	25	17	19	3	2	0	15	0	4	2	0
S22	8	4	53	15	28	10	1	10	10	2	48	13	2	3	4	1	4	5
S23	8	11	18	13	28	15	5	9	6	0	8	2	0	5	0	1	0	0
T-2	21	14	149	85	27	100	12	4	12	2	49	30	1	4	0	3	5	5
S25	9	11	11	95	37	15	3	25	11	1	11	6		2		6	3	7
S26	11	12	21	18	46	15	3	46	13	10	10	3		8		2	4	4
S27	8	11	33	22	30	17	3	6	3	11	13	0		35		2	3	0
S28	11	9	28	39	41	17	1	1	15	9	13	7		15		0	0	4
S29	15	23	29	15	26	26	0	11	14	8	14	27		32		0	0	0
S30	8	7	6	23	53	11	2	1	62	9	26	23		4		4	11	11
S31	9	6	11	14	71	13	10	26	17	11	11	3		20		0	0	0
S32	6	29	44	39	33	23	3	7	18	15	81	16		6		0	3	13
S33	1	172	148	13	69	23	39	4	27	21	19	2		28		3	8	2
S34	10	135	9	5	45	21	24	12	27	7	70	4		6		0	3	3
S35	6	900	28	32	48	21	27	20	60	8	35	57		5		3	0	3
T-3	3	37	84	17	87	130	3	9	14	9	42	52		0		1	1	4

YOKED STUDY
MORPHINE DELIVERED PER SESSION (mg)

	TRIPLET					
SESSION	1	2	3	4	5	6
S1	15	4	0	3	6	5
S2	5	4	2	8	8	3
S3	10	7	10	0	8	3
S4	10	8	9	1	6	6
S5	4	9	3	0	9	1
S6	8	9	4	3	7	6
S7	10	3	6	3	10	4
S8	5	8	10	14	7	3
S9	7	9	33	5	10	4
S10	4	0	10	6	7	6
S11	3	9	15	8	10	7
T-1	0	0	0	0	0	0
S12	5	5	7	8	19	6
S13	4	7	4	4	16	9
S14	2	6	7	3	7	7
S15	3	3	5	4	4	10
S16	3	5	13	7	11	9
S17	4	4	25	7	14	6
S18	9	8	70	8	6	8
S19	5	9	25	7	12	6
S20	4	8	10	12	23	9
S21	7	3	31	11	18	7
S22	6	7	8	8	20	11
T-2	0	0	0	0	0	0
S23	6	8	8	25	20	10
S24	8	5	10	13	19	10
S25	5	3	20	15	18	11
S26	7	8	15	28	21	13
S27	10	17	18	8	18	18
S28	6	6	4	15	28	11
S29	5	4	7	10	43	10
S30	4	14	27	14	24	15
S31	1	79	72	10	42	14
S32	8	53	12	3	30	11
S33	4	39	18	23	32	14
T-3	0	0	0	0	0	0

YOKED STUDY
WEEKLY FAILURES TO RIGHT

GROUP	WEEK 1	WEEK 2	WEEK 3	TOTAL
S-A	2	0	1	3
Y-M	7	0	3	10
Y-R	0	0	0	0

YOKED STUDY
PAW-LICK LATENCY (s)

GROUP	TRIPLET	LATENCY
S-A	3	9.9
S-A	4	13.5
S-A	5	10.6
S-A	6	11.3
Y-M	3	14.8
Y-M	4	13.4
Y-M	5	13.3
Y-M	6	16.9
Y-R	3	
Y-R	4	30
Y-R	5	30
Y-R	6	30

YOKED STUDY
TOTAL WITHDRAWAL SIGNS PER TEST

GROUP	TRIPLET	T-1	T-2	T-3	TOTAL
S-A	1	25	13	12	50
S-A	2	18	10	18	46
S-A	3	70	77	61	208
S-A	4	32	78	16	126
S-A	5	52	21	38	111
S-A	6	39	37	60	136
Y-M	1	13	2	5	20
Y-M	2	9	3	10	22
Y-M	3	15	31	14	60
Y-M	4	11	20	10	41
Y-M	5	23	19	25	67
Y-M	6	25	23	33	81
Y-R	1	5	1		6
Y-R	2	2	1	2	5
Y-R	3	4	7		11
Y-R	4	3	7	11	21
Y-R	5	6	14	5	25
Y-R	6	7	14	8	29

APPENDIX D: Data Tables (Signalled/Non-Signalled Study).

SIGNALLED / NON-SIGNALLED STUDY
DAILY WEIGHT (g)

DAY	S-M	S-M	S-M	S-M	S-M	S-M	S-M	N-M	N-M	N-M	N-M	N-M	N-M	N-M
D1	501	499	457	475	476	440	463	491	457	467	484	477	447	464
D2	503	494	450	473	474	424	454	499	457	464	487	473	441	467
D3	498	496	446	474	460	410	440	500	450	454	483	460	438	458
D4	486	485	430	463	454	428	437	489	441	451	473	448	427	458
D5	495	478	427	456	452	444	428	487	445	447	475	447	410	453
D6	492	469	433	452	453	444	418	486	442	449	472	459	416	455
T-1	483	459	423	446	442	424	413	467	439	436	464	448	399	441
D8	465	457	415	434	436	420	407	462	425	427	444	431	381	433
D9	479	460	407	440	445	424	416	451	429	436	456	438	386	434
D10	479	461	410	444	442	431	422	456	438	433	461	440	387	438
D11	483	453	413	436	452	428	431	461	435	443	463	448	384	445
D12	486	433	412	450	449	421	435	468	447	448	459	449	380	450
D13	490	400	395	449	447	439	435	475	448	447	466	455	377	453
T-2	485	399	375	447	442	426	429	466	441	429	454	449	370	437
D15	467	388	366	428	413	400	406	444	431	420	439	423	365	430
D16	482	386	361	446	435	412	426	458	420	438	458	446	388	447
D17	486	378	365	458	432	418	434	458	422	444	459	442	395	456
D18	491	375	335	445	431	423	432	471	416	437	464	455	402	463
D19	477	389	325	448	421	418	426	452	432	430	458	443	397	454
D20	484	399	337	445	418	415	424	454	433	435	454	445	396	451
T-3	481	420	353	436	419	412	423	448	434	430	459	449	400	453

SIGNALLED / NON-SIGNALLED STUDY
LEVER-PRESS RESPONSES PER SESSION

SESSION	S-M	S-M	S-M	S-M	S-M	S-M	S-M	N-M	N-M	N-M	N-M	N-M	N-M	N-M
S1	2	0	3	2	3	3	4	28	0	1	4	4	2	65
S2	0	0	17	2	1	9	0	1	0	1	1	1	0	5
S3	0	43	9	0	3	0	22	32	10	8	13	35	59	6
S4	10	8	50	6	2	2	5	5	2	8	13	12	75	10
S5	2	150	40	0	2	18	13	22	12	26	22	39	39	13
S6	0	0	13	1	1	0	1	11	4	22	42	2	19	9
S7	1	22	47	1	1	0	18	39	6	44	1	32	11	19
S8	0	2	4	0	0	0	5	0	2	0	3	7	0	0
S9	1	0	27	0	0	11	1	10	7	5	0	4	6	5
S10	0	1	26	0	6	0	6	3	0	19	18	20	55	15
S11	4	2	4	2	6	0	5	0	1	7	0	9	0	2
T-1	11	62	79	20	3	0	17	49	3	11	31	62	115	37
S13	11	5	10	2		14	0	5	5	9			6	3
S14	42	230	190	13	1	1	31	24	2	27	4	6	140	12
S15	1	23	125	53	0	2	10	25	6	52	3	6	25	30
S16	0	62	210	18	2	2	5	15	9	78	35	70	187	14
S17	2	101	84	62	4	0	0	4	8	103	7	16	162	2
S18	11	107	141	104	23	8	12	46	27	211	3	36	89	7
S19	9	42	227	117	32	3	5	64	8	71	26	19	169	40
S20	14	26	257	37	37	0	10	64	7	35	12	14	33	34
S21	25	3	268	45	19	4	39	19	21	46	44	50	88	20
S22	4	2	70	28	14	5	6	30	27	6	15	17	32	24
S23	21	1	57	23	25	6	8	38	1	27	15	19	36	26
T-2	8	47	109	0	9	0	22	27	1	19	41	41	135	15
S25	11	0	35	20	0	1	2	18	2	21	45	79	46	3
S26	8	6	8	12	4	6	1	18	15	3	81	11	19	18
S27	4	6	29	4	14	11	20	24	0	22	23	15	58	1
S28	3	9	5	1	7	0	4	8	2	9	13	22	17	5
S29	15	33	0	5	8	5	3	10	1	5	14	20	112	7
S30	14	1	3	4	2	3	6	53	6	4	13	10	99	21
S31	7	32	31	14	7	6	9	1	21	43	32	17	50	93
S32	20	4	3	4	2	3	3	18	1	2	61	21	19	10
S33	36	54	76	27	12	1	6	29	10	29	13	10	52	46
S34	56	42	236	1	36	8	3	31	9	18	121	35	225	66
S35	37	21	400	19	22	2	2	13	12	29	47	34	23	25
T-3	26	38	8	5	6	10	34	46	3	73	76	35	116	12

SIGNALLED / NON-SIGNALLED STUDY
INFUSIONS & MORPHINE (mg) DELIVERED PER SESSION

SESSION	INFUSIONS	MORPHINE
S1	4	3
S2	9	7
S3	0	0
S4	1	1
S5	0	0
S6	4	3
S7	2	2
S8	19	16
S9	7	6
S10	7	6
S11	11	10
T-1	21	0
S13	10	8
S14	7	6
S15	3	2
S16	6	5
S17	8	6.5
S18	8	6.5
S19	9	7
S20	8	6.5
S21	15	13
S22	15	12
S23	12	10
T-2	43	0
S25	35	30
S26	16	14
S27	21	18
S28	36	29
S29	12	10
S30	20	16
S31	13	10.5
S32	24	20
S33	13	10.5
S34	5	3.5
S35	31	26
T-3	8	0

SIGNALLED / NON-SIGNALLED STUDY
TILTOMETER: ANGLE OF SLIPPAGE (degrees)

SESSION	S-M	S-M	S-M	S-M	S-M	S-M	S-M	N-M	N-M	N-M	N-M	N-M	N-M	N-M
S1	37	39	35	45	40	39	35	40	41	40	36	33	32	46
S2	33	41	33	36	32	25	35	37	29	38	30	29	28	39
S3	45	43	38	42	41	40	39	41	38	45	40	37	33	35
S4	48	31	38	44	40	35	39	48	41	43	39	43	36	39
S5	45	32	45	46	37	36	41	49	42	40	40	41	32	41
S6	45	42	39	45	46	33	46	48	38	46	37	36	39	43
S7	45	45	48	52	40	40	42	49	45	44	44	42	39	40
S8	27	22	27	30	40	26	36	34	27	36	30	37	30	30
S9	35	42	36	41	37	30	40	42	31	35	36	36	34	33
S10	38	34	38	43	41	22	44	48	33	44	31	38	29	43
S11	37	32	38	39	37	25	41	50	31	36	29	34	21	33
T-1	45	37	35	45	45	31	35	49	35	41	33	36	36	39
S13	29	39	32	34	43	24	45	45	28	36	26	37	27	30
S14	49	28	32	41	40	26	41	41	33	35	30	40	30	34
S15	49	36	35	40	40	33	45	38	32	35	30	33	35	45
S16	38	44	31	40	39	29	42	48	30	37	36	39	37	39
S17	37	32	35	40	47	29	46	46	35	33	33	42	32	33
S18	33	35	31	47	42	33	51	51	35	37	32	36	33	39
S19	43	39	36	46	38	38	46	41	33	46	36	41	31	37
S20	40	35	36	39	48	29	53	48	32	32	44	41	35	36
S21	35	36	29	39	39	28	43	39	30	35	38	37	37	36
S22	32	30	33	42	43	26	49	39	35	34	33	36	32	36
S23	34	31	30	44	40	34	48	38	32	33	32	43	33	42
T-2	46	43	36	50	44	35	42	52	46	45	49	41	36	51
S25	28	28	28	37	36	25	34	35	27	31	31	34	29	39
S26	45	35	32	44	49	32	52	42	36	38	33	42	34	41
S27	31	32	30	39	38	32	34	42	28	32	40	35	36	34
S28	28	30	26	38	39	33	40	33	24	31	30	22	33	30
S29	39	28	29	36	42	30	41	38	32	30	30	30	33	43
S30	32	28	30	35	53	29	41	42	35	34	30	33	36	38
S31	31	28	27	40	37	28	46	37	31	42	36	35	29	36
S32	31	36	28	40	40	26	36	40	35	36	29	39	33	38
S33	33	30	33	41	40	25	45	48	31	35	28	47	29	40
S34	55	44	33	48	52	29	51	48	37	45	37	43	32	41
S35	32	32	30	36	39	26	32	45	33	35	35	43	33	42
T-3	55	36	44	47	52	32	36	55	38	46	40	44	30	39

SIGNALLED / NON-SIGNALLED STUDY
FAILURES TO RIGHT PER WEEK

GROUP	WEEK 1	WEEK 2	WEEK 3	TOTAL
S-M	4	1	9	14
N-M	11	1	10	22

SIGNALLED / NON-SIGNALLED STUDY
PAW-LICK LATENCY (s)

GROUP	LATENCY
S-M	30
S-M	21
S-M	24.5
S-M	13.2
S-M	30
S-M	20.1
S-M	11
N-M	12.1
N-M	22.3
N-M	20.8
N-M	16.8
N-M	30
N-M	14.8
N-M	16.6

SIGNALLED / NON-SIGNALLED STUDY
TOTAL WITHDRAWAL SIGNS PER TEST

GROUP	T-1	T-2	T-3	TOTAL
S-M	22	19	15	56
S-M	31	38	16	85
S-M	28	29	24	81
S-M	27	36	20	83
S-M	36	41	25	102
S-M	23	24	12	59
S-M	23	25	21	69
N-M	30	20	33	83
N-M	28	19	12	59
N-M	17	16	18	51
N-M	23	43	10	76
N-M	36	18	21	75
N-M	20	33	24	77
N-M	16	20	11	47

APPENDIX E: Data Tables ((S-A)-TO-Y Study).

(S-A)-TO-Y STUDY
DAILY WEIGHT (g)

	GROUP	S-A							(S-A)-TO-Y							
DAY	PAIR	1	2	3	4	5	6	X	1	2	3	4	5	6	X1	X2
D1		453	400	371	392	524	520	578	419	404	438	421	546	532	552	522
D2		445	408	365	382	510	502	569	409	405	442	409	548	514	548	509
D3		443	411	348	371	495	485	561	398	381	439	402	537	496	539	497
D4		432	405	341	368	483	471	560	396	379	430	405	537	486	535	482
D5		423	402	338	360	476	460	549	377	370	430	389	522	475	522	467
D6		407	389	338	360	468	451	546	375	374	425	379	512	460	517	459
T-1		409	402	336	363	459	445	515	372	376	428	381	505	445	508	455
D8		399	389	333	368	457	449	483	361	363	436	376	498	430	499	454
D9		402	384	327	369	462	454	481	377	379	430	384	506	426	493	457
D10		406	393	339	373	448	459	486	373	385	427	392	503	421	494	457
D11		402	399	349	374	452	458	495	375	386	427	403	499	413	480	471
D12		403	405	348	363	455	454	484	371	385	425	400	501	400	488	476
D13		404	406	341	370	440	461	457	365	385	420	409	510	387	475	457
T-2		383	396	347	365	432	457	477	373	381	413	409	516	380	476	469
D15		389	390	334	369	432	457	477	376	387	404	380	516	380	476	469
D16		401	411	347	385	435	460	462	388	393	414	394	529	400	453	441
D17		405	417	357	392	454	465	488	388	401	420	416	527	421	450	433
D18		411	420	364	385	464	470	479	382	396	423	420	527	430	445	447
D19		416	422	367	389	435	470	488	383	402	424	425	543	439	459	460
T-3		399	403	352	382	420	464	427	378	397	407	417	543	425	437	417

(S-A)-TO-Y STUDY
LEVER-PRESS RESPONSES PER SESSION

GROUP	S-A							(S-A)-TO-Y							
SESSION PAIR	1	2	3	4	5	6	X	1	2	3	4	5	6	X	X
S1	7	2	0	3	0	0	24	5	1	1	1	0	6	2	7
S2	1	3	1	0	2	22	24	24	1	0	2	0	0	4	42
S3	2	9	3	0	3	75	65	15	4	0	5	5	2	5	18
S4	11	10	1	0	3	9	161	13	8	3	7	0	1	9	20
S5	9	11	3	2	2	0	332	11	12	0	11	7	0	9	39
S6	10	6	2	1	3	20	29	7	17	0	4	4	10	5	13
S7	8	11	3	5	4	5	370	15	10	0	5	1	5	10	5
S8	13	6	3	0	5	8	639	16	12	2	9	1	12	12	25
S9	16	11	4	7	5	0	647	19	13	3	5	46	10	10	5
S10	19	10	6	8	10	7	135	5	10	1	7	0	8	11	15
S11	28	28	5	12	11	0	69	13	8	2	8	18	11	4	20
T-1	21	54	24	85	58	2	64	1	69	1	40	1	77	24	22
S13	27	11	6	3	47	8	30	0	9	2	32	8	4	21	74
S14	13	8	6	9	37	7	43	22	49	6	3	8	1	2	6
S15	16	9	17	14	3	0	28	10	16	5	0	15	9	0	1
S16	37	13	11	6	21	1	75	35	40	18	1	13	42	9	6
S17	18	7	20	15	32	2	350	13	77	1	0	1	37	0	1
S18	23	14	18	15	36	7	1566	79	50	1	4	4	24	2	4
S19	21	13	19	8	172	2	561	45	20	1	6	5	14	6	9
S20	16	12	13	17	31	10	12	10	35	25	1	3	65	4	45
S21	22	9	12	14	15	0	93	0	14	10	7	1	71	13	5
S22	19	13	15	23	62	10	168	14	16	25	1	0	65	9	60
S23	16	9	13	31	2	5	220	10	12	15	1	0	65	2	25
T-2	37	54	73	26	81	51	101	19	108	45	22	1	18	37	55
S25	13	13	8	13	20	15	119	24	8	9	3	2	5	30	34
S26	6	8	7	8	21	11	35	18	7	3	7	13	11	19	15
S27	11	14	9	10	62	0	169	60	12	64	5	4	16	17	28
S28	7	15	9	8	384	12	126	18	14	18	6	9	18	22	28
S29	13	16	17	8	231	13	105	19	6	34	11	9	83	16	46
S30	17	15	13	14	32	19	239	23	13	22	7	3	48	20	36
S31	21	21	9	13	40	17	180	29	2	39	6	2	43	30	##
S32	19	18	22	19	56	40	86	38	11	17	18	27	89	23	30

(S-A)-TO-Y STUDY
MORPHINE DELIVERED PER SESSION (mg)

GROUP	S-A							(S-A)-TO-Y							
SESSION PAIR	1	2	3	4	5	6	X	1	2	3	4	5	6	X	X
S1	5	2	0	2	0	0	7	4	1	1	1	0	2	2	7
S2	1	2	1	0	3	12	17	17	1	0	2	0	0	4	20
S3	1	7	3	0	2	18	35	11	3	0	4	4	1	3	6
S4	9	8	0	0	2	5	80	9	8	2	4	0	1	8	9
S5	5	7	3	2	2	0	133	7	9	0	9	6	0	4	11
S6	7	5	2	1	3	9	19	5	9	0	3	2	7	5	8
S7	7	8	2	3	2	3	141	10	8	0	4	1	3	7	4
S8	9	5	3	0	4	6	191	11	9	1	7	1	8	10	17
S9	9	7	2	6	4	0	294	6	10	2	2	12	6	7	3
S10	14	8	5	7	8	3	77	3	7	1	6	0	6	6	11
S11	18	22	3	8	6	0	59	8	6	1	7	14	9	4	17
T-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S13	15	6	5	1	3	5	15	15	6	5	1	3	5	15	15
S14	9	6	4	7	5	2	28	9	6	4	7	5	2	28	28
S15	12	7	12	7	5	0	16	12	7	12	7	5	0	16	16
S16	21	9	7	5	12	1	39	21	9	7	5	12	1	39	39
S17	9	7	10	6	18	2	133	9	7	10	6	18	2	133	133
S18	14	10	7	7	29	4	140	14	10	7	7	29	4	140	140
S19	13	7	13	5	79	2	209	13	7	13	5	79	2	209	209
S20	11	8	6	9	22	9	11	11	8	6	9	22	9	11	11
S21	11	9	7	10	11	0	39	11	9	7	10	11	0	39	39
S22	11	8	9	11	33	8	83	11	8	9	11	33	8	83	83
S23	11	7	9	9	2	5	90	11	7	9	9	2	5	90	90
T-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S25	9	11	6	9	14	9	50	18	5	7	2	1	4	12	21
S26	6	7	5	6	14	7	21	11	7	2	6	10	8	13	11
S27	9	9	7	8	29	0	57	16	8	8	5	2	10	10	19
S28	5	11	5	6	167	8	52	13	8	5	4	4	12	11	19
S29	10	12	10	6	111	9	45	12	4	23	6	5	52	11	30
S30	12	10	9	9	21	10	100	15	7	14	5	2	32	13	23
S31	14	16	7	9	27	15	60	19	2	23	4	1	27	18	35
S32	11	10	11	13	36	27	46	22	6	6	8	3	52	17	21
T-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

(S-A)-TO-Y STUDY
FAILURES TO RIGHT PER SESSION
{1=FAILURE TO RIGHT}

GROUP	S-A							(S-A)-TO-Y							
SESSION PAIR	1	2	3	4	5	6	X	1	2	3	4	5	6	X	X
S1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
S2	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
S3	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0
S4	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
S5	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
S6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S9	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
S10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S11	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
T-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S13	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
S14	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S15	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
S16	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S17	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
S18	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S19	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
S20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S21	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S22	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
S23	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
T-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S25	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
S26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S28	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
S29	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0
S30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S31	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
S32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T-3	0	0	0	0	0	0	0			0	0	0	0	0	0

(S-A)-TO-Y STUDY
TILTOMETER: ANGLE OF SLIPPAGE (degrees)

GROUP	S-A							(S-A)-TO-Y							
SESSION PAIR	1	2	3	4	5	6	X	1	2	3	4	5	6	X	X
S1	31	41	40	46	40	47	33	42	38	42	38	46	36	43	46
S2	30	39	37	43	36	35	31	36	46	43	33	39	41	38	50
S3	35	36	43	46	39	28	30	36	38	42	37	45	38	38	34
S4	31	33	44	44	35	42	31	42	32	43	32	46	45	34	33
S5	36	42	48	38	46	41	36	42	29	46	32	32	45	42	34
S6	32	40	50	37	41	33	34	40	35	41	36	38	41	41	38
S7	36	40	48	37	46	35	38	38	30	38	32	46	42	35	39
S8	32	35	40	40	35	35	42	37	34	40	33	39	42	35	33
S9	35	33	38	38	38	36	38	38	34	30	35	27	39	36	42
S10	24	39	42	28	33	36	45	41	30	40	28	36	38	34	36
S11	20	24	38	32	33	35	42	32	25	39	25	30	37	36	38
T-1	33	43	42	43	34	37	34	44	40	43	42	38	38	41	38
S13	25	28	41	38	33	38	30	32	24	38	35	36	38	30	30
S14	40	34	50	34	30	38	33	31	35	35	33	41	33	30	33
S15	33	37	42	47	31	37	31	38	31	24	32	40	40	29	29
S16	36	37	40	40	35	39	31	39	32	34	39	37	36	26	27
S17	39	35	41	36	32	38	28	42	31	28	35	46	37	26	26
S18	40	35	44	41	34	42	35	36	35	30	35	38	37	37	46
S19	39	43	40	41	33	41	28	35	31	32	46	42	37	30	26
S20	38	38	41	39	31	39	34	47	31	40	37	46	30	34	33
S21	38	45	43	39	30	47	34	35	33	39	31	50	39	35	38
S22	40	36	46	41	33	38	35	41	35	31	39	46	32	24	39
S23	40	46	44	37	32	39	35	35	33	33	45	37	31	29	38
T-2	39	49	43	46	31	42	37	46	45	39	41	37	37	42	41
S25	32	39	44	42	36	41	35	34	38	38	42	41	38	36	37
S26	43	42	40	45	39	42	42	42	43	41	42	41	42	38	46
S27	38	45	48	42	32	46	35	35	39	43	43	35	38	33	38
S28	42	42	42	42	28	42	33	40	45	39	43	45	32	34	41
S29	45	45	46	43	31	37	34	39	53	33	39	42	26	27	39
S30	34	43	39	41	39	39	41	39	40	36	38	37	38	32	41
S31	38	40	41	40	31	37	38	40	49	39	35	44	33	37	26
S32	40	39	39	43	39	37	40	37	48	40	39	33	31	36	37
T-3	49	45	43	54	37	35	27	42	55	36	43	49	38	41	30

(S-A)-TO-Y STUDY
TOTAL WITHDRAWAL SIGNS PER TEST

GROUP	PAIR	T-1	T-2	T-3	TOTAL
S-A	1	13	29	25	67
S-A	2	10	30	24	64
S-A	3	19	31	25	75
S-A	4	13	25	30	68
S-A	5	21	45	34	100
S-A	6	11	32	13	56
S-A	X	26	26	38	90
(S-A)-TO-Y	1	12	9	19	40
(S-A)-TO-Y	2	21	11	19	51
(S-A)-TO-Y	3	9	20	12	41
(S-A)-TO-Y	4	14	13	16	43
(S-A)-TO-Y	5	28	15	7	50
(S-A)-TO-Y	6	19	24	19	62
(S-A)-TO-Y	X	37	15	22	74
(S-A)-TO-Y	X	29	21	24	74

APPENDIX F: Data Tables (Quad Study).

QUAD STUDY
DAILY WEIGHT (g)

DAY	GROUP QUAD	S-A 1	S-A 2	Y-M 1	Y-M 2	Y-M-D 1	Y-M-D 2	Y-R 1	Y-R 2
D1		530	602	518	603	532	576	508	609
D2		527	587	511	591	531	568	506	603
D3		524	578	505	579	530	550	509	597
D4		519	568	497	564	523	537	503	602
D5		512	560	494	545	520	529	500	598
D6		507	548	489	544	510	518	505	601
D7		507	539	481	537	510	519	509	595
D8		499	529	473	528	497	507	499	588
D9		489	534	468	528	483	501	500	590
D10		480	529	457	510	490	506	501	591
D11		485	531	466	505	493	508	510	593
D12		487	520	468	495	504	498	504	586
D13		479	524	449	473	486	500	505	580
D14		479	520	461	474	484	489	501	592
D15		473	492	454	483	486	479	510	583
D16		472	512	458	489	487	499	514	586
D17		472	487	434	468	476	463	508	574
D18		473	498	445	478	493	484	510	575
D19		479	489	446	456	491	481	506	565
D20		473	490	447	444	492	483	505	558
D21		474	503	449	444	495	484	505	565
D22		479	489	441	441	486	468	507	542
D23		470	513	439	462	495	482	504	543
D24		466	475	441	464	492	488	505	529
D25		469	471	444	470	485	470	501	523

QUAD STUDY
LEVER-PRESS RESPONSES PER DAY

DAY	GROUP QUAD	S-A 1	S-A 2	Y-M 1	Y-M 2	Y-M-D 1	Y-M-D 2	Y-R 1	Y-R 2
D1		8	38	0	0	0	0	0	0
D2		9	40	0	0	0	0	0	0
D3		12	9	0	0	0	0	0	0
D4		20	23	0	0	0	0	0	0
D5		24	39	0	0	0	0	0	0
D6		35	27	0	0	0	0	0	0
D7		21	37	0	0	0	0	0	0
D8		21	76	0	0	0	0	0	0
D9		17	23	0	0	0	0	0	0
D10		29	30	0	0	0	0	0	0
D11		44	30	0	0	0	0	0	0
D12		24	141	0	0	0	0	0	0
D13		23	19	0	0	0	0	0	0
D14		36	38	0	0	0	0	0	0
D15		23	175	0	0	0	0	0	0
D16		0	0	9	231	1	8	0	9
D17		0	0	24	295	17	53	2	5
D18		0	0	20	41	5	24	2	9
D19		0	0	21	42	19	33	3	14
D20		0	0	71	33	11	27	0	11
D21		0	0	16	44	1	2	3	14
D22		0	0	17	111	32	155	3	17
D23		0	0	17	93	12	70	3	11
D24		0	0	31	625	10	4	8	13
D25		0	0	0	421	36	42	7	14

QUAD STUDY
MORPHINE DELIVERED PER DAY (mg)

	GROUP	S-A	S-A	Y-M	Y-M	Y-M-D	Y-M-D	Y-R	Y-R
DAY	QUAD	1	2	1	2	1	2	1	2
D1		6	13	6	13	6	13	0	0
D2		8	15	8	15	8	15	0	0
D3		8	5	8	5	8	5	0	0
D4		7	16	7	16	7	16	0	0
D5		17	24	17	24	17	24	0	0
D6		22	17	22	17	22	17	0	0
D7		14	24	14	24	14	24	0	0
D8		14	24	14	24	14	24	0	0
D9		12	16	12	16	12	16	0	0
D10		19	19	19	19	19	19	0	0
D11		22	21	22	21	22	21	0	0
D12		16	95	16	95	16	95	0	0
D13		14	16	14	16	14	16	0	0
D14		19	23	19	23	19	23	0	0
D15		18	99	18	99	18	99	0	0
D16		22	21	7	102	0	7	0	4
D17		16	95	11	116	15	36	1	6
D18		14	16	10	29	4	16	3	5
D19		19	23	13	27	12	28	1	8
D20		18	99	44	100	8	16	0	10
D21		22	21	10	21	1	1	2	9
D22		16	95	9	52	17	44	2	10
D23		14	16	10	64	11	42	1	9
D24		19	23	19	231	6	4	4	9
D25		18	99	0	192	11	3	3	8

QUAD STUDY
FAILURES TO RIGHT PER DAY
{1=FAILURE TO RIGHT}

DAY	GROUP QUAD	S-A 1	S-A 2	Y-M 1	Y-M 2	Y-M-D 1	Y-M-D 2	Y-R 1	Y-R 2
D1		0	1	0	1	0	1	0	0
D2		0	1	0	1	0	1	0	0
D3		0	0	0	0	0	1	0	0
D4		0	0	0	0	0	0	0	0
D5		0	0	0	0	0	1	0	0
D6		0	0	1	0	1	0	0	0
D7		0	0	0	0	0	0	0	0
D8		0	0	0	0	0	0	0	0
D9		0	0	0	0	0	0	0	0
D10		0	0	0	0	0	0	0	0
D11		0	0	0	0	0	0	0	0
D12		0	0	0	1	0	1	0	0
D13		0	0	0	0	0	0	0	0
D14		0	0	0	0	0	0	0	0
D15		0	0	0	1	0	1	0	0
D16		1	0	0	0	0	0	0	0
D17		0	0	0	0	0	0	0	0
D18		0	0	0	0	0	0	0	0
D19		0	0	0	0	0	0	0	0
D20		0	0	0	0	0	0	0	0
D21		0	0	0	0	0	0	0	0
D22		0	0	0	0	1	0	0	0
D23		0	0	0	0	0	0	0	0
D24		0	0	0	0	0	0	0	0
D25		0	0	0	0	0	0	0	0

QUAD STUDY
TILTOMETER: ANGLE OF SLIPPAGE (degrees)

DAY	GROUP QUAD	S-A 1	S-A 2	Y-M 1	Y-M 2	Y-M-D 1	Y-M-D 2	Y-R 1	Y-R 2
D1		28	29	33	26	36	25	42	32
D2		33	32	39	32	41	26	50	36
D3		35	32	33	31	41	25	52	41
D4		36	37	37	34	35	32	49	42
D5		38	30	32	28	30	26	46	40
D6		27	29	25	29	27	29	47	42
D7		29	30	24	30	31	31	49	43
D8		32	25	35	25	32	38	48	43
D9		32	32	33	29	38	26	48	43
D10		32	35	30	27	28	29	40	44
D11		29	30	29	23	26	24	47	43
D12		28	25	37	25	32	23	51	40
D13		33	29	38	28	35	32	55	40
D14		35	29	42	26	30	29	56	43
D15		32	23	41	18	30	22	54	42
D16		36	33	52	27	42	38	51	32
D17		28	33	28	26	27	33	44	27
D18		27	35	39	29	35	37	43	31
D19		32	33	48	27	30	29	46	31
D20		38	38	51	33	33	41	51	32
D21		30	31	47	32	38	40	43	29
D22		36	34	43	27	29	30	49	36
D23		29	25	45	32	31	26	51	36
D24		32	32	46	22	33	41	52	34
D25		28	38	46	26	38	42	42	36

QUAD STUDY
TOTAL WITHDRAWAL SIGNS PER TEST

GROUP / TEST	QUAD	S-A 1	S-A 2	Y-M 1	Y-M 2	Y-M-D 1	Y-M-D 2	Y-R 1	Y-R 2
T-1		17	19	7.5	15.5	5	5	4.5	5
T-2		34.5	34	9.5	22.5	18	14.5	7	3.5
T-3		30	21	11	9.5	15.5	13	6	4
T-4		32.5	11	17.5	39.5	16	29	18.5	14
T-5		28.5	8.5	15	9	10	15	6	13.5