The Discriminative Stimulus Properties of Morphine and U-50,488H in a Three-Key Assay: A MU and Kappa Opioid Discrimination in the Pigeon

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THE DISCRIMINATIVE STIMULUS PROPERTIES OF MORPHINE AND U-50,488H IN A THREE-KEY ASSAY: A MU AND KAPPA OPIOID DISCRIMINATION IN THE PIGEON

by

Malath Makhay

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Submitted to the
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Western Michigan University
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Opiate drugs have been classified in two-choice assays according to their ability to produce generalization in animals to the prototypic μ opiate, morphine, versus vehicle, or to the κ opioid, U-50,488H versus vehicle injections (Picker & Dykstra, 1987). A three-choice discrimination procedure, in which subjects discriminate among morphine, U-50,488H, and vehicle injections, might afford a greater degree of precision in characterizing the subjective effects of opioids. The feasibility of such a procedure was demonstrated in the present study, in which five pigeons were trained to discriminate among injections of 5.6 mg/kg morphine, 5.6 mg/kg U-50,488H, and saline. Reliable discrimination was attained by reinforcing injection-appropriate key-pecks under a schedule that required 20 consecutive responses on the injection-appropriate key. Orderly dose-response relations were obtained when doses of morphine and U-50,488H from 0.10 to 32.0 mg/kg were substituted for the training doses. Regardless of substitution dose, the subjects almost never responded on the U-50,488H-appropriate key when morphine was administered, or on the morphine-appropriate key when they received U-50,488H.
Pigeons were tested with various doses of naltrexone (0.01 to 1.0 mg/kg) in combination with morphine and U-50,488H in doses ranging from 5.6 to 56 mg/kg. High doses of naltrexone completely blocked the morphine stimulus cue, but failed completely to block the U-50,488H cue. d-Amphetamine primarily engendered saline-appropriate, not morphine- or U-50,488H-appropriate, responding.

Ethylketazocine produced mixed results in that moderate doses produced responding on both the morphine- and U-50,488H-appropriate keys. However, 3.2 mg/kg ethylketazocine completely substituted for the morphine cue.

In conclusion, pigeons can be trained to discriminate directly between \( \mu \) and \( \kappa \) opioids. Ethylketazocine, a compound that may be selective for both receptor subtypes, engendered both morphine- and U-50,488H-appropriate responding. Further studies are warranted in other species and with other opioid compounds to evaluate the discriminative stimulus properties of opioids.
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CHAPTER I

INTRODUCTION

For over three decades, behavioral pharmacologists have emphasized that drugs can affect behavior by acting as unconditional, conditional, reinforcing, punishing, and discriminative stimuli (e.g., Thompson & Schuster, 1968; Thompson, Pickens, & Meisch, 1971). All of these stimulus functions are interesting and potentially important for explaining how drugs affect behavior in humans and other animals. Consequently, considerable research interest has been directed towards exploring each of them. Physiologists, for example, characteristically examine drugs as unconditional stimuli, and substance abuse researchers explore drugs as positively reinforcing stimuli.

A large number of studies have explored the capacity of drugs to serve as discriminative stimuli. Research in this area has helped to explain the multiple and seemingly paradoxical effects of drugs in humans and has provided an avenue for examining relations between drugs' neuropharmacological actions and their behavioral effects (Poling, 1986). In the present study, a drug discrimination assay was used to examine the extent to which two opioid drugs with known differences in neuropharmacological actions, morphine and the experimental drug U50,488H, produce similar subjective effects. Prior studies using similar, but less complex and
apparently less sensitive assays, have found that research subjects can detect the presence versus the absence of both of these drugs, but no one has demonstrated that the same subjects can be trained to respond differentially in the presence of morphine, U50,488H, and drug vehicle. The present study used a three-response drug discrimination assay to demonstrate that pigeons can be trained to do so.

Drugs as Discriminative Stimuli

The term, "discriminative stimulus," has been defined in several different ways. Most definitions emphasize that (a) some kind of responding occurs more often in the presence of the discriminative stimulus than in its absence and, (b) because, historically, that kind of responding has been more successful (in producing reinforcement) in the presence of the discriminative stimulus than in its absence. A clear definition with these features is provided by Michael (1982), who defines the discriminative stimulus as follows:

It is a stimulus change which, (1) given the momentary effectiveness of some particular type of reinforcement (2) increases the frequency of a particular type of response (3) because that stimulus change has been correlated with an increase in the frequency with which that type of response has been followed by that type of reinforcement. (p.149)

To be established as a discriminative stimulus, a drug must produce detectable subjective effects (i.e., sensory consequences) and appropriate conditions of differential reinforcement must be arranged in the presence and absence of drug.
research in this area has yielded a wealth of information concerning the sensory consequences of drugs and the biochemical mechanisms that mediate these consequences (e.g., Ho, Richards, & Chute, 1978; Lal, 1977; Schuster & Balster, 1977, Seiden & Dykstra, 1977; Weissman, 1977; Kallman & Rosecrans, 1978; Colpaert, 1977, 1978, 1987; Lal, Glanutsos & Micksic, 1977; Lal, 1977). The typical procedure that is used is a two-choice discrimination in which a drug versus drug vehicle discrimination is trained. Another two-choice assay that has been employed is the drug\textsubscript{1} vs. drug\textsubscript{2} procedure. Here, one kind of responding (e.g., pecking the left key) is reinforced in the presence of drug\textsubscript{1} and a second kind of responding (e.g., pecking the right key) is reinforced in the presence of drug\textsubscript{2}.

As an alternative to two-choice procedures, three-choice assays employing a drug\textsubscript{1} vs. drug\textsubscript{2} vs. vehicle, drug-dose\textsubscript{1} vs. drug-dose\textsubscript{2} vs. vehicle, or drug\textsubscript{1} vs. drug\textsubscript{2} vs. drug\textsubscript{3} discrimination, may be preferred to afford a greater degree of precision in evaluating differences between drugs of different classes, or doses of the same drug. Three-choice discriminations are increasing in number, despite the rather extensive training that they require.

Species of subjects that have been used in drug discrimination assays include the rat, pigeon, monkey, dog, cat, gerbil, mouse, and human. No significant general difference between species has emerged (Lal, 1977). However, in some narcotic discrimination studies, differences between the pigeon, rat, and monkey have been observed (Picker & Dykstra, 1987). It seems that compounds such
as ethylketazocine act as \( \kappa \) agonists in monkeys and rats, but as \( \mu \) agonists in pigeons. Further studies are warranted to evaluate these results.

Drugs that are established as discriminative stimuli appear to operate similarly to other such stimuli (e.g., lights, tones, sounds). Generalization is observed when various doses of the same drug are administered and also with drugs of similar pharmacological specificity. Moreover, Overton (1964) and Harris and Balster (1971) have shown that drug stimuli are just as effective as shock stimuli or as visual stimuli in controlling behavior as discriminative stimuli.

One may surmise that drug discriminations would be learned more slowly than other discriminations since specialized receptors, sensory pathways, and brain mechanisms have evolved to mediate the perception of sensory stimuli, whereas correlative structures in the brain to detect drug effects are not known to exist. However, this does not seem to be the case. The speed of acquisition of drug discrimination is not obviously different than the speed of acquisition of stimulus-controlled responding with other kinds of stimuli (Overton, 1987).

Most drugs that are psychoactive are discriminable and virtually all abused drugs exhibit discriminative control. In all drug discrimination studies, it is important to recognize the need to separate discriminative control by drugs from control by reinforcers (e.g., food, water) used to maintain responding. To achieve this, the animal's responding is observed in periods of extinction (D'Mello & Stolerman, 1978). In the two-choice operant task, it has been suggested that the
operandum initially selected provides a better indication of drug-induced stimulus control than that obtained by comparing overall responses on the two bars. The latter may be confounded by a tendency to "probe" (p.243) for the correct operandum as the extinction schedule takes effect. D’Mello and Stolerman conducted three studies to evaluate whether this phenomenon was actually true. Overall, they found a high correlation between the bar initially selected and percentage responding on that bar. They concluded that the problem of probing may be less serious than previously assumed and they did not see any advantage of using only the initially selected bar as a criterion for drug-induced stimulus control. Drugs that are virtually indiscriminable include lithium, nicotinic blockers, and salicylates (Overton, 1987).

No unique analyses are required to explain how drugs control responding as discriminative stimuli. They do so by virtue of a unique history of differential reinforcement. It is that history alone that gives a drug the capacity to control a particular kind of behavior as a discriminative stimulus. Drugs acquire their discriminative stimulus properties through differential reinforcement, and maintain these properties only so long as differential reinforcement continues.

Historical Overview

In one of first studies of drug discrimination, Culler, Coakley, Shurrager, and Ades (1939) demonstrated that differential responding could be obtained under
curare and non-drug conditions. In 1951, Conger reported that ethanol could acquire discriminative stimulus control by demonstrating that rats could learn to run down a telescope-alley when given the drug and to withhold the same response when given vehicle. He postulated that ethanol produced sensory stimuli that provided the basis for the rats’ discriminated responding. In 1961, Overton conducted the first drug generalization tests. After drug vs. drug vehicle training was conducted, other drugs were substituted for the training stimuli. Not surprisingly, drugs that were reported previously as being similar to the training drug produced drug-appropriate responding.

In many early studies, maze-running systems (e.g., T-maze) were commonly used to establish differential response patterns in nonhuman subjects (Overton, 1964). A typical trial consisted of dropping the rat into the start box of the maze with electric shock already activated and allowing the rat to run freely in the maze until it reached the correct goal box. Reaching the unshocked grid floor of the correct goal box served as negative reinforcement. Usually, 10 trials were conducted each day in a single 10-min training session. The rat was typically trained to discriminate between two drug states. On successive days the imposed drug state alternated, as did the required choice. The T-maze was preferred because it was considered to be a simple task, and the rats were able to learn to run to the left (or right) goal box in a few trials. The rats were on a continuous reinforcement schedule in which each run through the maze was reinforced if the
rat reached the correct goal box.

This procedure eventually was modified so that responding was maintained by food presentation rather than escape from shock. Kubena and Barry (1969) employed a procedure in which rats were trained to press a lever that was followed either by food or shock, depending on whether the animal received drug or saline. For example, in one group of animals responding was followed by food in the presence of drug and by shock in the presence of saline. Following training with this procedure, the rats in this group generally responded on the lever on days on which they received drug, and withheld responding on days on which they received saline. Kubena and Barry (1969) also utilized a two-choice assay in which two levers were present in the box and lever pressing on only one of the two levers was followed by food. For example, right-lever responses were followed by food only when the animal had received drug. Left-lever responses were followed by food only when the animal received saline. In some sessions, no food was delivered during the first 5 min of each session to test for antecedent stimulus control.

Since the genesis of drug discrimination studies, modifications have been made that have fine-tuned the procedure. In general, the modern method of drug discrimination consists of the following experimental conditions: first, the animals are trained to discriminate a training drug at a given dose from the drug vehicle; second, the two responses are operant in nature; third, the two discriminated responses have a similar topography; fourth, the responses are mutually exclusive;
fifth, reinforcement for the two operant responses is the same; finally, the schedule of reinforcement is symmetrical (Colpaert, 1987). Procedures with these features should produce data that are not confounded by irrelevant training or drug effects, and provide a sensitive assay of the subjective effects (often termed “cue properties”) of drugs (Colpaert, 1977).

Results obtained under drug discrimination procedures are affected by pharmacological, procedural, and subject variables. Pharmacological variables include the training drug, the training dose, and the route and time of drug administration prior to training sessions. Kinetic properties of the drug (pharmacokinetics) determine the route and time of drug administration. When possible, the training dose is chosen to be within a clinically relevant and behavioral range. Quantitative differences between doses of the training drug have been found in terms of intensity of the set point for discriminability. For example, in studies in which a high dose of the drug is trained, the dose-response curve in generalization tests (described later) shifts to the right of the dose-response curve similarly obtained in animals trained on a lower dose. The dose-response curves are parallel and the ED$_{50}$ varies directly with the training doses (Lai, 1977).

The dependent variables that are typically measured in drug discrimination are overall response rate, response latency, and the percentage of responses on the treatment-appropriate lever (overall and prior to the first reinforcer).

Drugs established as discriminative stimuli include anesthetics, sedative-
hypnotics, anxiolytics, narcotic analgesics, muscarinic and nicotinic cholinergic agonists, cholinergic antagonists, dopamine-receptor agonists, amphetamines, psychotomimetics, marijuana constituents, antidepressants, and less readily, the neuroleptics (Lai, 1977; Overton, 1987; Colpaert, 1977, 1987; Seiden & Dykstra, 1977; Kamien, Bickel, Hughes, Higgins, & Smith, 1993).

After the pharmacological parameters have been established, the main features of the procedure are determined. The typical operant procedure that is employed by researchers in drug discrimination is the two-choice lever-press response assay with food, water, or escape from electric shock as reinforcement. This procedure appears to be more sensitive and more widely used than maze tasks, where locomotor responses are reinforced by the escape from or avoidance of electric shock or immersion in water. Several different schedules of reinforcement, such as fixed-ratio (FR), variable-interval (VI), fixed-interval (FI), variable-ratio (VR), differential-reinforcement-of-low-rate (DRL), random-interval, and chained random-interval. Maze procedures typically utilize continuous reinforcement. When generalization tests are conducted, results are similar under different schedules. Two factors are taken into account when determining schedule of reinforcement: the amount of responding that the schedule generates and its vulnerability to drug effects on rate. Overton (1979) concluded that FR schedules with food or water reinforcement generally yield relatively rapid acquisition and high asymptotic accuracy when compared with VI and DRL schedules.
To ascertain whether the drug is exerting stimulus control, the animal’s performance must meet criteria set by the experimenter. For example, subjects must achieve at least 80% accuracy on the injection-appropriate lever (Colpaert, 1987). That is, at least 80% of responding must occur on the injection-appropriate lever at two points in the session: at the beginning, when responses leading to the first reinforcer are generated, and throughout the entire session. Others have used a criterion of 70 or 90%. Also, it is common practice to record the number of daily sessions that are required to meet this criterion, which is termed sessions to criterion (STC). This measures drug discriminability in terms of speed of acquisition. Another method to measure drug discriminability is to determine the minimum dose of a drug that an animal can learn to discriminate from its vehicle. This is termed the ED$_{50}$, or the dose of the drug which produces drug-appropriate responding 50% of the time (Seiden & Dykstra, 1977). Another measure that is commonly recorded is the rate of response compared to control rate, that of the drug vehicle. This helps to determine at what dose to cease testing the drug of interest.

After the subjects have achieved the criteria for reliable drug discrimination, generalization tests are conducted in which the animal is essentially required to compare a test drug or different dose of the training drug with the two training conditions and then indicate which of the training conditions is most similar to the test drug or dose (Seiden & Dykstra, 1977). Allocation of responses on the choice
operand are measured as percent of drug-appropriate responding.

Two methods of stimulus generalization tests are utilized: one method is to conduct a single trial followed by extinction, and the other allows multiple trials to be reinforced. A concern with the latter strategy is that reinforcement may confound the results. Moreover, extinction testing is often thought to prevent new discrimination learning from taking place in test sessions. An advantage to arranging reinforcement in generalization tests is that the availability of reinforcement affords the experimenter the opportunity to analyze the possible effects of test treatments on overall rate of responding (Colpaert, 1987).

Generalization tests can be conducted by substituting other doses of the training drug for the training dose. In this case, the procedure resembles that used to generate generalization gradients with other kinds of stimuli (e.g., tones, lights), and similar gradients are obtained. That is, as doses grow progressively smaller than the training dose, progressively less drug-appropriate responding occurs. Test doses close to or somewhat greater than the training dose characteristically produce patterns of responding similar to those engendered by the training dose (Colpaert, 1987).

Generalization tests also can involve substituting other drugs for the training drug. When this is done, substitution drugs with neuropharmacological actions like those of the training drug characteristically engender training-drug-like patterns of responding. That is, at appropriate doses of the test drug, most responses are
emitted on the training-drug-appropriate key (Overton, 1987; Colpaert, 1977, 1985, 1987; Lal, 1977). Cross-generalization occurs with drugs from the same pharmacological classes (e.g., alcohol, benzodiazepines, and barbiturates; heroin, morphine, and opium), but not with drugs from different pharmacological classes (e.g., alcohol, LSD, and cocaine), and this finding is taken as evidence of the sensitivity of the procedure, as well as its utility in classifying drugs.

Antagonism tests can also be arranged for subjects trained in a drug discrimination assay. In an antagonism test, one drug (a putative antagonist) is given in combination with the training drug (or a test compound) to determine whether the antagonist alters the degree of stimulus control maintained by the drug (or test compound). When the neuropharmacological actions of the putative antagonist are known, results of antagonism tests can help to reveal the neuropharmacological actions through which drugs produce their discriminable effects. If, for example, pretreating animals with a drug that blocks dopamine receptors causes them to emit vehicle-appropriate responses when they receive the training drug, it is plausible that dopamine receptors play an important role in mediating the detectable (and, by virtue of operant history, discriminative stimulus) effects of the training drug. Of course, if the putative antagonist has sensory consequences of its own, such an interpretation is oversimplified. Giving two drugs together may change the subjective state of the organism, and hence weaken the stimulus control exerted by the training drug, even though no actual
pharmacological antagonism occurs. Fortunately, many antagonists that are used as research tools have weak or nonexistent sensory consequences at the doses used.

When the results of generalization (substitution) and antagonism tests are combined with training data, it is often possible to provide a reasonable profile of the neuropharmacological mechanisms through which drugs produce their discriminable effects. Because of this, drug discrimination assays have become a favored tool of neuroscientists (Branch, 1984; Stolerman, 1993).

Applications of Drug Discrimination Procedures

Researchers using drug discrimination procedures have attempted to address a number of questions. The most obvious are (1) which drugs can and cannot be discriminated by animals; (2) when a drug is established as a discriminative stimulus, which drugs do and do not generalize to this stimulus; and (3) when a drug is established as a discriminative stimulus, which drugs do and do not block its effects? (Weissman, 1977).

Drug discrimination studies that address such questions provide an animal model to evaluate the subjective effects that may be experienced by humans. Discriminative stimuli produced by drugs are related to their subjective effects in many instances. For example, nonhumans trained to discriminate LSD from vehicle emit LSD-appropriate responding when tested with mescaline or psilocybin, and people report that the three drugs provide similar (i.e., hallucinogenic)
subjective effects (Hirschhorn & Rosecrans, 1976).

Discriminative stimulus assays can be used to classify new compounds into various pharmacological classes as well as to establish their mechanism of action (Lal, 1977). The drug discrimination procedure can be reliably used for predicting drug classification, particularly in the case of narcotic analgesics, sedatives, psychotomimetics, nicotinics, muscarinics, and CNS stimulants. The use of the drug vs. drug vehicle discrimination is beneficial with any drug class for establishing pharmacological specificity. Two doses of the same drug can be established as discriminative stimuli in the same animal, and this can provide information on qualitative and quantitative aspects of the drug's subjective effects. Two drugs of the same class can also be established as discriminative stimuli. Information can be obtained on differences in pharmacological properties of two drugs belonging to the same class (Colpaert, 1987).

An important question in pharmacological research is determining the underlying biochemical mechanism of each of the various actions of a drug. Because discriminative stimulus effects can be reliably measured, the drug discrimination assay is a good tool to establish drug mechanisms. For instance, techniques are available to detect antagonistic properties of drugs at the biochemical level. The drug discrimination assay can be used to determine specifically if the potential antagonist blocks subjective effects of the psychoactive drugs. If so, it is likely that the drug cue is mediated by the neurochemical system affected by the
antagonist although, as discussed previously, this is not foregone.

A valuable property of drug discrimination is the ability to quantify the degree of discriminability. Three indices of performance have been used to indicate degree of discriminability: (1) asymptotic accuracy, (2) speed of acquisition, and (3) ability to substitute for another discriminable drug (Overton, 1987). Asymptotic accuracy refers to the relative frequency of correct responses after a prolonged period of drug discrimination training. The speed of acquisition refers to the number of training sessions before some criterion level of accuracy is achieved or the average accuracy during early drug discrimination training sessions. The substitution test can indicate the relative ability of various test drugs to produce discriminable effects that are similar to those of the training drug. Substitution tests are only useful, according to Overton (1987), when the test stimulus is virtually identical to those of the training drug. Others, however, are more optimistic concerning the value of such tests.

Problems With Drug Discrimination

An advantage to the drug discrimination assay is the measurement of behavioral responses that are readily observed and quantified. However, the dependent variables in drug discrimination research must be considered as derived variables. The dependent variables are constructed by the experimenter and are designed to accommodate some a priori concept or theory (Colpaert, 1987). The
two most important variables used to measure discrimination and generalization are (1) the percentage of drug-appropriate responding, and (2) response selection. The former measure is widely used but no justification is given in most studies (Colpaert, 1987). The percentage of responses as opposed to the absolute number of drug-appropriate responses is an "apparent attempt to accommodate possible drug effects on total response output" (Colpaert, 1987, p.352). Total response output involves a comparison of the occurrence of drug-appropriate responding as opposed to saline-appropriate responding, and is nominal in nature. Colpaert argues (1987) that drug-appropriate responding as opposed to saline-appropriate responding, constitutes the only variation in behavior which can reflect the discriminative stimulus effects of drugs. The statistical analysis of nominal data for each subject is very simple and often not reported. The total error rate is very low (10% or less) and as a result, response selection data can be accepted with the 0.10, 0.05, or < 0.05 level of significance. When testing different doses of the training drug or other drugs, generalization proceeds in orderly fashion from 0 to 100% as a function of the test dose. In the nominal approach, percentage generalization refers to the percentage of animals selecting the drug-appropriate lever; the quantitative approach refers to the mean percentage of drug-appropriate responding. Partial generalization is simply responding that is intermediate between those produced by the treatments which the animals are trained to discriminate. It can be interpreted in two ways: it may represent the ceiling level of a drug's effect or it can occur
along a dose-response curve progressing orderly from 0 to 100% level of effect (Colpaert, 1987).

In the generalization test procedure, a novel stimulus is administered to the animal and responses are observed and measured. If the novel stimulus engenders saline-appropriate responding, it may indicate that the effects induced by the stimulus were not similar to those of the training stimulus, but this does not carry any information regarding the potential discriminative stimulus effects of the compound (Jarbe, 1988). Essentially, the subject is forced to make a response, even if the novel stimulus differs significantly from both training conditions (Seiden & Dykstra, 1977). This problem has been somewhat alleviated with the use of three-choice assays in which two drugs or two doses of the same drug and saline are trained (White & Holtzman, 1981; France & Woods, 1985), but remains an important issue for drug discrimination researchers.

One disadvantage of generalization testing is that each substitution test on each animal produces relatively little information. Fortunately, the development of cumulative dosing procedures that engender dose-response curves within a single day has reduced the amount of time needed to collect meaningful data. Such procedures are, however, not yet fully validated or universally employed (Overton, 1987). Three disadvantages have been found with the cumulative dosing procedure. First, after a series of injections, the drug blood level is not always predictable. Second, the results obtained during a particular test trial within a test
session may not be independent of the results obtained during the previous test trial. Third, this type of series of test trials may disrupt accurate response control under training drug conditions. However, if cumulative dosing procedures are used in the training sessions, this problem may be alleviated.

Drug discrimination has been purported to be useful in classifying psychoactive drugs, especially those that are abused. However, it is unknown if the discriminative stimulus effects are related to the sensory effects that seem to contribute to drug abuse. In other words, the neural mechanisms underlying the reinforcing and discriminative stimulus effects may be dissimilar. The face validity of drug discrimination as a method for studying drug abuse is limited. According to Overton (1987), little effort has been made to determine which drug discriminations are based on effects that are related to drug abuse and which are not.

In conclusion, it can be stated that drug discrimination is a useful method for classifying psychoactive compounds. However, it must be noted that the drug discrimination procedure is limited by its very nature and there is no other procedure with which to measure its validity and reliability. Further studies and reviews are warranted to answer some of the basic questions and concerns of drug discrimination research.
Opioid Receptors and Opioid Effects

As Ross (1990) relates,

The effects of most drugs result from their interaction with macromolecular components of the organism. Such interaction alters the function of the pertinent component and thereby initiates the biochemical and physiological changes that are characteristic of the response to the drug (p.33).

The macromolecular component of the organism with which the drug interacts is termed the “receptive substance,” or “receptor.” The concept of drug receptors originated with the work of Ehrlich and Langley roughly 100 years ago, and is now a cornerstone of pharmacological theory. Many drug receptors are proteins that normally serve as receptors for endogenous regulatory substances (e.g., neurotransmitters). Drugs that mimic the effects of endogenous regulatory compounds are termed agonists. Drugs that bind to receptors but have no regulatory effect are termed antagonists; such compounds inhibit the effects of agonists (Ross 1990).

The behavioral effects of drugs are determined, in part, by their actions at particular receptor sites. The relation between behavioral functions and receptor actions has been studied especially well with opioid drugs. As Jaffee and Martin (1990) point out,

Studies of the binding of various ligands in brain and other organs suggest the existence of a multitude of distinct types of receptors that can interact with opioid drugs or endogenous peptides. There is reasonably firm evidence for three major categories of opioid receptors in the central nervous system,
designated $\mu$ (mu), $\kappa$ (kappa), and $\delta$ (delta)...

The various opioid receptors are found in different densities in different parts of the brain (e.g., Chang, Hazum, & Cuatrecasas, 1984; Goodman, Snyder, Kuhar, & Young, 1980) and control different physiological processes (e.g., Jaffee & Martin, 1990; Pasternak, 1988).

By studying interactions between drugs with known neuropharmacological properties, it is possible to determine the receptor systems involved in producing the behavioral and physiological effects of these compounds. For example, $\kappa$ agonists such as ketazocine, bremazocine, and ethylketazocine increase urinary output (Leander, 1983, 1985). Oxilophon and butorphanol also increase urinary output in the rat (Miller, 1975). It is known that $\mu$ agonists produced antidiuretic effects, thus implying that oxilophon and butorphanol might be $\mu$ antagonists. However, naloxone, an antagonist with high affinity for the $\mu$ receptor, does not produce diuresis. This led to the conclusion that the diuretic effect of oxilophon and butorphanol was an agonist action.

Leander (1983) reported that the agonist/antagonists, nalorphine, cyclazocine, and butorphanol were not as efficacious as the $\kappa$ agonists in producing diuresis. These compounds have antagonist action at $\mu$ receptors but agonist action at $\kappa$ receptors. It was postulated that increased urination is a $\kappa$ opioid effect because ethylketazocine, ketazocine, and bremazocine produced diuresis, whereas $\mu$ agonists such as morphine and $l$-methadone did not. Moreover, opioid antagonists
were able to block the diuretic effects of the \( \kappa \) compounds. Clonidine and phencyclidine were also tested for diuretic effects and, in fact, increased urinary output. However, this effect is not blocked by naloxone. It is of interest that higher doses of ethylketazocine and ketazocine produced less urinary output than lower doses. It appears that this effect is due to some \( \mu \) receptor agonist activity at high doses of the two compounds. These findings indicate that opioid drugs often have multiple and complex receptor actions, and it is not necessarily easy to relate these effects to actions observed at another level of analysis.

Studies of schedule-controlled behavior provide further evidence of the complex relations between neuropharmacology and overt behavior. In a study by Leander (1982), three benzomorphans (ethylketazocine, ketazocine, and phenazocine) were evaluated in pigeons responding under a multiple fixed-ratio 30/fixed-interval 5-min schedule of food presentation. Ethylketazocine and ketazocine are putative \( \kappa \) receptor agonists, whereas phenazocine is a reputed \( \mu \) agonist. All three agonists produced rate reductions in a dose-dependent manner. Phenazocine was more potent than the two \( \kappa \) agonists in suppressing responding. Ethylketazocine, ketazocine, and phenazocine were tested in combination with naloxone, an antagonist with high affinity for the \( \mu \) receptor. Naloxone was most effective in blocking phenazocine's rate-decreasing effects, and more effective in blocking ethylketazocine's effects than those of ketazocine. These results may suggest that ethylketazocine interacts with the \( \kappa \) receptor as well as the \( \mu \) receptor.
sites, and ketazocine may interact exclusively with $\kappa$ receptor sites.

Studies of the various opioid drugs utilizing drug discrimination assays have revealed clues to the receptor mechanisms through which various agents produce their detectable effects, and several studies in this area have appeared. In one such study, White and Holtzman (1981, 1983) demonstrated that cyclazocine (a $\sigma$ agonist, morphine (a $\mu$ agonist), and vehicle could be established as discriminative stimuli in rats using a three-lever assay. These drugs are thought to have primary efficacy at different receptor subtypes, thus it is perhaps not surprising that no cross-generalization occurred between morphine and cyclazocine. Moreover, in previous studies, cyclazocine (a reputed $\sigma$ agonist) did not substitute for morphine in squirrel monkeys (Teal & Holtzman, 1980a), rats (Hirschhorn & Rosecrans, 1976; Shannon & Holtzman, 1976a, 1979) or pigeons (Herling et al., 1980) trained to discriminate between morphine and saline. Conversely, morphine was shown not to substitute for cyclazocine in either monkeys (Schaefer & Holtzman, 1978) or rats (Hirschhorn, 1977; Rosecrans et al., 1978; Teal & Holtzman, 1980b) trained to discriminate between cyclazocine and saline.

Agonists that are active at the $\kappa$ receptor site have been studied extensively in drug discrimination assays, especially in comparison with $\mu$ agonists. Comparisons have been made because of an ongoing search for opioids that possess analgesic properties, but do not have abuse potential. Most often, researchers compare two groups of subjects, one trained to discriminate a $\mu$ agonist from its
vehicle, and the other trained to discriminate a κ agonist from its vehicle. For example, Shearman and Herz (1982) compared ethylketazocine (EKC), a reputed κ agonist, and fentanyl, a prototypic μ agonist. Rats were trained to discriminate either fentanyl or ethylketazocine from saline. They investigated these drugs to evaluate if the mechanism of action of opioid compounds was receptor-mediated. The EKC-trained rats generalized to cyclazocine and bremazocine in a dose-dependent manner. Fentanyl and morphine did not engender EKC-appropriate responding. Similarly, EKC, cyclazocine, and bremazocine did not produce fentanyl-appropriate responding.

The antagonists naloxone, MR 2266 and SKF 10,047, each antagonized fentanyl and EKC in a dose-dependent manner. However, the drugs differed in their relative potency in regard to their antagonism of the agonists trained. Naloxone was eight times more potent in antagonizing the discriminative stimulus effects of fentanyl than in antagonizing those of EKC. SKF 10,047 was less potent than naloxone in antagonizing the stimulus effects of EKC and fentanyl. And MR 2266 was less potent than naloxone in antagonizing fentanyl but more potent than naloxone in antagonizing ethylketazocine. From these data, naloxone was purported to be a highly selective μ antagonist, and SKF 10,047 and MR 2266 to be κ antagonists. In a related study, Herling, Coale, Valentino, Hein, and Woods (1980) evaluated the effects of narcotics in pigeons trained to discriminate morphine from saline. Drugs that produced morphine-appropriate responding included
codeine, EKC, and ketazocine. Those that did not engender drug-appropriate responding were SKF 10,047, cyclazocine, and UM 1046 (a benzazocine compound structurally related to the mixed narcotic agonist-antagonists cyclazocine and SKF 10,047). However, in rhesus monkeys trained to discriminate EKC from saline, morphine, pentazocine, and codeine all failed to substitute for the training drug stimulus (Hein, Young, Herling, & Woods, 1981).

Other reputed \( \kappa \) agonists have been studied in comparison to \( \mu \) opiate receptor agonists. Römer, Büscher, Hill, Maurer, Petcher, Welle, Bakel, & Akkerman (1980) suggested that bremazocine is an agonist that interacts with the \( \kappa \) receptor site. Their studies investigating the properties of bremazocine in comparison to morphine found that bremazocine was not self-administered and it did not substitute for morphine in morphine-dependent monkeys. Bremazocine and ketazocine did not cause mydriasis or the Straub tail phenomenon in mice. Moreover, bremazocine did not cause respiratory depression, a common effect of morphine. However, in analgesic assays, bremazocine was three to four times as potent as analgesic morphine in the mouse hot plate and tail flick assays. In contrast, when bremazocine was administered orally, it was only half as potent as morphine in the hot plate test but again, three times as potent in the tail flick procedure. In the rhesus monkey, bremazocine was 180 times more potent than morphine when evaluated under a shock titration assay of analgesia. In antagonism tests in which Mr 2266 and WIN 44,441-3 were given in combination with
bremazocine, it was found that these antagonists are κ-selective and bind with high affinity to the bremazocine site in rat brain homogenates. Naloxone did not antagonize the actions of bremazocine in the tail flick assay as well as the other antagonists tested. This reinforces the notion that bremazocine is active at a receptor site other than μ. Bremazocine seems to be similar to EKC, a κ agonist, in that MR 2266 binds to the bremazocine site.

In pigeons, either bremazocine or fentanyl and saline were established as discriminative stimuli in a two-choice procedure (Picker & Dykstra, 1989). In the bremazocine-trained birds, EKC produced intermediate levels of drug-appropriate responding whereas in the fentanyl-trained birds, EKC engendered full generalization to the drug-appropriate key. This is consistent with other findings indicating that EKC has selectivity for both μ and κ receptor sites in the pigeon.

To compare more closely the discriminative stimulus effects of EKC and bremazocine, Shearman and Herz (1982b) trained two groups of rats to discriminate either EKC or bremazocine from saline. They proposed that EKC interacted with the κ receptor subtype, and that bremazocine interacted with both κ and σ receptors. In generalization tests, reputed κ agonists (nalorphine, MRZ-2033, and pentazocine) were given in substitution for either bremazocine or EKC. Both groups displayed similar results in that they generalized the effect of MRZ-2033, and biphasically generalized nalorphine. Some rats also generalized the effect of pentazocine. The biphasic effect (i.e., nalorphine produced saline-appropriate
responding at low doses, drug-appropriate responding at intermediate doses, and saline-appropriate responding again at high doses) that occurred when nalorphine was tested was observed previously when nalorphine was tested in morphine- and fentanyl-trained rats (Shannon & Holtzman, 1976; Colpaert, Niegemeers, & Janssen, 1976). This may be attributed to the low affinity and low efficacy of nalorphine at the \( \kappa \) receptor site. Etorphine was reported to have much more selectivity for the \( \mu \) receptor than for the \( \kappa \) receptor (Wood, Charleston, Lane, & Hudgin, 1981). Also, Hein et al. (1981) reported that in rhesus monkeys trained to discriminate EKC from saline, etorphine engendered saline-appropriate responding. In both the EKC- and bremazocine-trained rats, etorphine again produced responding to the lever appropriate for saline. This reinforces the postulation that etorphine is a highly specific \( \mu \) agonist.

Another opioid that is purported to be highly selective for the \( \kappa \) receptor is U-50,488H [trans-3,4-dichloro-N-methyl-N(2-(1-pyrrolidinyl)cyclohexyl)benzeneacetamide]. It is antagonized by naloxone, but the dose required for antagonism is higher than that required to antagonize morphine. U-50,488H causes sedation, diuresis, and corticosteroid elevations (Katz, Woods, Winger, & Jacobson, 1982; VonVoigtlander, Lahti, & Ludens, 1983; Leander, 1983). Lahti, VonVoigtlander, and Barsuhn (1982) assessed the analgesic and sedative properties of U-50,488H in mice by testing two antagonists in combination with U-50,488H.

Naloxone was more potent in antagonizing morphine than U-50,488H;
conversely, MR 2266 was more potent in antagonizing the analgesic effects of U-50,488H than those of morphine. In mice, no cross-tolerance was observed between morphine and U-50,488H. Also, mice did not become physically dependent upon U-50,488H. VonVoigtlander et al. (1983) examined analgesia, physical dependence, and tolerance when U-50,488H was administered to rats. Naloxone antagonism of morphine was higher than that of U-50,488H; 36 times as much naloxone was required to block the analgesic effects of U-50,488H as was required to block morphine-induced analgesia. In fact, naloxone's ability to antagonize U-50,488H's analgesic effects were almost identical to naloxone's ability to antagonize bremazocine's effects. MR 2266 was much more potent at blocking U-50,488H than at blocking morphine. These results are consistent with previous findings (Lahti et al., 1982). Mice made tolerant to U-50,488H were not cross-tolerant when tested with morphine or EKC. Some cross-tolerance was evident with ketazocine, and marked cross-tolerance observed with bremazocine. These results suggest that U-50,488H and bremazocine interact exclusively with \( \kappa \) receptor sites; ketazocine and EKC do not seem solely to affect either receptor. U-50,488 was found to be highly specific to the \( \kappa \) receptor site (Lahti et al., 1982; VonVoigtlander et al., 1982; Tang & Collins, 1985).

To compare the effects of \( \kappa \) opioid agonists in the rhesus monkey, Dykstra, Gmerek, Winger, and Woods (1987) tested U-50,488H, bremazocine, ethylketazocine, tifluodam, and MR 2033. These compounds were chosen because
of their slight differences in activity. In monkeys trained to discriminate ethylketazocine from saline, all of the \( \kappa \) agonists tested produced responding on the lever appropriate for the training drug. These results are consistent with previous findings (Hein et al., 1981; Young & Stephens, 1984). Also, the discriminative stimulus effects of ethylketazocine were antagonized by quadazocine, a long-acting antagonist that is purported to be highly specific for the \( \kappa \) opioid receptor subtype. It is like naloxone in that it may have higher affinity for the \( \mu \) receptor than the \( \kappa \) receptor. In the discrimination studies, very low doses of the compounds produced discriminative stimulus effects in these subjects, as observed in previous studies using rats and squirrel monkeys (Teal & Holtzman, 1980; Shearman & Herz, 1982).

Most of the drug discrimination studies discussed above employed a two-choice discrimination assay to compare \( \kappa \) and \( \mu \) opioid agonists. For example, in the study by Picker and Dykstra (1987), pigeons were trained in a two-choice discrimination task with U-50,488H and vehicle as stimuli; another group was trained to discriminate between morphine and vehicle. After generalization tests with a variety of opioids, antagonism tests were conducted for the purpose of evaluating whether morphine and U-50,488H were differentially sensitive to naltrexone antagonism. The decision to test pigeons in this manner was based on previous studies that found inconsistencies in the data when pigeons were compared to other species, such as monkeys and rats (Teal & Holtzman, 1980; Hein et al.,

For example, in pigeons trained to discriminate EKC from saline, morphine and other \( \mu \) agonists occasioned EKC-appropriate responding. Similarly, in pigeons trained to discriminate morphine from water, EKC and other reputed \( \kappa \) agonists engendered morphine-appropriate responding (Hein et al., 1981; Herling et al., 1980). Drugs that are highly selective for the \( \mu \) receptor subtype, morphine, fentanyl, and \( l \)-methadone, produced predominantly vehicle-appropriate responding in the U-50,488H-trained pigeons and produced rate-decreasing effects at high doses. In the morphine-trained pigeons, the same drugs produced dose-related increases in drug-appropriate responding and never decreased rates by more than 25 percent. U-50,488H and bremazocine produced dose-related increases and rate-decreasing effects in drug-appropriate responding in the U-50,488H-trained pigeons. These same drugs engendered vehicle-appropriate responding in the morphine-trained pigeons. Ethylketazocine and ketazocine produced intermediate levels of responding on the drug-appropriate key for the U-50,488H-trained pigeons (65 and 40\%, respectively).

In the morphine-trained pigeons, however, EKC and ketazocine produced dose-related increases in drug-appropriate responding with no rate-decreasing effects at the doses that substituted for the morphine stimulus. The results of the EKC and ketazocine tests suggested that in pigeons, these compounds seem to be selective for the \( \mu \) receptor subtype. However, EKC and ketazocine produced
intermediate levels of drug-appropriate responding in the U-50,488H-trained group. This may indicate that the two compounds have some affinity for the κ receptor.

Evidence for EKC and ketazocine's lack of high selectivity for either κ or μ receptors was shown in studies of diuresis in the rat and schedule-controlled behavior in the pigeon (Leander, 1982, 1983). In the antagonism tests, the dose of naloxone that decreased drug-appropriate responding in the morphine-trained group by 50% was 100 times smaller than the dose required to decrease drug-appropriate responding by 50% in the U-50,488H-trained group. These results are consistent with previous studies that demonstrated naloxone's higher affinity for μ receptors than for κ receptors (Leander, 1982, 1983; Shearman & Herz, 1982; Katz et al., 1982; VonVoigtlander et al., 1983; Lahti et al., 1982).

The discrepant results in generalization and antagonism tests in pigeons trained to discriminate either a κ or μ opioid agonist from saline may have been due to the training doses of the drug. To compare training doses of U-50,488H and morphine, Picker, Doty, Negus, Mattox, and Dykstra (1990) trained two groups of rats to discriminate either 3.0 mg/kg or 5.6 mg/kg U-50,488H from vehicle; two other groups were trained to discriminate either 3.0 mg/kg or 10 mg/kg morphine from vehicle. Substitution and antagonism tests with various μ and κ agonists and with naloxone were conducted. Essentially, the μ agonists, l-methadone, morphine, and fentanyl, failed to substitute for the high dose of U-50,488H; conversely, the same compounds substituted completely for the high training dose of morphine. In
the group of rats trained with the low dose of U-50,488H, the \( \mu \) agonists produced complete generalization to the drug-appropriate lever. In the morphine group trained with a low dose, the \( \kappa \) agonists, ethylketazocine and bremazocine, engendered high levels of drug-appropriate responding; ketazocine completely substituted for the morphine stimulus. U-50,488H produced vehicle-appropriate responding in rats trained with low and high training doses of morphine. It is of interest that the \( \mu \) agonists substituted for the low training dose of U-50,488H in rats. This asymmetrical cross-substitution pattern warrants further evaluation of the role that training dose plays in establishing drugs as discriminative stimuli.

Because it has been established through the studies discussed above that pigeons can discriminate between \( \kappa \) and \( \mu \) agonists, a three-choice discrimination task might afford a greater degree of precision in characterizing the discriminative stimulus effects of opioids (White & Holtzman, 1981). As already noted, some discrepancies have been found in the results of discrimination and antagonism tests in pigeons compared to other species. In a study conducted by White and Holtzman (1983), rats were trained to discriminate morphine, cyclazocine, and saline. The lack of cross-generalization between cyclazocine and morphine in this study suggests that stimulus control was based on qualitative differences in the stimulus effects of the two drugs.

Drugs that possess mixed agonist-antagonist effects or have low selectivity for either \( \mu \) or \( \kappa \) receptor subtypes can be tested in a three-choice paradigm. In a
two-choice discrimination task, a drug may be tested on a subject that is trained on a κ or μ agonist and the test drug does not share the same characteristics as the training drug, the subject will most likely produce vehicle-appropriate responding although the test drug is similar to vehicle in its subjective effects. The three-choice discrimination amplifies the dissimilarities in the stimulus effects of morphine and U-50,488H, two drugs believed to have overlapping but not identical neuropharmacological actions.

The objective of the present study was to train pigeons in a three-choice task with 5.6 mg/kg morphine, 5.6 mg/kg U-50,488H, and vehicle as discriminative stimuli. A dose-response curve was conducted for cross-generalization of both U-50,488H and morphine. Substitution tests with d-amphetamine were conducted to determine if the subjects were responding to the opiate cue. Also, ethylketazocine was given in substitution for the training drugs to evaluate whether it would engender vehicle-, morphine-, or U-50,488H-appropriate responding. Previous investigations have raised the question of ethylketazocine's opioid receptor selectivity. Finally, the opiate antagonist, naltrexone, was tested for antagonism of the discriminative stimulus effects of morphine and U-50,488H. Differential sensitivity to naltrexone has been demonstrated in subjects trained to discriminate κ and μ agonists from vehicle. It has been posited that naltrexone has higher affinity for the μ receptor, hence, the drug should be more effective at blocking the effects of morphine than at blocking the effects of U-50,488H.
CHAPTER II

METHOD

Subjects

Five experimentally-naive White Carneau pigeons, maintained at 85-90% (470-510 g) of their free-feeding weights, served as subjects. The subjects were housed individually in a colony room with a 12-hour light/dark cycle. Water and grit were freely available in each bird's home cage.

Apparatus

Experimental sessions were conducted in three-key operant chambers, each approximately 38 cm high, 30 cm wide, and 37 cm long. The inside front panel of each chamber contained three translucent response keys (2.5 cm diameter), located about 25 cm above the floor of the chamber and 5.5 cm apart, which were transilluminated during the experimental sessions by white 7-w lights located behind the keys. A 7-w light above the keys provided general illumination throughout the experimental session, except during food delivery. When desired, mixed grain was delivered into a receptacle located beneath the center key and approximately 10 cm above the chamber floor. Programming of events and data
were recorded by a IBM-compatible computer equipped with MED-PC software (MED Associates, Wilmington, VT).

Procedure

For all birds, pecking on each of the three keys initially was engendered through the use of autoshaping procedures similar to those described by Picker and Poling (1984). Discrimination training was begun immediately after keypecking was established. During discrimination training, subjects received an intramuscular (IM) injection of 5.6 mg/kg morphine, 5.6 mg/kg U-50,488H, or isotonic saline solution (vehicle) 20 min before behavioral testing. A random sequence was used to determine which injection was administered, with the restrictions that the same substance was not given on more than two consecutive occasions and the number of morphine, U-50,488H, and saline injections were approximately equal.

Immediately after injection, the birds were placed in the experimental chambers, under conditions where all lights were extinguished and keypecks had no programmed consequences. Twenty minutes later, the keylights and houselight were illuminated, and the experimental session began. During training, food delivery (4 sec) was dependent upon responding on a particular key. The key on which responses produced food depended on the substance that was injected prior to the session. For two birds, the right key was designated as morphine-appropriate, the center key as saline-appropriate, and the left key as U-50,488H-appropriate.
During the first three training sessions, every peck on the appropriate key produced food. Over six sessions, the number of consecutive responses on the injection-appropriate key required to produce food (4-sec access) was gradually increased to 20, which was the value used in all subsequent sessions. If a bird responded on one of the other two keys before emitting 20 consecutive responses on the injection-appropriate key, the response requirement was reset to 20, no matter how many responses had been emitted on the appropriate key. All training sessions lasted for 30 min, during which responses on each key was recorded. Also recorded was the allocation of responses prior to the first food delivery. The study was approved by Western Michigan University’s Institutional Animal Care and Use Committee (see Appendix).

Dose-Response Determinations

Discrimination training continued until subjects met three criteria. First, they had to earn two or more food deliveries over 10 consecutive sessions. Second, at least 90% of their total responses during those sessions had to occur on the injection-appropriate key. Third, at least 90% of their responses prior to the first food delivery had to occur on the injection-appropriate key. After these criteria were met, dose-effect determinations were conducted with both morphine and U-50,488H. During dose-response testing, doses ranging from 0.10 to 32 mg/kg in quarter-log units were administered. Test doses were given in a random sequence
of doses and drugs. Dose-effect determinations were separated by training sessions, which were arranged until a subject met the criterion of emitting at least 90% of their total responses on the injection-appropriate key during three consecutive sessions. Test sessions differed from training sessions in that no food was delivered, and sessions ended after 20 consecutive responses on any of the three keys or 30 min, whichever occurred first. As in discrimination training, a 20-min presession injection interval was used during dose-effect determinations.

Generalization Testing

To determine whether the pigeons were responding specifically to the opioid cue, the drug d-amphetamine was administered following dose-effect testing with the training drugs. d-Amphetamine sulfate (Sigma, St. Louis) was tested at doses of 0.32, 1.0, and 3.2 mg/kg. Conditions for testing with d-amphetamine were equivalent to those described for dose-effect testing. d-Amphetamine was administered by IM injection 20 min prior to behavioral testing.

Following testing with d-amphetamine, generalization tests were conducted with ethylketazocine at doses of 0.10, 0.32, 1.0, and 3.2 mg/kg. Ethylketazocine was injected 20 minutes prior to behavioral testing.

Antagonism Testing

In the last phase of the study, injections of naltrexone were given in
combination with injections of morphine or U-50,488H under conditions similar to those described for dose-effect testing. During antagonism testing, 0.01 mg/kg naltrexone was administered in combination with 5.6 mg/kg morphine and in combination with 5.6 mg/kg U-50,488H. Moreover, 0.10 mg/kg and 1.0 mg/kg naltrexone doses were administered in combination with doses of morphine ranging from 5.6 to 56 mg/kg, and in combination with doses of U-50,488H ranging from 5.6 to 32 mg/kg. Each combination dose was given to every subject on one occasion. Naltrexone was injected into the side of the breast opposite that in which morphine or U-50,488H was administered. Naltrexone injections were given immediately before injections of morphine or U-50,488H.

Data Analysis

The data are expressed as the average percentage of responses made on each of the injection-appropriate keys per session ± 1 S.E.M. Response data are shown as the mean response rate ± 1 S.E.M., expressed as responses per second, on all three keys.
CHAPTER III

RESULTS

All birds eventually mastered the three-key discrimination. For the birds as a group, a mean of 121 sessions was required to meet the three criteria for mastery; the range across subjects was 74 to 160 sessions. On average, 98, 98, and 99% of responses prior to the first food delivery were injection-appropriate with morphine, U-50,488H, and saline, respectively. Mean response rates for sessions when morphine, U-50,488H and saline were administered were 1.25, 1.32, and 1.69 responses per second, respectively. For individual pigeons, the training dose of morphine (5.6 mg/kg) reduced the response rate to between approximately 50 and 90% of the control (i.e., saline) rate. The training dose of U-50,488H affected response rates similarly. During the course of the study, which lasted approximately 1.5 years, no apparent tolerance developed to the rate-decreasing effects of the training doses of morphine or U-50,488H.

Figure 1 shows the results of dose-response testing with morphine. For the subjects as a group, morphine produced generally dose-dependent reductions in response rates relative to the control (saline) level. Almost all responding occurred on the saline-appropriate key at morphine doses at or below 1.0 mg/kg, whereas almost all responding occurred on the morphine-appropriate key at morphine doses.
Figure 1. Dose-Effect Curve of Morphine Measured in Percentage of Responding and Response Rate in a Morphine-U-50,488H-Saline Discrimination.
of 5.6 mg/kg or above. Morphine doses of 1.8 and 3.2 mg/kg occasioned responding on both the saline-appropriate and morphine-appropriate keys, although for subjects as a group both doses were associated with more saline-appropriate than morphine-appropriate responding. Examining the performance of individual pigeons indicated that two birds engendered at least 90% morphine-appropriate responding at the 1.8 and 3.2 mg/kg morphine doses, whereas three birds produced only saline-appropriate responding. At 1.8 mg/kg morphine, an average of 40% of the group's responses occurred on the morphine-appropriate key. All other doses of morphine occasioned no responding on the U-50,488H-appropriate key. The ED_{50} dose for morphine was 3.18 mg/kg.

Figure 2 shows the results of dose-response testing with U-50,488H. For the subjects as a group, U-50,488H produced generally dose-dependent reductions in response rates relative to the control (saline) level. No dose of U-50,488H engendered more than occasional responding on the morphine-appropriate key. At U-50,488H doses at or below 1.8 mg/kg, almost all responses were emitted on the saline-appropriate key, and at doses of 5.6 mg/kg or above, almost all responses occurred on the U-50,488H-appropriate key. At 3.2 mg/kg, a mean of 43 and 57% of the responses occurred on the U-50,488H-appropriate and saline-appropriate keys, respectively. At this dose, two birds emitted over 80% of responses on the U-50,488H-appropriate key, one bird had emitted 33% of its responses on the U-50,488H-appropriate key and the remainder of responses on the saline-appropriate key.
Figure 2. Dose-Effect Curve of U-50,488H Measured in Percentage of Responding and Response Rate in a Morphine-U-50,488H-Saline Discrimination.
key, and 2 birds failed to emit any responses on the drug-appropriate keys. No responding on the morphine-appropriate key occurred during dose-effect determinations with U-50,488H. The ED$_{50}$ dose for U-50,488H was 3.66 mg/kg.

Figure 3 shows the results of generalization testing with $d$-amphetamine. $d$-Amphetamine at the doses tested (0.32, 1.0, and 3.2 mg/kg) reduced the mean group response rate relative to the control level and engendered primarily saline-appropriate responding. At 0.32 mg/kg $d$-amphetamine, one bird completely generalized to the U-50,488H-appropriate key, whereas all other subjects emitted only saline-appropriate responding. Two birds produced morphine-appropriate and U-50,488H-appropriate responding at 1.0 mg/kg $d$-amphetamine while all birds completely generalized to the saline-appropriate key at 3.2 mg/kg $d$-amphetamine.

Results of generalization testing with ethylketazocine are depicted in Figure 4. This drug produced generally dose-dependent reductions in response rates. The lowest dose tested (0.10 mg/kg) engendered only saline-appropriate responding, whereas the highest dose tested (3.2 mg/kg) engendered only morphine-appropriate responding. At intermediate doses (0.32 and 1.0 mg/kg), subjects as a group allocated responses among the morphine-, saline-, and U-50,488H-appropriate keys. At 0.32 mg/kg ethylketazocine, three out of five birds emitted at least 95% of responses on the morphine-appropriate key. At 1.0 mg/kg ethylketazocine, two birds completely generalized to the morphine-appropriate key, and two birds emitted over 83% of their responses on the U-50,488H-appropriate key.
Figure 3. Substitution Tests of d-Amphetamine as Measured in Percentage of Responding and Response Rate in a Morphine-U-50,488H-Saline Discrimination.
Figure 4. Substitution Tests of Ethylketazocine as Measured in Percentage of Responding and Response Rate in a Morphine-U-50,488H-Saline Discrimination.

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Table 1 lists the effects of naltrexone given in combination with either morphine or U-50,488H. On average, the birds emitted 57% of their responses on the U-50,488H-appropriate key when tested with 0.01 mg/kg naltrexone in combination with the training dose of U50,488H (5.6 mg/kg). A dose of 0.01 mg/kg naltrexone completely blocked the stimulus effects of the training dose of U-50,488H in two birds, whereas it failed to antagonize the stimulus effects in three birds. A dose of 0.10 mg/kg naltrexone completely blocked the training dose of U-50,488H in all birds except one. The dose of naltrexone which reduced U-50,488H-appropriate responding at the training dose (5.6 mg/kg) to 50% (i.e., A\textsubscript{50}) was 0.013 mg/kg.

In tests with morphine, 0.01 mg/kg naltrexone failed to block the training dose (5.6 mg/kg) in all birds, as listed in Table 2. The next highest dose tested, 0.032 mg/kg, completely antagonized the training dose of morphine. Higher doses of naltrexone with either morphine or U-50,488H were tested for antagonism. A dose of 0.10 mg/kg decreased morphine- and U-50,488H-appropriate to 44 and 50% responding at the 10 mg/kg doses of morphine and U-50,488H, respectively. A stronger differential sensitivity to naltrexone occurred at the higher doses of morphine and U-50,488H. When 1.0 mg/kg naltrexone was given in combination with 17.8 mg/kg morphine or U-50,488H, an average of all the birds produced 0 and 39% responding, respectively. Moreover, in three birds tested with 1.0 mg/kg naltrexone and 32 mg/kg morphine, none responded on the drug-appropriate keys.
The same dose of naltrexone failed completely to block the stimulus effects of 32 mg/kg U-50,488H in the same three birds. At 56 mg/kg morphine, two birds emitted an average of 50% morphine-appropriate responding when tested with 1.0 mg/kg naltrexone. The same dose of U-50,488H was not tested because the lower dose tested engendered complete U-50,488H-appropriate responding. The dose of naltrexone which decreased morphine-appropriate responding at the training dose (5.6 mg/kg) by 50% was 0.032 mg/kg.

Table 1

Mean Percent Morphine-Appropriate (M-A) Responses and U-50,488H-Appropriate (U-A) Responses (and Standard Error) During Naltrexone Antagonism Tests

| Morphine (mg/kg) | Naltrexone (mg/kg) | M-A | U-A | M-A | U-A | M-A | U-A | M-A | U-A | M-A | U-A |
|------------------|--------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                  | 5.6                |     |     |     |     |     |     |     |     |     |     |     |
|                  | 10.0               |     |     |     |     |     |     |     |     |     |     |     |
|                  | 17.8               |     |     |     |     |     |     |     |     |     |     |     |
|                  | 32.0               |     |     |     |     |     |     |     |     |     |     |     |
|                  | 56.0               |     |     |     |     |     |     |     |     |     |     |     |
| 0.01             | 100(0)             | 0(0) |     |     |     |     |     |     |     |     |     |     |
|                  | 5                  |     |     |     |     |     |     |     |     |     |     |     |
| 0.032            | 0(0)               | 0(0) | 54(41) | 0(0) |     |     |     |     |     |     |     |
|                  | 5                  |     | 3     |     |     |     |     |     |     |     |     |
| 0.10             | 0(0)               | 0(0) | 44(22) | 0(0) | 33(29) | 0(0) | 52(34) | 0(0) |     |     |     |
|                  | 5                  |     | 5     | 3     |     |     |     |     |     |     |
| 1.0              | 0(0)               | 0(0) | 0(0) | 0(0) | 22(18) | 0(0) | 50(36) | 0(0) |     |     |     |
|                  | 3                  |     | 3     | 3     |     |     |     |     |     |

Note: The Value Below the Means Indicates the Number of Subjects Tested.
Table 2

Mean Percent U-50,488H-Appropriate (U-A) Responses and Morphine-Appropriate (M-A) Responses (and Standard Error) During Naltrexone Antagonism Tests

<table>
<thead>
<tr>
<th>Naltrexone (mg/kg)</th>
<th>5.6</th>
<th>10.0</th>
<th>17.8</th>
<th>32.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-A</td>
<td>57(21)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-A</td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-A</td>
<td>100(0)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-A</td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-A</td>
<td>40(21)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-A</td>
<td>100(0)</td>
<td>0(0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U-A</td>
<td>0(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-A</td>
<td>4(0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: The Value Below the Means Indicates the Number of Subjects Tested.
CHAPTER IV

DISCUSSION

The present data demonstrate that two compounds selective for different opioid receptor subtypes, morphine and U-50,488H, can be established as discriminative stimuli in a three-key assay. Subjects in this study required substantially more sessions to master the discrimination than pigeons trained on a two-choice task with morphine versus saline or U-50,488H versus saline as training stimuli. One factor that may have contributed to the relatively slow task mostly in the present study is the use of relatively low training doses of morphine and U-50,488H. A second factor is the use of a criterion of 90% response accuracy, which is higher than the criterion used in other studies (France & Woods, 1985; Picker & Dykstra, 1987).

The dose-effect curves achieved in the present study demonstrate that doses as low as 1.8 and 3.2 mg/kg morphine and U-50,488H, respectively, evoked about 50% drug-appropriate responding. In other assays using two-choice discriminations between a μ agonist versus vehicle or κ agonist versus vehicle, doses between a quarter-log or half-log lower than the training dose also evoked about 50% drug-appropriate responding (Picker & Dykstra, 1987). The data in the present study are consistent with previous studies assessing the extent of stimulus control exerted by
morphine and U-50,488H.

In general, the substitution and antagonism data obtained with other opioid agonists and antagonists in pigeons trained to discriminate among two opioids and saline were similar to data from previous studies employing two-choice discriminations. In the present study, ethylketazocine produced intermediate responses on the U-50,488H-appropriate key at doses of 0.32 and 1.0 mg/kg whereas a dose of 3.2 mg/kg produced responses on the key that was correlated with the morphine stimulus. In other assays, ethylketazocine has produced morphine-like effects. For example, Leander (1983) demonstrated that \( \kappa \) agonists produced diuresis. When ethylketazocine was tested in this assay, higher doses produced less urinary output. Because \( \mu \) agonists are known to have antidiuretic effects, higher doses of ethylketazocine may interact with \( \mu \) receptors more so than with \( \kappa \) receptors, thus, the effect of less urinary output at higher doses.

In a study examining the effects of ketazocine, ethylketazocine, and phenazocine on schedule-controlled behavior, naloxone was given in combination with the training drugs to determine if it could block their rate-decreasing effects. Naloxone was most effective in blocking phenazocine’s rate-decreasing effects, yet more effective in blocking ethylketazocine’s effects than those of ketazocine (Leander, 1982). Naloxone is a putative \( \mu \) antagonist because it has high affinity for the \( \mu \) receptor. Herling et al. (1980) tested ethylketazocine in pigeons trained to discriminate 10 m/kg morphine from saline. Both ketazocine and ethylketazocine
engendered morphine-appropriate responding. In birds trained to discriminate either morphine or U-50,488H from saline, ethylketazocine produced intermediate levels of U-50,488H-appropriate responding, whereas in morphine-trained pigeons, ethylketazocine produced dose-dependent increases in drug-appropriate responding (Picker and Dykstra, 1987). In another study in which pigeons were trained to discriminate either bremazocine or fentanyl from water (Picker and Dykstra, 1989), ethylketazocine produced intermediate levels of drug-appropriate responding in the bremazocine-trained birds. In the fentanyl-trained birds, ethylketazocine substituted completely for the fentanyl stimulus in a dose-dependent fashion. The data obtained from the present study and from previous investigations provide compelling evidence that ethylketazocine has affinity for both \( \mu \) and \( \kappa \) receptor sites.

The antagonism data derived from this study provide more evidence to support findings from earlier studies suggesting that naltrexone has higher affinity for \( \mu \) receptor sites than for \( \kappa \) receptor sites (Harris, 1980; Holtzman, 1983; Dykstra, 1985; Picker and Dykstra, 1987, 1989). In the present study, a dose of 0.01 mg/kg naltrexone reduced drug-appropriate responding to 57% when given in combination with the training dose of U-50,488H, whereas the same dose of naltrexone failed to antagonize the stimulus effects of the training dose of morphine. However, at higher doses of naltrexone and the training drugs, morphine appeared to be more sensitive than U-50,488H to the antagonizing effects.
of naltrexone. For instance, a dose of 1.0 mg/kg naltrexone completely blocked the
effects of 32 mg/kg morphine, whereas it failed to block the stimulus effects of 32
mg/kg morphine, whereas it failed to block the stimulus effects of 32 mg/kg U-
50,488H. In a previous study, fentanyl and bremazocine were shown to be
differentially sensitive to the effects of naloxone in pigeons trained to discriminate
either fentanyl or bremazocine from saline. Naloxone completely antagonized
fentanyl and bremazocine at 1.0 and 0.30 mg/kg, respectively (Picker and Dykstra,
1989). At 1.0 mg/kg naltrexone in the present study, the stimulus effects of 32
mg/kg morphine were blocked in 4 of 5 birds whereas the same dose
of U-50,488H still engendered full drug-appropriate responding. Picker and
Dykstra (1987) showed that complete antagonism of U-50,488H’s and morphine’s
stimulus effects was achieved with doses of 1.0 and 0.10 mg/kg naloxone,
respectively.

Attributing differential sensitivity to interaction with different receptor
populations may be an incomplete or even incorrect assumption in explaining
naltrexone’s effects on μ and κ agonists. The level of stimulus control exerted by
the drugs, as Picker and Dykstra postulated (1987), may account for the
differences. The doses of the training drugs used for discrimination training may
not be equal in potency. However, in the present study, the response rates for
morphine and U-50,488H, on average for all birds, were very similar, suggesting
that comparable doses were used. It is of interest that U-50,488H was more
sensitive to the antagonizing effects of naltrexone than morphine at the training dose but less sensitive at higher doses in this study. Clearly, U-50,488H and morphine can be established as discriminative stimuli in a three-key assay. Possibilities for further research include employing different compounds that are selective for μ and κ receptor sites and different training doses of morphine and U-50,488H. Testing other antagonists, such as Mr 2266, which is purported to be very κ-selective, may assist in understanding the neuropharmacological mechanisms that determine in opioids’ differential sensitivity to antagonists.
APPENDIX
IACUC Investigator Certification Form
INVESTIGATOR CERTIFICATION

Title of Project: The Discriminative Stimulus Properties of Mu and Kappa Opioid Agonists: A Three-Choice Discrimination Task in Pigeons

If any of the above procedures are changed, I will submit a new protocol.

I understand that any failure to comply with the Animal Welfare Act, the provisions of the DPHS Guide for the Care and Use of Laboratory Animals, and requirements set down by the IACUC may result in the suspension of my animal studies.

Signature:（签名）  Date: 9/9/91

REVIEW BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

Disapproved   Approved   Approved with provisions listed below

Provisions or Explanation:

Injection must include a 1cc syringe, including or exclude saline, morphine, or a tranquilizer injection.

Dr. Malade, I think I made the appropriate revisions on page 8-7. Please call me if you have any questions. Thanks, Malade.

IACUC Chairperson

Researcher's Acceptance of Provisions:

Signature:  Date:  Sept 24, 1991

IACUC Chairperson Final Approval

Approved IACUC Number

Revised February 12, 1991
BIBLIOGRAPHY


