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Modulation of hyperactivity and reward by excitatory amino acid, serotonin, and opiate receptors in nucleus accumbens

Layer, Richard Thomas, Ph.D.
The Ohio State University, 1992
MODULATION OF HYPERACTIVITY AND REWARD
BY EXCITATORY AMINO ACID, SEROTONIN, AND OPIATE RECEPTORS
IN NUCLEUS ACCUMBENS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of the Ohio State University

By

* * * * *

The Ohio State University

1992

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The Secret Sits

We dance round in a ring and suppose,
But the Secret sits in the middle and knows.

-Robert Frost
To Lynda, my partner, who stoically endured both my rantings and ravings, and the chaotic appearance of our home while this dissertation was in preparation, and who loved me throughout. I couldn't have done it without you.
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CHAPTER I
INTRODUCTION

Drug abuse is a major health problem affecting society. In 1988, the abuse of alcohol and drugs by Americans was estimated to have cost over $144 billion, including cost of treatment, prevention, lost productivity and violence (Carey, 1990). Furthermore, this figure does not address the cost in human suffering born by both addicts and their loved ones. Despite the considerable progress that has been made in recent years towards understanding the phenomena of addiction (for review see Koob and Bloom, 1988), effective strategies for treating drug addicted persons remain elusive.

In the past, theories of addiction were based on the observation that upon cessation of drug intake, a painful withdrawal occurred. Thus, further use of the drug was seen as an attempt by the user to alleviate the pain and distress of withdrawal. In recent years, however, evidence has mounted which suggests that addicts maintain drug use due to the positively reinforcing effects of the drug. Many researchers have therefore focused their efforts on the mechanisms of reward. While much progress has been made, the neural substrates of drug reward remain poorly understood. A better understanding of the interaction of
drugs with the neural substrates of reward will undoubtedly result in novel therapies for the treatment of drug addiction. The purpose of this dissertation, therefore, is to contribute to the growing database from which, hopefully, an understanding of the neural substrates of drug reward will come.

THEORY AND TERMINOLOGY

Any discussion of theories of drug addiction requires the use of terminology which is often loosely defined. It is therefore important to provide definitions prior to any discussion. Jaffe (1980) defines the phrase drug abuse as the self-administration of any drug in a manner that deviates from the approved medical or social patterns within a given culture. While this definition is not useful scientifically, it is useful in a sociological context. The phrase abuse potential refers to drugs whose habitual use could result in addiction, and abused drugs are those which are compulsively used by humans and which have addiction potential. The term drug addiction refers to "a behavioral pattern of drug use, characterized by overwhelming involvement with the use of a drug (compulsive use), the securing of its supply, and a high tendency to relapse after withdrawal" (Jaffe, 1980). Traditional theories concerning drug addiction involve the development of drug dependence, involving both physical and psychological dependence. Physical dependence refers to "an adaptive state that manifests itself by intense physical disturbances when the administration of the drug is suspended" (Eddy et al, 1965) and is typical of opiate withdrawal. It is often, but not always, associated with
tolerance, in which increasingly larger doses of a drug must be administered in order to obtain the effects observed with the original dose (Jaffe, 1985). Psychological dependence, on the other hand, is "a condition in which a drug produces a feeling of satisfaction and a psychic drive that requires periodic or continuous administration of the drug to produce pleasure or avoid discomfort" (Eddy et al, 1965). The Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1987) defines drug dependence, however, as "a cluster of cognitive, behavioral, and physiologic symptoms that indicate that the person has impaired control of psychoactive substance use and continues use of the substance despite adverse consequences."

In operant conditioning, the term reinforcement refers to the occurrence of an event, such as obtaining food or alleviating pain, following a desired response, such as pressing a lever. A positive reinforcer, as defined by Skinner (1938), is a stimulus, such as the presentation of a food pellet to a rat, which increases the probability of the response (lever press) which preceded its presentation. In contrast, a negative reinforcer is a stimulus, such as an electric shock, which, when removed following a response (i.e. a lever press), increases the probability of the response. Skinner (1938) labeled these phenomena as operant reinforcement.

Older theories of addiction emphasized the ability of abused drugs such as opiates to produce physical or psychological dependence and associated noxious withdrawal symptoms and suggested that avoidance of this dysphoric state was
the primary motivation for drug administration (Bishop, 1920; Collier, 1968; Dole and Nyswander, 1967; Lindesmith, 1947; Lindesmith, 1968; Wickler, 1952). Thus, in the case of compulsive drug use, withdrawal served as a negative reinforcer, removed following drug administration. Furthermore, tolerance to the euphoria elicited by certain drugs was proposed (Lindesmith, 1968; Wikler, 1973). These observations led to the opponent process theory of motivation (Solomon, 1977). This theory holds that a reinforcer arouses positive hedonic processes that are opposed by negative anhedonic processes (in a sort of neural tug of war). A drug user initially experiences a stimulation of the hedonic process. Repeated stimulation of the reward substrate which elicits the hedonic process, however, results in its desensitization, and tolerance develops to the positive hedonic process. Negative hedonic processes (aversive withdrawal symptoms) thus become more pronounced. Dependence results when negative hedonic processes predominate in the normal, drug free state, and the drug user must repeatedly take the drug just to maintain a normal opposition between these opposing processes.

An important tenet of this theory (the negative reinforcement model) is that the negative reinforcement mechanism is the result of the desensitization of the positive reinforcement mechanism. Several lines of evidence, however, do not support the negative reinforcement model, in which physical dependence is necessary for addiction. First, behaviors can be reinforced by opiates and psychostimulants in the absence of physical dependence (Mucha et al, 1982; Koob et al, 1984; Bozarth and Wise, 1984). Second, the opioid analgesic buprenorphine
is self-administered and elicits place preference (measures of reward) in monkeys (Mello et al., 1981; Lucas et al., 1986; Young et al., 1984; Brown et al., 1991) and is reported to have morphine-like euphoric effects in humans (Lewis, 1985) but produces only a very mild withdrawal syndrome (Jasinski et al., 1978); while U-50,488, an experimental opioid agonist, is not rewarding but produces physical dependence (Tang and Collins, 1985). Third, brain sites which mediate reward (nucleus accumbens and ventral tegmental area) and physical dependence (periaquiductal grey region and dorsal thalamus) appear to be anatomically distinct (Bozarth and Wise, 1983; Bozarth and Wise, 1984; but also see Baumeister et al., 1989; Koob et al., 1989). Fourth, repeated exposure to some abused drugs appears to intensify their rewarding effect, not produce tolerance to it (Lett, 1989; Vezina and Stewart, 1984).

While negative reinforcement may be involved to some extent (Koob et al., 1989; Koob and Bloom, 1988; Wise, 1990), in recent years evidence has accumulated which suggests that it is the positive reinforcing effect of drugs which is the critical determinant of their abuse (Wise, 1987; Wise, 1990; Negus and Dykstra, 1989; Lett, 1989; Stewart et al., 1984). Thus, the drug-elicited euphoria serves as a positive reinforcer, and drug use is explained as a repeated attempt by the user to regain that euphoria.

In humans, euphoria can be defined as a feeling of extreme pleasure, happiness and well-being. All drugs which elicit the feeling of euphoria as reported by human users, most notably the opiates (morphine, heroin) and psychostimulants
(amphetamine, cocaine), are also addicting and have abuse potential. Animals will also readily self-administer these drugs. However, as euphoria is a human, subjective, inner experience, one must resist the inappropriate and anthropomorphic conclusion that an animal is experiencing euphoria. The term reward, referring vaguely to an emotional state thought to be pleasurable, is preferable, and is best defined as an environmental stimuli which elicits approach (White, 1989b; Bozarth, 1991). For example, detecting a food pellet activates the neural substrates of reward in a rat, which then approaches the pellet. Thus the pellet is considered rewarding. Drugs such as opiates and psychostimulants, when given to animals, bypass the sensory organs, but nonetheless activate the neural substrates of reward, and are therefore considered rewarding.

METHODS OF ASSESSING DRUG REWARD

Drug addiction is characterized by compulsive drug administration. In the positive reinforcement model, drug-induced reward is the motivating factor for repeated drug use. Therefore, the study of drug reward will ultimately provide valuable insight into the phenomena of drug addiction.

There are currently several methods used for assessing the rewarding properties of drugs in animals (for review see Bozarth, 1987; Evenden, 1990; Woolverton and Nader, 1990). These methods have a high degree of correlation with data collected from humans. The most widely employed methods include direct drug self-administration, facilitation of electrical brain stimulation, drug
discrimination, which all involve operant responding, and conditioned reinforcement and conditioned place preference, which do not.

**Operant Reinforcement Techniques**

Operant reinforcement, as originally defined by Skinner (1938), is typically studied using operant conditioning methods in which a reward is contingent upon an appropriate response by the subject. These methods are reviewed in the following section.

**Self-Administration**

The self-administration paradigm typically involves implanting an intravenous catheter into an animal and subsequently allowing the animal to self-administer a drug. The administration of drug is typically contingent upon a behavioral response such as lever pressing and is easily understood utilizing the principles of operant psychology. The ability of the drug to reinforce the behavior that led to its administration is then assessed. Most of the drugs abused by humans (but not all, hallucinogens such as LSD are not, see Jaffe, 1985) are self-administered by animals, and self-administration studies can be used to assess the abuse potential of novel drugs (see Woolverton and Nader, 1990).

The rate or responding (how often a rat will press a lever for a drug injection) for rewarding drugs generally follows an "inverted U" dose-effect curve. Thus, animals do not respond for low doses of a drug, as it is not reinforcing. The rate
of responding increases up to a certain level, and then as the dose increases further, responding drops off again. This may reflect an attempt by the animal to titrate the amount of drug it gets, or it may reflect motor impairment.

The two most common techniques for assessing the ability of a drug to reinforce a behavior are cross substitution and the direct acquisition. The direct acquisition technique involves using the proposed drug initially, and determining if an animal will learn to self administer it. With the cross substitution method, on the other hand, animals are trained to self-administer a standard rewarding drug such as cocaine. A new drug is then switched with the standard drug (i.e. cocaine), and the ability of the new drug to maintain the behavior is assessed.

The self-administration technique can be used to determine which brain areas are important in reward mechanism. For example, animals will self-administer drugs directly into specific brain regions associated with reward. Intracranial self-administration techniques are advantageous in that specific anatomical questions can be addressed.

In another paradigm, attempts to disrupt self administration are made using antagonists or lesions. For example, if destruction of dopaminergic cell bodies disrupts amphetamine self administration, then these cell bodies must be involved in maintaining self administration behavior. Typically, disruption of reward results in an initial increase in lever pressing (presumably the animal is trying harder to get what is no longer there) followed by a decrease in responding, known as extinction (presumably, the animal realizes that its efforts are futile).
Facilitation of Electrical Brain Stimulation

Similar to the drug self administration paradigm, animals can be trained to work for electrical brain stimulation. Typically, an electrode is implanted in the medial forebrain bundle where it passes through the lateral hypothalamus. A behavioral event (lever press) is then followed by the administration of small current (in the form of a train of electrical pulses) into the median forebrain bundle. This current activates the reward pathways and thus reinforces the behavior just like a food pellet might.

Some drugs enhance or facilitate the ability of electrical stimulation to elicit a response interpreted as reward. This can be demonstrated in two ways; either a given dose of a drug can increase the rate of responding for electrical brain stimulation (animal will press the lever more often), or a given dose of a drug will lower the current threshold required to maintain responding for electrical brain stimulation. There is a high correlation between drugs which have abuse potential in humans and drugs which enhance or facilitate electrical brain stimulation.

Drug Discrimination

The drug discrimination paradigm is used to study the subjective effects of drugs. In this paradigm, animals are trained to make one of two alternate responses when under the influence of a specific drug. For example, a hungry rat will learn to press a lever for a food pellet. Next, the rat is administered a drug, such as morphine, in a chamber with two levers and trained to press one particular
lever (the right lever) for the pellet. When administered saline, the rat learns to press the left lever for the pellet. After a number of daily trials, the rat learns to discriminate the morphine from the saline. At this point, a novel drug is administered. If after its administration the rat presses the lever associated with the morphine experience, then the novel drug is said to have similar (or, in this case, morphine-like) discriminative stimulus properties. If the rat presses the lever associated with the saline, then the drug is considered not to substitute for the test drug. Animals can distinguish many different classes of psychoactive drugs (e.g. ethanol, benzodiazepines, psychostimulants, opiates), and drugs which have similar subjective effects in human studies typically generalize in animal drug discrimination studies.

Non-Operant Techniques

A qualitatively different type of conditioning was described by Pavlov (1927). When rewarding stimuli are paired with neutral stimuli (those stimuli which elicit neither approach nor avoidance), then the formerly neutral stimuli gain the capacity to elicit approach and therefore become rewarding. Skinner (1938) referred to this as respondent conditioning. The ability to predict a goal object, such as food or a sexual partner, represents an enormous evolutionary advantage, and neural systems have developed which make this possible. The intrinsic properties of the goal, which make it rewarding (capable of eliciting approach), are referred to as the unconditioned stimulus (US). Any arbitrary stimulus with which it is associated
is termed the conditioned stimulus (CS). Thus, if there is a predictive relationship between the CS and the US (such as a dinner bell signaling the arrival of food), then the CS will come to elicit the central representation of the US (Robbins et al, 1989; Mackintosh, 1983).

In the methods described above, administration of the reinforcing stimulus (i.e. food or drug reward) is contingent upon the expression of an appropriate behavior. The methods described below, in which operant responding may play some role, emphasize the assessment of the behavior of an animal after it has been presented with a conditioned stimulus associated with reward (see Bozarth, 1987).

**Conditioned Place Preference**

In the conditioned place preference paradigm, rats are trained by pairing distinct environmental stimuli or cues with the administration of a rewarding stimulus. This occurs over the course of several training periods in which an animal is administered a rewarding stimulus such as food or a dose of a rewarding drug and placed in one chamber of a place preference apparatus (see Figure 1) or given a neutral stimulus such as saline or vehicle and placed in another distinct chamber. An association between the environmental cues in the first chamber and the rewarding stimulus then develops. Theoretically, the environmental cues in the first chamber are now capable of eliciting approach. The animal is tested later to see if it then spends more time in the chamber paired with the rewarding stimulus. Animals will form a place preference with a number of rewarding stimuli, including
Figure 1. The conditioned place preference apparatus. The drug is paired with the least preferred side, usually white (CS+ for conditioned stimulus +). Saline is given in the preferred side, usually black (CS- for conditioned stimulus -).
food (Spyraki, 1982a), sucrose solutions (White and Carr, 1985), electrical brain stimulation (Ettenberg and Duvauchelle, 1988), and many drugs abused by humans (see Hoffman, 1989; Swerdlow et al, 1989).

A major advantage of the conditioned place preference paradigm is that animals are (in most cases) drug free during the actual test. This is in contrast to the procedures for measuring reward, which described above, involve operant responding by an animal while under the influence of a drug. The conditioned place preference paradigm has numerous other advantages; and was the method used in the studies described in this dissertation. First, the rewarding effects of drugs are assessed in the drug free animal. Thus complications arising from increases or decreases in motor activity are avoided. Second, the procedure is rapid, in one case place conditioning was reported after only one pairing with morphine (Bardo and Neisewander, 1986). Third, the apparatus is simple to construct and maintain. Fourth, no complicated surgeries are required (intravenous catheters are notorious for being difficult to maintain). Fifth, the method is sensitive and easy to quantitate.

There are two basic types of place preference procedure: the balanced and unbalanced paradigms. In the balanced paradigm, animals initially spend an equal amount of time in both chambers. In the unbalanced paradigm, the animals initially prefer one side over the other, and the rewarding drugs are paired with the non-preferred side. Each method has advantages and disadvantages (see van der Kooy, 1987; Fibiger, 1987; Bozarth, 1987). For example, in the unbalanced
paradigm measures of aversion cannot be done, as there may be a ceiling effect. That is, it may be that one cannot get an animal to dislike the least preferred side more than it does initially. Also, since in the unbalanced paradigm the drug must be paired with the least preferred side (for reasons of ceiling effect, i.e. one may not get an animal to like the preferred side more than it does initially), counter balanced controls (i.e. morphine in the preferred side) cannot be run. On the other hand, using an unbalanced paradigm is easier, and it allows one to maximize the potential shift in place preference following drug pairing with the least preferred side.

The unbalanced procedure used in some of the experiments for this dissertation project is described here and is typical of most conditioned place preference paradigms (Figure 2). The place preference apparatus used was a 3-compartment apparatus arranged so that a small central compartment separates 2 larger compartments. (Figure 1). Guillotine doors separate the compartments. The small compartment is grey, while the one larger compartment is painted white and the other black. The white compartment has a floor composed of steel rods, and the black compartment has a floor composed of wire mesh. On the day prior to training animals were allowed to freely explore the apparatus for 15 minutes with the guillotine doors open, thus establishing a baseline preference, (some investigators use more than one pre-training session). As this apparatus provides an unbalanced paradigm, the chamber they spend the least time in is considered the non-preferred side. On alternate days, rats were injected with either the drug
Figure 2. The conditioned place preference paradigm (adapted from Swerdlow et al, 1989).
and confined to the non-preferred side for 35 minutes or were injected with saline and confined to the preferred side for 35 minutes. Rats were tested for place preference on the day following training. Rats were placed in the small grey compartment with the doors closed. The doors were then opened, and the amount of time spent in each compartment during a 15 minute test period was recorded (see Figure 2).

Conditioned Reinforcement

In the conditioned reinforcement paradigm, an animal is trained to respond for a rewarding drug injection. The drug administration is accompanied, however, by another neutral stimulus, such as a light or a tone. An association is then presumably formed by the animal between the rewarding drug injection and the secondary stimulus (e.g. the tone). The animal is then tested to determine whether it will respond for the tone only. If the drug was indeed rewarding, and an appropriate association was made, the animal should lever press for the tone alone. This technique, along with conditioned place preference, has the advantage that animals are tested in a drug free state, and other effects of the drug such as motor disturbances will not directly interfere (Davis and Smith, 1987).

THE LOCOMOTOR ASSAY AND ITS RELATIONSHIP TO REWARD

Psychostimulants such as cocaine and amphetamine are characterized both by their positively reinforcing effects and their ability to increase locomotor activity
(Evenden and Ryan, 1990). In addition, other classes of rewarding drugs (at appropriate doses), including methylxanthines such as caffeine and opiates such as heroin (Swerdlow et al., 1986), and electrical stimulation of the medial forebrain bundle (Glickman and Schiff, 1967) are simulators of locomotor activity. Furthermore, treatments which reduce the locomotor response to psychostimulants frequently also reduce their ability to elicit reward. These correlations have led to the hypothesis that increased locomotor activity is intimately involved in the reward process.

It has been proposed that all positive reinforcers (Glickman and Schiff, 1967), including rewarding drugs (Wise and Bozarth, 1987), will activate approach responses. Locomotor activity may therefore represent an approach response in the absence of a specific object to be approached. Thus a relationship between the neural substrates of locomotor activity and reward seems to exist. While all stimuli that activate locomotor activity are not positively reinforcing (as avoidance is also a form of locomotor activity), it is possible that all positively rewarding stimuli (including abused drugs) will activate locomotor activity. The locomotor activity assay is therefore useful for analyzing the motor activating properties of abused drugs.

Locomotor activity is typically assessed by measuring either the breaking of infrared beams in a photocell cage or directly measuring distance traveled using a digitized image analysis. Combined with stereotaxic microinjection techniques, the locomotor activity assay is useful for analyzing the anatomical substrates
necessary for motor activation elicited by many classes of abused drugs (for review see Swerdlow et al, 1986; Geyer, 1990).

NEURAL SUBSTRATE OF DRUG REWARD AND LOCOMOTOR PHARMACOLOGY

The previous sections of this dissertation have reviewed some of the more common methods used in studying the behavioral effects of rewarding drugs, (in a loose and anthropomorphic sense, animal models of human, drug-elicited pleasure). To elicit reward, however, a drug must gain access to some critical part of the central nervous system, which mediates natural reward. In recent years, the central sites which mediate the rewarding effects of abused drugs have begun to be elucidated. The following sections, therefore, will review what is currently known about the neural substrates of reward. Recent research involving the neurobiology of drug reward has suggested that the three major classes of abused drugs, the psychostimulants, the opioids, and ethanol, trigger activity in a common reward system (Wise and Bozarth, 1982; Wise and Bozarth, 1984; Koob and Bloom, 1988; Di Chiara et al, 1991; Bozarth, 1991). Early studies suggested the presence of what came to be known as a pleasure center (Olds, 1956); however, it has become apparent in recent years that multiple elements in a circuit are involved (Olds, 1972). This common reward circuit involves neurons which interconnect the ventral tegmentum, nucleus accumbens, ventral pallidum, and frontal cortex (Koob and Bloom, 1988). Elements of this circuit also appear to
comprise the neural substrate of locomotor activity. The overlap of the neural substrates for both locomotor activity and reward has led to the tempting hypothesis that this common substrate may mediate goal-directed behavior (Swerdlow et al., 1986). The following sections will review the anatomy and possible functions of these areas, with a special emphasis on the nucleus accumbens.

Motivation and Drive

Motivation has been defined as the set of processes that enable an organism to control the availability, probability or proximity of stimuli (Salamone, 1991). These processes underlying the initiation and termination of goal-directed behavior. Two schools of thought have been developed to explain the phenomenon of goal directed behavior. The first emphasizes what came to be known as drive reduction theory, and the second emphasizes incentive motivation theory.

Drive reduction theory suggests that the internal conditions that arouse and direct voluntary goal-directed motor behaviors are known as motivational states, and specific motivational states are known as drives (Hull, 1943; Kupfermann, 1985). Animals tend to maintain homeostasis, and when homeostatic imbalances occur, animals act in such a way as to correct them. Thus there are specific drives for things such as food, water, and sex, and the biological incentive is to reduce that drive. For example, a hungry rat can be described as being in a drive state for food. Thus, drive states tend to "push" an animal to act, if hungry then
seek food.

In contrast, incentive motivational theory (see Bindra, 1968) emphasizes the ability of stimuli associated with a goal object to energize motivated behavior. Thus, the animal is "pulled" toward the goal object because of its rewarding value. If a rat is hungry it simply adds to the incentive value of stimuli associated with food. One factor which contributes to rewarding value and controls motivated behavior is pleasure. As we are all well aware, eating a delicious meal when one is hungry elicits the feeling of pleasure, it is a pleasurable experience. Although it is impossible to know if an animal such as a rat feels pleasure; it seems likely that they do. For instance, rats will eat more of a palatable meal (including chocolate chip cookies and salami) than an equally nutritious but bland meal of rat chow (Sclafani, 1976). While this investigator can attest to the palatability of chocolate chip cookies and salami, I must let the reader assume that rat chow is bland, as I have never sampled it.

Anatomy of the Reward Substrate

In 1954 it was demonstrated that electrical stimulation of specific areas associated with the hypothalamus could reinforce operant behavior (Olds and Milner, 1954). Thus, electrical brain stimulation of this area seemed to be rewarding, similar to food. However, only a hungry rat will respond for food, while electrical brain stimulation of certain brain areas is effective without a drive state. This lead to the hypothesis that electrical brain stimulation both evokes a drive
state, and activates reward or "pleasure centers", (Deutsch and Howarth, 1963).

Since the discovery by Olds and Milner in 1954, evidence has been mounting that a single "pleasure center" does not exist in the brain. Rather a system of interacting areas seems to underlie reward (Olds, 1972). It has recently been proposed that neurons which form a ventral tegmentum-nucleus accumbens-ventral pallidum-frontal cortex pathway form the substrate for natural reward (Koob and Bloom, 1988). A diagram of this circuit is shown in Figure 3.

The first element of the reward circuit is the medial forebrain bundle, electrical stimulation of which appears to be rewarding (Liebman, 1989). While presumably most of the many fibers which course through the medial forebrain bundle are stimulated by an electrode, electrophysiological and histological studies have revealed that the critical fibers for the rewarding effect of electrical stimulation represent myelinated axons, with a diameter of 0.5 and 2.0 \( \mu \text{m} \) and with cell bodies in the lateral hypothalamus or the nucleus of the diagonal band (for review see Wise and Bozarth, 1984; Carlson, 1986). This suggests that it is not the small and unmyelinated monoaminergic fibers (i.e. dopaminergic or noradrenergic which also course through the medial forebrain bundle) which are activated by electrical stimulation. These fibers then synapse at the ventral tegmental area of the ventral mesencephalon, where they activate dopaminergic (and possibly other) neurons which project to the nucleus accumbens.

The dopaminergic cells of the ventral tegmental area (A10) then send projections (back through the medial forebrain bundle, see Figure 3) to the nucleus
Figure 3. The proposed reward circuit. Abbreviations: (CTX) cortex; (PFC) prefrontal cortex; (HIP) hippocampus; (VTA) ventral tegmental area; (NDB) nucleus of the diagonal band; (NA) nucleus accumbens; (RN) raphe nuclei; (VP) ventral pallidum; (PPN) pedunculopontine nucleus; (DMT) dorsomedial thalamus; (mfb) medial forebrain bundle; (DA) dopamine; (GLU) glutamate; (5HT) serotonin.
The nucleus accumbens, a region of the basal forebrain. The nucleus accumbens then sends projections (believed to be GABAergic and enkephalinergic) both to the ventral pallidum and back to the ventral tegmental area. The ventral pallidum, as used in this text, includes the subcommissural substantia innominata and the more rostral sublenticular ventral pallidum (Heimer and Alheid, 1991). The ventral pallidum sends projections to the frontal cortex, a site associated with reward, and to both the dorsomedial thalamus and the pedunculopontine nucleus (considered the rat homolog of the mesencephalic locomotor region), sites associated with the control of locomotor activity. The dopaminergic cells of the ventral tegmental area also project to the prefrontal cortex, which then sends a projection to the nucleus accumbens (for a comprehensive review, see Wise, 1990; Heimer and Alheid, 1991; Groenewegen et al, 1991).

The Nucleus Accumbens

Figuring prominently in the above circuit (see Figure 3) is the nucleus accumbens, a structure of the basal forebrain. This nucleus, and especially the dopaminergic synapses within it, appears to be the critical substrate for a number of abused drugs. Recent advances in the anatomy and histology of the nucleus accumbens have raised questions about its precise definition. The questions arise; what is the nucleus accumbens, and what does it do? In the following section and in the subsequent chapters, I will try to address these questions.

The nucleus accumbens, part of the ventral striatum, is located strategically
within the proposed reward circuit such that it receives afferent projections (input) from limbic structures and sends efferent projections (output) to areas involved with motor behavior. This has led to the intriguing suggestion that the nucleus accumbens may serve as an interface between limbic and motor areas, translating relevant motivational-emotional information into motor behavior (Mogenson and Yim, 1981).

The anatomy, histochemistry, and synaptic organization are similar in nucleus accumbens and striatum (Groenewegen, 1991; Voorn et al, 1986). In fact, while the nucleus accumbens is considered to lie ventral to the striatum, dorsal to the olfactory tubercle, and lateral from the medial septal area (see Figure 4), its borders are difficult to discern. The majority of the neurons of the striatal complex (>90%) are medium spiny neurons (Cajal, 1911; Kemp and Powell, 1971). These cells are primarily GABAergic, and may also contain enkephalin or substance P (Kita and Kitai, 1988; Gerfen and Young, 1988). They are the primary output cells of the striatal complex (Fruend et al, 1984; Chang and Kitai, 1985; Grofova, 1975; Bolam et al, 1981a; Bolam et al 1981b; Smith and Bolam, 1990) and receive many convergent inputs (Groenewegen 1991). Other cell types include large, aspiny cholinergic interneurons, medium aspiny GABAergic and somatostatinergic interneurons, and possibly glutamatergic interneurons (Fonnum and Walaas, 1981; Bolam et al 1984; Phelps et al 1985; Graybiel and Ragsdale, 1983; Parent, 1986; Bolam et al, 1983; Difiglia and Aronin, 1982). Furthermore, although the anatomy of the accumbens is similar to that of the striatum, important differences are also
The nucleus accumbens receives afferent (incoming) projections from the following: neocortex; regions of the allocortex including entorhinal, pyriform, and prefrontal cortex, and hippocampus; amygdala; thalamus; septum; olfactory tubercle; mesencephalic dopaminergic neurons including the A8 group, A9 group (substantia nigra pars compacta), and those originating in the ventral tegmental area (A10 group); and dorsal raphe nuclei (Domesick, 1981; Parent et al, 1981).

It is now apparent, however, that the accumbens is not a homogeneous nucleus. Recently, it has been shown via anatomical and histological means that the accumbens can be divided into two distinct compartments: a central core and a partially surrounding shell which lies on the ventral and medial border of the core (Zaborsky et al, 1985; Heimer and Alheid, 1991) (see Figure 4). The efferent projections from the core of the accumbens resemble those of the more dorsal striatum in cytoarchitecture and anatomy. Whereas both the core and the shell send efferent projections predominantly to the ventral pallidum and the substantia nigra/ventral tegmental area, the shell sends additional projections to the hypothalamus and extended amygdala (Heimer and Alheid, 1991). It has been suggested that the nucleus accumbens may represent a zone of transition between the ventral elements of the striatum, and the lateral portions of the extended amygdala (Heimer and Alheid, 1991).

Additionally, anatomical projections and neurochemical markers are heterogeneously distributed within the nucleus accumbens (and similarly in the striatum) in a mosaic-like pattern referred to as the patch and matrix pattern.
Figure 4. The nucleus accumbens. This cross section (1.2 mm anterior to bregma) demonstrates the major anatomical features of the accumbens and surrounding structures. Note the regions of core and shell denoted by dashed lines, and patch/striosome compartments (filled with dots) as opposed to the surrounding matrix. Abbreviations: (AbS) shell of nucleus accumbens; (AbC) core of nucleus accumbens; (ac) anterior commisure; (cc) corpus callosum; (CP) caudate-putamen or dorsal striatum; (CTX) cortex; (IC) islands of Calleja; (OT) olfactory tubercle. (Adapted from Paxinos and Watson, 1982).
(Herkenham, 1984; Gerfen, 1984; Gerfen, 1987; Groenewegen et al, 1987; Jimenez-Castellanos and Graybiel, 1987). This heterogeneity is similar in several species including rat, cat, monkey, and human (Graybiel, 1984). The geographic relationship between these two compartments has been likened to a piece of swiss cheese, in which the holes represent the patch or striosome compartment and the cheese represents the matrix compartment (Goldman-Rakic, 1982). While 95% of striatal neurons are of the medium spiny type (Kemp and Powell, 1971), and the cells of patch and matrix have very similar morphology and repetitive firing characteristics (Kawaguchi et al, 1989), there are important differences. The patch compartment is characterized by dense μ opiate receptor binding and high enkephalin and substance P immunoreactivity (Graybiel, 1990), and patches are more numerous in the ventral striatum and nucleus accumbens (White, 1989a). The matrix is characterized by high acetylcholinesterase activity (Graybiel and Ragsdale, 1978; Graybiel, 1990), tyrosine hydroxylase, cytochrome oxidase, and calbindinD28k-like immunoreactivity (Agwood and Emson, 1992). These studies suggest that matrix cells may be more metabolically active. Neurons containing somatostatin seem to connect the two compartments, and communication between the compartments may occur through these cells (Gerfen, 1984; Gerfen, 1987).

The anatomical connections of these two compartments are similarly distinct. While dopaminergic cells from the ventral mesencephalon project to both compartments, distinct subdivisions of these cells differentially innervate both patch and matrix. In rats, cells from the ventral tegmental area (A10) project principally
to the matrix in the nucleus accumbens and ventral striatum. Cells from the dorsal part of the substantia nigra pars compacta (A9) project to the matrix of the dorsal striatum, whereas cells from the ventral part of the substantia nigra pars compacta project to patches in the dorsal and ventral striatum and nucleus accumbens. Cells from a cluster in the ventral substantia nigra pars reticulata project to patches in the dorsal striatum (Gerfen, 1987; Jimenez-Castellanos and Graybiel, 1987; also see White, 1989a).

Interestingly, a recent study (Gerfen et al, 1987) demonstrated that dopaminergic innervation of the patch appears to develop earlier than that of the matrix. 6-OHDA was administered unilaterally into the striatum of one day old neonatal rats. After thirty days, the rats were sacrificed, and their brains were stained for tyrosine hydroxylase activity (a marker for dopaminergic axon terminals). It was discovered that dopaminergic innervation of the patch was absent, but that of the matrix was normal. These and supporting findings (see Fishell and van der Kooy, 1989) may explain much puzzling data involving neonatally lesioned rats.

Non-dopaminergic inputs to the patch and matrix compartments of the striatum and nucleus accumbens are equally distinct. The matrix compartment receives projections from areas including the hippocampus, sensory and motor cortex, and the cingulate gyrus, while the patch compartment receives projections from the amygdala and prefrontal cortex. Thus, a functional difference between these compartments has been proposed. The patch compartment is associated with
structures mediating affect related learning and memory, while the matrix compartment is associated with spatial memory and sensory-motor processing (Graybiel, 1990). In addition, it was recently demonstrated that modulation of dopamine release by NMDA in the patch compartment is functionally distinct from that of the matrix (Krebs et al., 1991). NMDA-evoked release appears to be greater in the matrix compartment, relative to patch (Krebs et al., 1991).

Efferent projections from patch and matrix compartments are equally distinctive. Whereas outputs from the patch compartment project to the area of the dopaminergic cell bodies of the ventral mesencephalon (A8, A9, A10) (Gerfen, 1984; Jimenez-Castellanos and Graybiel, 1989), outputs from the matrix project mainly to the pallidum and the substantia nigra pars reticulata (Jimenez-Castellanos and Graybiel, 1989).

Metabolic responses to drugs also vary between the two compartments. Recently, it has been shown that the psychostimulants amphetamine and cocaine induce distinct patterns of activation of the c-fos gene in the two compartments, patch and matrix, of both dorsal striatum and nucleus accumbens (Graybiel et al., 1990). Thus, while c-fos induced by amphetamine was more pronounced in striosomes, c-fos induced by cocaine occurred in both compartments. A pharmacological distinction between effects of the two stimulants was also observed as induction of c-fos by cocaine was inhibited by reserpine, whereas induction of c-fos by amphetamine was not. Induction of c-fos by both stimulants, however, was blocked by a D1 dopaminergic antagonist, suggesting that the D1
receptor mediates this effect and further suggesting that the adenylate cyclase/A
kinase or phospholipase/C kinase second messenger systems are involved. In
support, D1, but not D2, selective agonists have been shown to induce c-fos in the
6-OHDA dopamine depleted striatum of the rat (Robertson et al, 1989).

Role of Dopamine in Nucleus Accumbens

Dopaminergic synapses within in the nucleus accumbens are critically involved
in the phenomenon of reward. The following sections will review the anatomy,
biochemistry, electrophysiology; and behavioral role of dopamine in the nucleus
accumbens.

Anatomy of Dopaminergic Projections

The dopaminergic cell bodies of the ventral mesencephalon have ascending
projections to several forebrain areas (for review see Bjorkland and Lindvall, 1984;
Fuxe, 1965). The mesolimbic system originates in the ventral tegmental area
(A10) and projects mainly to the nucleus accumbens and amygdala. The
nigrostriatal system originates in the substantia nigra pars compacta (A9) and
projects predominantly to the striatum. The mesocortical system also originates
in the ventral tegmental area and projects to the prefrontal, cingulate and
entorhinal cortices. Grouped together, these systems form the mesotelencephalic
dopamine system. Projection patterns from this system are similar in rodents,
primates and man.
The large majority of cells in the nucleus accumbens and striatum are medium spiny neurons, that is, neurons with large spiny dendrites (Cajal, 1911; Kemp and Powell, 1971; Freund et al, 1984; Smith and Bolam, 1990). While some dopamine axons synapse on the cell bodies and dendritic shafts of the medium spiny output neurons, most synapse on the spines themselves (Fruend et al, 1984). Axons from the cortex (Bouyer et al, 1984), hippocampus (Totterdell and Smith, 1989; Groenwegen, 1991), and possibly amygdala (see Yim and Mogensen, 1986), which may use glutamate as a neurotransmitter (Fonnum et al, 1981; Christie et al, 1987; Fuller et al, 1987; also see Yim and Mogensen, 1989) synapse in close apposition to the dopamine terminals. Thus the dopamine synapses are in an ideal position to modulate information coming from the cells projecting from cortical regions (see Smith and Bolam, 1990; Groenewegen, 1991).

Biochemical Pharmacology of Dopamine

It now appears that there are five dopamine receptors, designated D1, D2, D3, D4 and D5 (see Strange, 1991; Snyder, 1990). Each of these receptor subtypes have now been cloned (Bunzow et al, 1988; Deary et al, 1990; Zhou et al, 1990; Sunahara et al, 1990; Strange, 1990; and Sokoloff et al, 1990). These dopamine receptors display an amino acid sequence consistent with the motif of seven transmembrane hydrophobic regions typical of receptors linked to a G protein. The D2 and D3 are closer in homology than with the D1 receptor, with the major differences lying in the third intracellular loop (shorter in D1) and carboxy-terminal
tail (longer in D1). Additionally, two new dopamine receptors have been cloned, D4 and D5 (Sunahara et al, 1991; Van Tol et al, 1991). Interestingly, the D4 receptor appears to have a high affinity for the atypical antipsychotic clozapine, and the D5 receptor appears to have a higher affinity for dopamine than the D1 receptor.

D1 receptors have been shown to activate the enzyme adenylate cyclase (Kebabian and Calne, 1979). The molecular structure, similar to the β2-adrenergic receptor which interacts with Gs, is consistent with this finding. D2 receptors are associated with dopamine nerve terminals and may function as autoreceptors and may regulate dopamine synthesis and release (Roth et al, 1978).

D1 and D2 receptors are found in most of the areas innervated by dopaminergic cells, including the nucleus accumbens (Wamsley et al, 1989). Interestingly, the D3 receptor has a limited distribution and appears to be enriched in limbic areas including the nucleus accumbens, olfactory tubercle, hypothalamus and the islands of calleja (Strange, 1991).

**Electrophysiology of Dopamine in Nucleus Accumbens**

Electrophysiological studies have revealed a complex role for dopamine. Activation of dopaminergic projections to the nucleus accumbens typically inhibits accumbens neurons (Yim and Mogenson, 1982) in anesthetized rats, probably by increasing the membrane potassium conductance. Studies using specific agonists have revealed that activation of D1 receptors by SKF-38393 and activation of D2
receptors by LY-141865 inhibit both spontaneous and glutamate-induced firing of accumbens neurons (White and Wang, 1986). On the other hand, studies using specific antagonists have revealed a different picture. Exogenous dopamine in the presence of a specific D2 antagonist, sulpiride, hyperpolarized accumbens cells via activation of D1 receptors; however, exogenous dopamine in the presence of a D1 antagonist, SCH-23390, lead to depolarization of accumbal neurons via activation of D2 receptors (Uchimara and North, 1990). In separate studies in the striatum, it was found that dopamine inhibited the basal firing rate of striatal cells without greatly affecting the firing rate of the same cells responding during the performance of a specific task (Rolls et al, 1984; Chiodo and Berger, 1986). It is becoming apparent that the electrophysiological actions of dopamine, although predominantly inhibitory, are more complex than previously thought.

Role of Nucleus Accumbens Dopamine in Behavior

There are basically three ways to study the contribution of dopaminergic systems in behavior (Evenden and Ryan, 1990). First is to destroy dopaminergic cells with the selective neurotoxin 6-hydroxydopamine (6-OHDA). 6-OHDA is taken up specifically by and selectively destroys catecholaminergic neurons. By using an appropriate uptake blocker, one can protect either dopaminergic or noradrenergic cells. For example, desmethylimipramine prevents the uptake of 6-OHDA into noradrenergic cells, thus sparing them. The second method involves directly administering dopaminergic antagonists into dopamine terminal fields, and
the third involves the direct administration of agonists into the terminal fields.

Studies using the techniques described above have revealed that dopamine in the nucleus accumbens plays an important role in the control of cognitive processes and emotional behavior. Lesions of the dopaminergic projection from the ventral tegmental area to the nucleus accumbens with 6-OHDA have been shown to reduce motivated behaviors such as exploratory behavior in a novel environment without producing motor impairment (Kelley and Stinus, 1983; Kelley et al, 1989) and to reduce food pellet hoarding (Kelley and Stinus, 1985). 6-OHDA lesions of the dopaminergic projection to the nucleus accumbens have also been shown to reduce displacement behavior (Robbins and Koob, 1980). In this paradigm, hungry rats were presented with a food pellet every 60 seconds, (more time than it takes for a rat to consume a food pellet). The displacement behavior, drinking while waiting for the next pellet, was increased in sham treated controls but not in 6-OHDA treated rats. The theory holds that the motivational excitement accompanying the delivery of a food pellet outlasts its consumption, in which case alternative (displacement) behaviors are evoked by available environmental stimuli (in this case a water bottle). Thus the 6-OHDA seems to produce a deficit not in the consumption of a pellet, but in the level of motivational excitement accompanying the pellet (Robbins and Koob, 1980).
INTERACTION OF DRUGS OF ABUSE WITH THE REWARD SUBSTRATE

As stated above, to elicit reward initially and ultimately result in addiction according to the positive reinforcement model, rewarding drugs must gain access to elements of the proposed reward system. The following sections will review the evidence that the major drugs abused by humans do indeed interact at some level with the proposed system.

Psychostimulants

Psychostimulants are characterized by their ability to increase locomotor activity and their ability to elicit reward. The prototype psychostimulants are amphetamine and cocaine, and these will be discussed in the following section. Psychostimulants are readily self administered intravenously by rats, monkeys, and humans (Petit et al, 1984; Roberts and Koob, 1982; Wilson and Schuster, 1972; Zito et al, 1985) and lower reinforcement thresholds for brain stimulation reward (Koob and Bloom, 1988; Kornetsky and Esposito, 1981). Cocaine blocks the reuptake of dopamine into the presynaptic terminal (Heikkila, 1975; Kuhar et al, 1991). Amphetamine acts similarly but also stimulates dopamine release (Axelrod, 1970; Carlsson, 1970; Kuczenski, 1983). Furthermore, psychostimulants increase extracellular concentrations of dopamine in the nucleus accumbens as measured by in vivo microdialysis (Di Chiara and Imperato, 1988). That this increase in synaptic dopamine is involved in the rewarding effects of psychostimulants is evidenced by the finding that dopamine antagonists inhibit the reward elicited by
both amphetamine (Wilson and Schuster, 1972; Yokel and Wise, 1975; Yokel and Wise, 1978) and cocaine (de Witt and Wise, 1977; Ettenberg et al, 1982) in animals. In addition, the euphoric effects of amphetamine are blocked by dopamine antagonists (Gunne et al, 1972) and dopamine depletion (Jonsson et al, 1971) in man.

Several lines of evidence indicate that these psychostimulants elicit locomotor activity and reward from augmentation of dopaminergic activity within the nucleus accumbens. Injection of psychostimulants into the nucleus accumbens (Pijnenburg et al, 1976; Kaddis et al, 1991), but not the caudate (Jackson et al, 1975), elicits increased locomotor activity in rats. Blockade of dopamine receptors (Lyon and Robbins, 1975; Swerdlow et al, 1986) or 6-OHDA lesions of the nucleus accumbens (Kelly et al, 1975) block psychostimulant induced locomotor activity.

A role for the nucleus accumbens in mediating amphetamine reward is well established. Indeed, amphetamine is readily self-administered into the nucleus accumbens (Hoebel et al, 1983). Administration of amphetamine into the nucleus accumbens elicits a conditioned place preference (Carr and White, 1983; Carr and White, 1986). 6-OHDA lesions of the nucleus accumbens attenuate amphetamine self-administration (Lyness et al, 1979) and $\alpha$-flupenthixol co-injected into the nucleus accumbens with amphetamine on training days inhibits the conditioned place preference elicited by amphetamine (Aulisi and Hoebel, 1983).

The role of dopamine in the nucleus accumbens in mediating cocaine reward is less well established. 6-OHDA lesions of the nucleus accumbens (Petit et al,
1984) or ventral tegmental area (Roberts and Koob, 1982; Zito et al, 1985) attenuate or block cocaine-induced reward as measured by self-administration. Cell body lesions of the nucleus accumbens or ventral pallidum attenuate cocaine reward as well (Zito et al, 1985).

In contrast, lesions with 6-OHDA of the nucleus accumbens (Spyraki et al, 1982b) and systemic administration of dopamine antagonists (Spyraki et al, 1982b; Mackey and van der Kooy, 1985) have been reported not to inhibit a conditioned place preference elicited by intraperitoneally administered cocaine. However, dopamine antagonists do block the place preference elicited by intravenous (Spyraki et al, 1987) and intracerebroventricular (Morency and Beninger, 1986) cocaine. Furthermore, intraperitoneally administered procaine, which shares cocaine's local anesthetic effect but not central stimulant effect, elicits place preference (Spyraki et al, 1982b). Thus Spyraki et al have concluded that cocaine elicits place preference by enhancing dopamine transmission in the nucleus accumbens, and by producing local anesthetic effects in the periphery (Spyraki et al, 1982b; Spyraki et al, 1987).

While cocaine is readily self administered into the prefrontal cortex (which receives innervation from the mesocortical dopamine system originating in the ventral tegmental area), it is not self administered into nucleus accumbens (Goeders and Smith, 1983). On the other hand, a separate but elegant study suggests a role for the nucleus accumbens (Wood and Emmett-Oglesby, 1989). Rats were trained to distinguish a rewarding, systemic dose of cocaine. When rats
were trained sufficiently, cannulae were implanted into either the nucleus accumbens or the prefrontal cortex. Intra-accumbens cocaine generalized with systemic cocaine, but intra-prefrontal cortex cocaine did not. One hypothesis which integrates these two sets of conflicting data suggests that the prefrontal cortex mediates the initiation of cocaine action, while the nucleus accum bens is necessary for maintenance of cocaine action.

The studies reviewed above suggest that the psychostimulants, amphetamine and cocaine, interact at some level in the proposed system to elicit locomotor activity and reward. Additionally, the above studies implicate the nucleus accum bens (and possibly the prefrontal cortex) as substrates of locomotor activity and reward. The finding that cell body lesions of the ventral pallidum, the main projection area of the nucleus accum bens in the proposed pathway, attenuate cocaine reward (Zito et al, 1985) suggests that lesions "downstream" from the site of action will similarly attenuate reward.

**Opiates**

Opiates, morphine and heroin are prototypes, have been used (and abused) by humans for centuries. The use of opium (the juice derived from the opium poppy *Papaver somniferum* and source of morphine) was first recorded by Theophrastus in the third century B.C., and its use probably started much earlier. A great deal of evidence indicates that opiates may also interact with the proposed reward circuit. It further appears that opioid µ and δ receptors are involved: both are
found in the nucleus accumbens and ventral pallidum, and µ receptors are found in the ventral tegmental area (Atweh, 1983; Mansour et al, 1987). At appropriate doses, opiates increase locomotor activity when injected systemically (Swerdlow et al, 1986) or into the ventral tegmental area (Joyce and Iversen, 1979). Opiates are readily intravenously self-administered (Bonese et al, 1974; Van Ree et al, 1978; Weeks and Collins, 1964; Thompson and Schuster, 1964; Petit et al, 1984), lower thresholds for brain stimulation reward (Marcus and Kornetsky, 1974; Bush et al, 1976; Koob et al, 1975; Reid, 1987), and elicit place preference (Bozarth and Wise, 1981; Phillips and Le Piane, 1982; van der Kooy et al, 1982; Spyraki et al, 1983; Bechara and van der Kooy, 1989; Hoffman, 1989). The precise neural substrate within the reward circuit, however, is a matter of some debate.

A substantial body of evidence indicates that opiates may act at the level of the ventral tegmental area to increase firing rate of dopaminergic cells that project to the nucleus accumbens. Thus, opiates administered into the ventral tegmental area elicit place preference (Phillips and LePiane, 1980; Phillips and LePiane, 1982; Bozarth and Wise, 1981; Bozarth, 1987). Opiate antagonists administered into the ventral tegmental area inhibit intravenous heroin administration (Britt and Wise, 1983; Vaccarino et al, 1985). Furthermore, opiates increase the extracellular concentration of dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988). Morphine administered into the ventral tegmental area elicits dopamine dependent locomotor activity (Joyce and Iversen, 1979). Finally, neuroleptics inhibit intravenous heroin self-administration and heroin induced place preference.

On the other hand, opiates may also act in the nucleus accumbens to elicit reward. Morphine (Olds, 1982) and methionine enkephalin (Goeders et al, 1984) are self administered into the nucleus accumbens, and intra-accumbens morphine elicits place preference (van der Kooy et al, 1982). Intra-accumbens α-flupenthixol (a dopaminergic antagonist), at a dose that blocks amphetamine locomotor activity, did not block heroin locomotor activity (Vaccarino et al, 1986). Administration of an opiate receptor antagonist into the nucleus accumbens attenuates heroin self-administration (Vaccarino et al, 1985) and locomotor activity (Amalric and Koob, 1985). While dopamine receptor blockade or 6-OHDA lesions of the nucleus inhibit cocaine or amphetamine self administration, these treatments spare heroin self administration (Petit et al, 1984; Zito et al, 1985). Neuroleptics block amphetamine elicited place preference but not morphine elicited place preference (Mackey and van der Kooy, 1985). Cell body lesions of the nucleus accumbens or ventral pallidum attenuate opiate reward as well (Zito et al, 1985).

It is likely that some of the seemingly contradictory data described above reflect procedural differences. While further studies will be required to resolve this issue, it is clear that opiates act at a site in the proposed circuit to elicit reward. The results reviewed above suggest that opiates may interact with the circuit at two levels; at the μ receptor in the ventral tegmental area (activating dopaminergic projections to the nucleus accumbens via disinhibition) and/or in the nucleus accumbens (possibly at μ or δ receptors) via a mechanism independent of
dopaminergic activity (Koob and Bloom, 1988).

**Ethanol**

Ethanol is widely abused by humans. Ethanol has also been shown to be self-administered intravenously by rats (Smith and Davis, 1974; but see also Collins *et al*, 1984) and rhesus monkeys (Deneau *et al*, 1969).

Ethanol increases firing of the A10 dopaminergic neurons of the ventral tegmentum which project to the nucleus accumbens (Gessa *et al*, 1985), resulting in an increase in the extracellular concentration of dopamine in the nucleus accumbens (Di Chiara and Imperato, 1988). These results also suggest an interaction with the proposed pathway.

**Nicotine**

Nicotine is self-administered intravenously in several species including rat, dog, rhesus monkey, squirrel monkey and baboon (Lang *et al*, 1977; Risner and Goldberg, 1981; Deneau and Inoki, 1967; Goldberg *et al*, 1981; Ator and Griffiths, 1983). Furthermore, nicotine elicits locomotor activity in rats (Museo and Wise, 1990), increases the firing rate of dopaminergic neurons of the ventral tegmental area (Merey *et al*, 1987), and increases dopamine levels in the nucleus accumbens (Imperato *et al*, 1986). Nicotine appears to produce these effects either through action at nicotinic receptors at cell bodies in the ventral tegmental area which cause an increase in firing or by action at terminal fields which
enhance the release of dopamine (Misfud et al., 1989; Rowell et al., 1987). Indeed, nicotine binding sites have been located autoradiographically in the ventral tegmental area and the nucleus accumbens (Clarke and Pert, 1985).

**Δ9-Tetrahydrocannabinol**

The active ingredient in marijuana, Δ9-tetrahydrocannabinol (THC) has been shown to be habit forming in humans and has been used for many centuries for its effects. THC facilitates brain stimulation reward in rats (Gardner et al., 1988), is self-administered in monkeys (Pickens et al., 1973) and rats (Takahashi and Singer, 1979), and enhances dopamine efflux from nucleus accumbens (Chen et al., 1989) and prefrontal cortex (Chen et al., 1990).

Thus it appears that drugs of abuse with different primary mechanisms of action initiate drug dependence by interacting with elements of a common reward pathway (Koob and Bloom, 1988; Wise and Bozarth, 1984). This reward circuit may become modified through chronic use, giving rise to opposing processes as the homeostatic mechanisms of the brain counter the drug effects (Koob and Bloom, 1988). The opposing processes may result in the aversive effects (i.e., dysphoria, malaise) of abstinence and may be a major motivating force in the maintenance of behaviors associated with drug addiction (Koob and Bloom, 1988).
OTHER NEUROTRANSMITTER SYSTEMS INVOLVED IN REWARD AND LOCOMOTION

Although a great deal of evidence implicates a role for dopamine in mediating reward and locomotion, other neurotransmitter systems certainly must play a role as well. Of particular interest with respect to the following chapters, neural systems utilizing excitatory amino acids, endogenous opioids, and serotonin as neurotransmitters appear to interact with dopamine systems and thus play a role in reward and locomotor activity. The following sections will review the anatomy, pharmacology, and possible functional role (with regards to reward and locomotion) of these systems.

Pharmacology of The Excitatory Amino Acid System

Endogenous Ligands

Excitatory amino acids, L-glutamate and related acidic amino acids, are considered to be the predominant excitatory neurotransmitters in the mammalian brain (Fagg and Foster, 1986; Fonnum, 1984). Aspartate, L-cysteine sulfinate, and quinolinate (all excitatory amino acids) are found endogenously and may be neurotransmitters as well. A great deal of evidence suggests that excitatory amino acids function as neurotransmitters (for review see Meldrum, 1985; Boldry, 1990). Not surprisingly, it has been demonstrated that fibers utilizing excitatory amino acids as neurotransmitters are found in the nucleus accumbens. By using a combination of $[^3H]$D-aspartate and wheat germ agglutinin-horse-radish peroxidase
(WGA-HRP) and studying that subset of cells labelled by WGA-HRP also labelled by the \[^{3}H\]D-aspartate, Fuller et al (1987) demonstrated that the nucleus accumbens receives prominent glutamatergic projections from the prefrontal and olfactory cortex, the subiculum, various nuclei of the amygdala (most prominent the lateral and basolateral nuclei), and the midline nucleus of the thalamus. In addition, glutamatergic interneurons have been demonstrated in the nucleus accumbens (Fonnum and Walaas, 1981). Finally, all three of the functionally defined ionotropic receptors - AMPA, kainate, and NMDA - have been reported in the nucleus accumbens (Cotman et al 1987).

**Receptors and Pharmacology**

Several distinct receptor subtypes have been established at which excitatory amino acids act. Unfortunately, different methods of analyzing function (electrophysiology, pharmacology) have resulted in different classification schemes and some degree of confusion. At present, however, excitatory amino acid receptors are characterized by the agonist which activates them, and they appear to occur in three or four distinct groups; 1) the ionotropic NMDA receptor, 2) the ionotropic AMPA and Kainate receptors, 3) the metabotropic receptor, and possibly 4) an AP4 sensitive autoreceptor (see Table 1).

The most extensively studied is the NMDA receptor (see Stone and Burton, 1987) at which NMDA is a selective agonist in electrophysiological studies. Activation of this receptor leads to the opening of an ion channel that is permeable
### Table 1. Pharmacological Classification of Excitatory Amino Acid Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist</th>
<th>Antagonist</th>
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<tr>
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<td>NMDA Site:</td>
<td>NMDA Site:</td>
</tr>
<tr>
<td></td>
<td>Ibotenate</td>
<td>AP-5</td>
</tr>
<tr>
<td></td>
<td>L-Glutamate</td>
<td>AP-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPP</td>
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<tr>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>LY-233053</td>
</tr>
<tr>
<td>Glycine Site:</td>
<td>Glycine Site:</td>
<td>Glycine Site:</td>
</tr>
<tr>
<td></td>
<td>Glycine</td>
<td>Cycloleucine</td>
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<tr>
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<td>Cycloserine</td>
<td>ACPC</td>
</tr>
<tr>
<td>Channel Site:</td>
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<tr>
<td></td>
<td></td>
<td>MK-801</td>
</tr>
<tr>
<td>AMPA</td>
<td>AMPA</td>
<td>DNQX</td>
</tr>
<tr>
<td></td>
<td>Quisqualate</td>
<td>CNQX</td>
</tr>
<tr>
<td></td>
<td>Willardine</td>
<td>NBQX</td>
</tr>
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<td></td>
<td>L-Glutamate</td>
<td>GAMS</td>
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<tr>
<td></td>
<td></td>
<td>GDEE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Joro Spider Toxin</td>
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<td>Kainate</td>
<td>Kainate</td>
<td>DNQX</td>
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<tr>
<td></td>
<td>Domoate</td>
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<td>GAMS</td>
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<td></td>
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<td>GDEE</td>
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<td>Metabotropic</td>
<td>Quisqualate</td>
<td>AP-3</td>
</tr>
<tr>
<td></td>
<td>Trans-ACPD</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: (ACPC) 1-aminocyclopropanecarboxylic acid; (ACPD) 1-amino-1,3-cyclopentane-dicarboxylic acid; (ADCP) cis-1-amino-1,3-dicarboxycyclopentane; (AMPA) α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; (AP-3) 2-amino-3-phosphonopropionic acid; (AP-5) 2-amino-5-phosphonovaleriv acid; (AP-7) 2-amino-7-phosphonoheptanoic acid; (CNQX) 6-cyano-7-nitroquinoxaline-2,3-dione; (CPP) 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate; (DNQX) 6,7-dinitroquinoxaline-2,3-dione; (GAMS) D-glutamylamino-methyl sulfonate; (GDEE) glutamic acid diethylester; (MK-801) [(+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine maleate]; (NBQX) 2,3-dihydroxy-6-nitro-7-sulfamoyl benzo(f)quinoxaline; (NMDA) N-methyl-D-aspartate.
to sodium, potassium and calcium (MacDermott et al, 1986). This conductance, however, is voltage dependent and inhibited by magnesium (Ascher and Nowak, 1988). In the absence of Mg\(^{2+}\) the channel is not voltage dependent (Nowak et al, 1984; Mayer et al, 1984). Thus, the channel is closed at or below resting membrane potential. As the cell is depolarized, the Mg\(^{2+}\) mediated channel block is lost, and current (carried mostly by sodium but also by potassium and calcium ions) flows through the channel. Recently, a receptor has been cloned with pharmacology consistent with the NMDA receptor (Moriyoshi et al, 1991). This NMDA receptor shares some homology with the AMPA/kainate receptors. It is of similar size and has a large N-terminal region and four transmembrane domains. A negatively charged region of glutamate residues, which precedes the second transmembrane domain, may be involved in voltage-dependent Mg\(^{2+}\) block (Moriyoshi et al, 1991). Although this receptor is fully functional, it probably represents a subunit in a homomeric molecular complex. Since depolarizations elicited in Xenopus oocytes by RNA synthesized in vitro from the clone were much smaller than depolarizations elicited from RNA isolated from rat brain, it is likely that other receptor subunits exist. Furthermore, additional NMDA receptors have been proposed (Monaghan et al, 1989; Stone and Burton, 1987), and another possible NMDA receptor subunit has been cloned with different sequence (Kumar et al, 1991; see also Mayer, 1991).

Several sites on the NMDA receptor-ionophore complex exist at which conductance can be regulated. Glycine, acting at a strychnine insensitive binding
site which colocalizes with the NMDA site, appears to be required for activation of NMDA receptors (Kleckner and Dingledine, 1988). In fact, two classes of NMDA receptor based on their differential regulation by glycine have been proposed (Monoghan et al, 1988). The polyamines spermine and spermidine appear to enhance NMDA receptor function, while the polyamine putrescine (which may be a spermine antagonist) appears to inhibit receptor function (Sacaan and Johnson, 1990). The transition metal zinc has also been shown to block the action of NMDA on cortical neurons (Peters et al, 1987).

The pharmacology of the NMDA receptor has been extensively reviewed elsewhere, (see Meldrum, 1985; Boldry, 1990; Watkins and Collingridge, 1989; Willetts et al, 1990), and NMDA is the agonist of choice for this receptor. Antagonists are of three types, competitive antagonists at the NMDA recognition site, non-competitive antagonists which appear to act as channel blockers, and glycine antagonists. Among competitive antagonists, AP5, AP7, CPP, CGS-19755, NPC12626, and LY233053 have been used, while among noncompetitive channel blockers, MK-801 (dizocilpine) is the drug of choice (see Willetts et al, 1990). As the channel blockers such as MK801 and PCP appear to actually bind in the ion channel, they are use dependent; thus, the channel must be open in order for them to act as antagonists. D-Cycloserine is an agonist, while cycloleucine and ACPC appear to function as antagonists at the strychnine-insensitive glycine site.

The second excitatory amino acid receptor group is made up of the AMPA and kainate ionotropic receptors, often defined negatively as non-NMDA receptors.
Activation of these receptors appears to open a voltage independent ion channel permeable to sodium and potassium (See Fagg et al, 1986). Furthermore, these receptors (AMPA/kainate) are hypothesized to be the primary mediators of rapid excitatory synaptic transmission (Fagg et al, 1986). While agonists seem to have some degree of selectivity in distinguishing between AMPA and kainate activated responses, antagonists for the AMPA and kainate excitatory amino acid receptors - including GAMS and the quinoxilinediones DNQX, CNQX, and NBQX - do not. Anatomical data suggest that the AMPA and kainate receptors are distinct, as these receptors appear to have different distributions in mammalian brain as measured by autoradiography (Monaghan et al, 1984). Functional data indicate that in primary afferent C fibers kainate evokes responses whereas AMPA does not (Agrawal and Evans, 1986). Much electrophysiological data seems to suggest that the AMPA and kainate receptors are actually the same (Jahr and Stevens, 1987; Dingledine, 1991). The use of molecular cloning techniques may, however, have resolved the issue.

Recently, a number of non-NMDA excitatory amino acid receptor subunits have been cloned and sequenced (Barnard and Henley, 1990; Miller, 1991; Dingledine, 1991). They are termed GluR1 through GluR6, in order of their discovery, and KA-1. These subunits may be arranged in a manner similar to that of the nicotinic acetylcholine receptor. Recall that the nicotinic receptor is composed of five subunits, arranged to form a channel. Thus the non-NMDA excitatory amino acid receptors may have a similar structure. Different combinations of the recently
discovered subunits may account for their functional properties. When expressed in various combinations in *Xenopus* oocytes, GluR1 through GluR4 appear to have the characteristics of an AMPA receptor: more sensitive to AMPA than kainate, rapid desensitization after AMPA, and competitive blockade by CNQX. GluR5, GluR6, and KA-1 appear to have the characteristics of kainate receptors: bind kainate with high affinity, insensitive to or only marginally activated by AMPA. Thus the molecular biology data suggests the existence of distinct receptor subtypes.

A third type of excitatory amino acid receptor is the metabotropic receptor. This receptor is sensitive to pertussis toxin and appears to be linked via a G protein, Gi, to the phosphoinositide second messenger system (Anwyl, 1991). Thus, activation of phospholipase C and subsequent liberation of inositol 1,4,5-triphosphate leads to calcium mobilization from internal stores. Quisqualic acid and trans-ACPD appear to be agonists at this receptor, while AP3 is an antagonist. A fourth possible excitatory amino acid receptor is sensitive to AP-4. While AP-4 antagonizes electrically evoked excitatory responses at glutamatergic synapses (measured as inhibition of synaptic transmission) in the cortex, it doesn’t block responses elicited by exogenous glutamatergic agonists. Therefore, AP-4 appears to act presynaptically (Davies and Watkins, 1982; Forsythe and Clements, 1988). Thus there is some speculation that this receptor may represent a glutamatergic autoreceptor.

The interaction between these various receptor subtypes leads to interesting
functional implications. In many brain regions, the excitatory post synaptic potential (EPSP) is composed of two components: a fast, rapidly decaying component associated with AMPA/kainate receptors; and a slower NMDA mediated component. Furthermore, AMPA and NMDA receptors appear to be colocalized in many brain regions (Monoghan et al, 1984; Rainbow et al, 1984). This evidence suggests that these receptors may lie on the same neurons. By working together, the excitatory amino acid receptors confer unique abilities on the cells that express them.

First, an amplification system is achieved. As glutamate is released, it acts at AMPA/kainate receptors initially thereby depolarizing the cell. At higher levels of depolarization, magnesium block is relieved at the NMDA receptor, and further depolarization is achieved. Thus, the NMDA receptor acts as an amplifier of large EPSPs.

Second, a filtering or selective-activation system may be achieved. As stated above, the NMDA-elicited component of the EPSP is slow, and several factors may account for this. The NMDA receptor has a lower dissociation constant for glutamate, thus, glutamate associates with the receptor longer. The rate of desensitization may also be different (longer for the NMDA receptor). Thus, if depolarizations mediated by AMPA/kainate receptors are occurring frequently, a cumulative depolarization occurs sufficient to open the NMDA channel. In fact, responses in cells of the ventrobasal thalamus evoked by high frequency electrical stimulation of an afferent pathway were blocked by AP5, but responses evoked by
low frequency electrical stimulation of the afferent pathway were not blocked by AP5 (Salt, 1986). Thus it seems that cells expressing the NMDA and non-NMDA receptors are able to distinguish low and high frequency stimulation.

Third, expressing both receptors may enable cells to act as pacemakers (Wallèn and Grillner, 1987). Following an initial depolarization by sodium influx (possibly by AMPA/kainate receptors), NMDA receptors will open, allowing the influx of calcium. This depolarization is initially opposed via voltage gated potassium channels; however, as calcium enters the cell, calcium-dependent potassium channels will also open, which gradually repolarize the cell. At a sufficient level of repolarization, NMDA receptors will close, accelerating repolarization. Because calcium ions mediate repolarization, when NMDA receptors close (due to voltage dependent Mg$^{++}$ block) and intracellular calcium decreases, a gradual depolarization occurs. If this reaches a level which relieves Mg$^{++}$ block, the cycle is ready to begin again (Wallèn and Grillner, 1987).

Fourth, the entry of calcium through the NMDA receptor is probably responsible for the induction of long term potentiation (LTP), (for review see Cotman and Monaghan, 1988). LTP was first described in hippocampal cells, in which brief but high frequency electrical stimulation of hippocampal neurons was followed by a long-lasting enhancement of synaptic function (Bliss and Lomo, 1973). Following this stimulation, possibly occurring via non-NMDA ionophore receptors, sufficient depolarization is reached to relieve magnesium blockade and open the NMDA channel, allowing the influx of calcium. The increase in the intracellular
concentration of calcium causes the activation of kinase enzymes and calmodulin. These events may cause induction of gene expression in the post synaptic neuron, such as induction of c-fos, and subsequent transcription of proteins which may elicit structural changes at active synapses or, upon secretion, elicit changes in presynaptic terminals. These changes ultimately result in a long term enhancement in the way the cell responds to a subsequent stimulation, or, in other words, a long term modification at the synaptic level. LTP has been implicated in learning and memory, synaptic plasticity, and satisfies the definition of a Hebbian synapse (Hebb, 1949). A Hebbian synapse is one in which the strength of the synapse will be increased when pre and post synaptic activity occur simultaneously. Neurons rich in NMDA receptors fit this description, as both postsynaptic depolarization (sufficient to relieve magnesium blockade) and presynaptic transmitter release (glutamate) must both occur.

Furthermore, the metabotropic receptor may be involved. There is evidence suggesting that it plays a role in both LTP and the down sensitization of AMPA receptors seen in the cerebellum during another form of neuronal memory, long term depression (see Anwyl, 1991).

**Behavioral Functions of the System**

There are numerous glutamatergic projections to the accumbens (Fuller et al, 1987), and three functionally defined ionotropic receptors (see below) have been reported in the accumbens (Cotman et al 1987). Behaviorally, direct injection of
excitatory amino acids into the nucleus accumbens results in increases in locomotor activity (Arnt, 1981; Donzanti and Uretsky, 1983). The locomotor activity elicited by excitatory amino acids can be inhibited by intra-accumbens administration of excitatory amino acid antagonists, including GDEE, GAMS, and DNQX (Donzanti and Uretsky, 1984a; Donzanti and Uretsky 1984b; Shreve and Uretsky, 1988; Boldry, 1990). Interestingly, increases in locomotion elicited by psychostimulants can also be reduced by excitatory amino acid antagonists including AP5, GDEE, and DNQX (Pulverenti et al, 1991; Willins et al, 1992; Kaddis et al, 1991), and locomotor activity elicited by excitatory amino acids is reduced by treatments which impair dopaminergic transmission (Boldry, 1990; Donzanti and Uretsky, 1984a). These data suggest interactions at the level of the nucleus accumbens between dopaminergic and excitatory amino acid systems (see Chapter II).

**Pharmacology of Opioids**

**Endogenous Ligands**

Endogenous opioids (i.e., compounds that are made in the brain and act on the same receptors as morphine-like compounds) derive from one of three precursors which comprise the opioid peptide family. These precursors include proopiomelanocortin (source of β-, and γ-endorphin), proenkephalin (source of the enkephalins), and proenkephalin B (source of dynorphin). All the opioid peptides contain the sequence Tyr-Gly-Gly-Phe-X, with X being Met or Leu (for review see
Receptors and Pharmacology

Opiate binding sites were first discovered in 1973 (Pert and Snyder, 1973). Since then a number of receptors have been characterized, including the μ1, μ2, δ, κ, and ε receptors (for review see Chang, 1984). Of these μ, δ, and κ receptors are the best characterized, with morphine, enkephalin, and ketocyclazocine acting as respective agonists. All of these receptors have more selective agonists and antagonists (see Table 2), but all are antagonized by naloxone. These receptors display a regional distribution within mammalian brain (for review see Atweh, 1983; Mansour et al, 1987). The neurophysiological role of these receptors appears to be activation of a G protein, which results in either an increase in potassium current (thus hyperpolarizing the cell and decreasing firing rate); or a decrease in calcium influx (thus accounting for a decrease in transmitter release).

Behavioral Functions of the System

Opiate receptors appear to play a major role as neuromodulators, affecting the release or post-synaptic response to a wide variety of neurotransmitters including dopamine, epinephrine, serotonin, acetylcholine, and substance P. Opiate receptors have been implicated in a host of physiological and behavioral functions. Physiological roles include analgesia and pain perception, stress regulation, and respiratory and temperature regulation. Opioid receptors also appear to play a role
Table 2. Pharmacological Classification of Opioid Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist</th>
<th>Antagonist</th>
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<tbody>
<tr>
<td>μ</td>
<td>Morphine</td>
<td>Naloxone</td>
</tr>
<tr>
<td></td>
<td>DAMGO</td>
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<tr>
<td></td>
<td></td>
<td>Naltrindole</td>
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<td>κ</td>
<td>Ketocyclazocine</td>
<td>Naloxone</td>
</tr>
<tr>
<td></td>
<td>U69593</td>
<td>nor-binaltorphimine</td>
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<td>ICI197067</td>
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Abbreviations: (CTAP) D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂; (DAGO) Tyr-D-Ala-Gly-MePhe-Glyol; (DPDPE) Tyr-D-Pen-Gly-Phe-D-Pen; (DTLET) [D-Thr²]leucine enkephalin-Thr; (ICI74864) N,N-bisallyl-tyr-Aib-Aib-Phe-Leu-OH {Aib: aminoisobutyrate}; (U50488H) trans-(±)-3,4-dichloro-n-methyl-[2-(1-pyrrolidiny]cyclohexyl] benzeneacetamide methane sulphonate
in behavioral functions including feeding, movement regulation including both catalepsy and locomotor activity, and emotional behavior involving regulation of stress and reward. Disorders of opioid systems may play a role in the etiology of schizophrenia and depression (for a general review, see Simon and Hiller, 1989; Hughes et al, 1984).

Pharmacology of the Serotonin System

Endogenous Ligands

Serotonin (5-hydroxytryptamine, 5-HT) neurons originate in raphe nuclei of the mesencephalon. The firing of serotonin cells of the raphe nuclei is normally regular and appears to correlate with the state of arousal. Thus, raphe neuron activity is high during waking hours and low during REM sleep (Aghajanian and Vandermaelen, 1986). On the other hand, lesions of the serotonergic system generally produce behavioral activation (Fibiger and Campbell, 1971). The nucleus accumbens receives serotonergic projections from the medial and dorsal raphe nuclei (Parent et al, 1981).

Receptors and Pharmacology

Serotonin exerts its actions by binding to any of several distinct receptors, including the 5-HT₁ (subdivided into 5-HT₁ₐ, 5-HT₁₈, 5-HT₁c), 5-HT₂, 5-HT₃, and 5-HT₄ receptors, each having distinct agonists (see Table 3). The antagonist pharmacology is still in its early stages, and few selective antagonists are known
Table 3. Pharmacological Classification of 5-HT Receptors

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Agonist</th>
<th>Antagonist</th>
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<td>5-HT$_{1A}$</td>
<td>8-OH-DPAT</td>
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<td>5-HT$_{1C}$</td>
<td>(+)S-α-Methyl-5-HT</td>
<td>Mesulergine</td>
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<td>5-methoxytryptamine</td>
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Abbreviations: (5-HT) 5-hydroxytryptamine; (CGS-12066B) (±)-12,5-dimethoxy-4-iodo-2-aminopropane; (DOI) (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl; (8-OH-DPAT) 8-hydroxy-2-(di-n-propylamino)-tetralin HBr; (mCPP) 1-(3-Chlorophenyl)piperazine HCl; (MDL-7222) 3-tropanyl-3,5-dichlorobenzoate; (NAN-190) 1-(2-methoxyphenol)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide; (TFMPP) m-trifluoromethylphenylpiperizine.
(for review see Peroutka, 1988; Sanders-Bush, 1989). The 5-HT$_1$, 5-HT$_2$, and 5-HT$_4$ receptors all appear to act through a G protein linked to second messenger systems and mediate slow modulatory responses. The 5-HT$_{1A}$, 5-HT$_{1C}$, and 5-HT$_2$ receptors have been cloned and display the seven transmembrane domains typical of receptors in the G-protein coupled superfamily (see Hartig, 1989). While the 5-HT$^{1A}$, 5-HT$_{1B}$, and 5-HT$_4$ receptors appear to be linked to adenylate cyclase, 5-HT$_{1C}$ and 5-HT$_2$ receptors (which display great homology) are linked to the phosphoinositide second messenger system (Hartig, 1989).

In contrast, the 5-HT$_3$ receptor - cloned recently (Maricq et al, 1991) - activates fast depolarizing currents in neurons. While only one subunit has thus far been cloned (termed 5-HT$_3$R-A), it is likely that there are others, characteristic of the excitatory ligand-gated ion channel superfamily. It is also likely that these subunits form a heteromeric complex; however, 5-HT$_3$R-A alone expressed in oocytes was able to form a functional receptor-ionophore with pharmacology and electrophysiology consistent with the 5-HT$_3$ receptor. 5-HT$_3$R-A has 27% homology with the nicotinic acetylcholine receptor and has four transmembrane domains (termed M1 through M4), a large amino-terminal extracellular domain, and a large intracellular domain connecting M3 to M4 (Maricq et al, 1991).

**Behavioral Functions of the System**

Serotonin is thought to play a role in many behavioral and physiological events, such as the regulation of body temperature, appetite and affective state.
Serotonin neuronal systems have also been shown to modulate locomotor activity mediated by the dopaminergic system (for review see Gersen and Baldessarini, 1980; Soubrie et al, 1984; see also chapter IV).

Serotonin systems may also be involved in the reward pathway. Lysergic acid diethylamide (LSD), a hallucinogen which decreases the firing of raphe neurons (see Aghajanian and Vandermaelen, 1986) possibly by acting as a 5-HT autoreceptor agonist, is used by humans to induce a state of altered perception (Jaffe, 1985). LSD and related indolamines may be agonists at 5-HT₁₅ and 5-HT₂ receptors (Aghajanian et al, 1988; Rasmussen and Aghajanian, 1990). While this drug is not rewarding in animal models (Jaffe, 1985), it does have discriminative stimulus properties. Simply, although animals will not work to get LSD, they can tell the difference between LSD and saline (Broadbent and Appel, 1990). The nucleus accumbens may be involved in the subjective effects of LSD. Animals cannot discriminate between systemic injections of LSD and injections of LSD in the nucleus accumbens, but can discriminate between systemic injection and injection into the caudate nucleus (Neilsen and Scheel-Kruger, 1986).

As is quite often the case, literature involving serotonin and drug reward in animal models is contradictory and confusing. Serotonin antagonists block amphetamine self-administration (Lecesse and Lyness, 1984; see also Lyness and Moore, 1983) and both amphetamine- and morphine-induced place preference (Nomikos and Spyraki, 1988). On the other hand, increasing serotonin transmission also decreases self-administration of (Lecesse and Lyness, 1984) and
conditioned place preference elicited by amphetamine (Kruszewska et al, 1986). 5-HT\textsubscript{3} antagonists block morphine- and nicotine-induced place preference, but not amphetamine-induced place preference (Carboni et al, 1989). Lastly, a recent study has proposed that the serotonin system may play a role in setting limits on the "value" of certain reinforcers (Woger et al, 1991). These studies suggest that 5-HT plays a complex role in drug reward, and more study is required to understand the substrates at which serotonin exerts its effects.

**STATEMENT OF THE PROBLEM**

Recent research involving the neurobiology of drug reward has suggested that abused drugs trigger activity in a common reward circuit (Koob and Bloom, 1988; Wise and Bozarth, 1984). This common reward circuit involves neurons which form a ventral tegmentum-nucleus accumbens-ventral pallidum-frontal cortex pathway (Koob and Bloom, 1988). Elements of this circuit also appear to comprise the neural substrate of locomotor activity. The overlap of the neural substrates for both locomotor activity and reward has led to the tempting hypothesis that this common substrate may mediate goal-directed behavior (Swerdlow et al, 1986).

The positive reinforcement model of addiction holds that it is drug reward which motivates an addict to use a drug. The nucleus accumbens, an integral part of the proposed reward substrate, appears to be an interface between limbic and motor areas of the brain (Mogenson and Yim, 1981). A pathway through the nucleus accumbens and incorporating dopamine (Kuczenski, 1983) forms the neural
substrate of opiate and psychostimulant action (Swerdlow, 1986; Evenden and Ryan, 1990) and seems to mediate both the rewarding and locomotor effects of several classes of drugs. Thus, the purpose of the present research was to study the chemoanatomy of the reward substrate (with emphasis on the nucleus accumbens) by examining the effects on locomotor activity and reward (as measured by conditioned place preference) of various classes of drugs which might interact with this pathway.

While a great deal is known about the role of dopamine systems in the nucleus accumbens, relatively little is known about the role of other systems in reward and locomotor activity. Thus the specific aims of this project are trifold:

1) While several studies have evaluated the role of excitatory amino acid receptors in locomotor activity, few have examined their role in drug reward. The first aim of this set of experiments was therefore to examine the role of excitatory amino acid receptors in drug reward, using the conditioned place preference paradigm as a measured response.

2) In contrast to the above, the role of opiate receptors in the nucleus accumbens in drug reward has received much attention, while the role of these receptors in locomotion has not. Thus the aim of this set of experiments was to examine the role of opiate receptors, and especially the μ receptor, in the expression of locomotor activity.
3) The role of serotonin systems in the nucleus accumbens in locomotor activity has been studied in the past. However, recent advances in the characterization of different serotonin receptors, and the development of selective ligands for each, makes a reevaluation of the role of serotonin necessary. Thus the aim of this set of experiments was to determine the contribution of the different serotonin receptor subtypes in the nucleus accumbens in the regulation of locomotor activity.

Together, these studies shed new light on the roles and chemoanatomy of the various neurotransmitter systems in the nucleus accumbens. Thus, these studies may contribute to the growing understanding of reward substrates. It seems likely that a better understanding of the reward substrate will lead to better treatments for the tragic problem of drug addiction. In addition, a better understanding of the reward substrates will ultimately lead to an understanding of affective states.
CHAPTER II
THE ROLE OF EXCITATORY AMINO ACID RECEPTORS IN DRUG REWARD

The mesolimbic dopamine system and especially its terminal fields within the nucleus accumbens (NA) comprise a critical substrate for the locomotor activating and rewarding properties of amphetamine (Carr and White, 1983; Swerdlow et al, 1986; Koob and Bloom, 1988). The NA, described as a functional interface between limbic and motor systems, may mediate goal directed behavior (Mogenson and Yim, 1981; Swerdlow et al, 1986). Recent studies suggest that glutamatergic and dopaminergic systems may interact in the nucleus accumbens (Arnt, 1981; Donzanti and Uretsky, 1983). Furthermore, glutamatergic afferents from limbic structures such as amygdala and hippocampus may modulate within the nucleus accumbens the behavioral effects of psychostimulants (Mogenson and Yim, 1981).

The following sections present the results of studies designed to examine the role of NMDA receptors and AMPA/kainate receptors in a measure of reward, conditioned place preference. Additionally, effects on locomotor activity were assessed.
THE NMDA RECEPTOR ANTAGONIST MK-801 ELICITS CONDITIONED PLACE PREFERENCE IN RATS

Introduction

The N-methyl-D-aspartate (NMDA) receptor, the best characterized of the excitatory amino acid receptors, has been implicated in such physiological functions as learning, memory, and locomotor activity. Overstimulation of this receptor has been implicated in neuronal cell death and associated neurological conditions (for review see Wroblewski and Danysz, 1989). MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate] is a potent noncompetitive antagonist of central NMDA receptors (Wong et al. 1986), which attenuates neurological damage induced by ischemia (Gill et al., 1987) and from high doses of methamphetamine (Sonsalla et al., 1988). Such observations suggest that drugs like MK-801 might be useful in minimizing neurological damage associated with certain pathologies.

Even though MK-801 prevents biochemical changes induced by high doses of methamphetamine, the NMDA antagonist produces behavioral effects that resemble the effects of amphetamine. Thus, after systemic injection, MK-801, like amphetamine, stimulates locomotor activity (Clineschmidt et al., 1982) and increases the release of dopamine in limbic areas (Löschler et al. 1991; Rao et al. 1990). As these effects are often associated with rewarding drugs, it follows that MK-801, like amphetamine, might also be rewarding. Indeed, it has recently been
demonstrated that MK-801 facilitates brain stimulation reward (Corbett, 1989; Herberg and Rose, 1989) and is self-administered in rhesus monkeys (Beardsley et al. 1990).

One complication of self-administration studies with drugs which stimulate hypermotility, such as amphetamine or MK-801, is separation of the influence of motility from the reward process. One technique that separates these effects is the conditioned place preference paradigm. In this model, animals are injected with drug during the conditioning procedure but are tested for conditioning in the absence of drugs. Consequently, the conditioned place preference would not be influenced by the direct motor effects of drugs. In the present study we have evaluated the ability of MK-801 to produce a conditioned place preference to determine if it is similar to amphetamine in its ability to elicit a place preference. Furthermore, the effect of MK-801 on locomotor activity was assessed during the training period. Amphetamine was administered as a positive control for both behavioral activation and conditioned place preference.

Methods

Male rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 250 to 350 g, were housed 4 to a cage in a temperature controlled (23 ± 1° C) room with a 12 hour light-dark cycle. A 3-compartment conditioned place preference apparatus with a small central compartment (10 x 10 cm and 56 cm high) separating 2 larger compartments (38 x 76 cm and 56 cm high) was used. Guillotine doors separated
the compartments. The small compartment was painted grey while the two larger compartments were painted either white or black. The white compartment had a floor composed of steel rods (1.5 cm apart), and the black compartment had a floor composed of wire mesh. Distance traveled and time spent in each compartment were monitored with a Videomex-V tracking system (Columbus Instruments, Columbus, OH). All training or test sessions were done between 8:00 am and 4:00 pm in an isolated environmental room maintained at a temperature of 23 ± 1°C.

Place preference conditioning occurred over a 4 day period. On the day prior to training animals were allowed to freely explore the apparatus for 15 minutes with the guillotine doors open, thus establishing a baseline preference, with the chamber they spent the least time in considered the non-preferred side. On days 1 and 3 of training, rats were injected with drugs or saline and confined to the non-preferred side for 35 minutes, while on days 2 and 4 all groups were injected with saline and confined to the preferred side for 35 minutes. Rats were tested for place preference on day 5. Rats were placed in the small grey compartment with the doors closed. The doors were then opened, and the amount of time spent in each compartment during a 15 minute test period was recorded.

Place preference data are expressed as the difference in minutes between time spent in the drug paired side (CS+; least preferred initially) and the time spent in the saline paired side (CS-). Values from both pretest baseline and test conditions are shown, with animals that acquire a place preference shifting their preference
to the drug paired side. Locomotor data from the first and third training days (in which the animals received the drug) are given as distance traveled (cm). All data are expressed as the mean and the standard error of the mean (SEM). The data were evaluated statistically using the nonparametric Kruskal-Wallis one-way analysis of variance followed by the one-tailed Mann-Whitney U-test, with p<0.05 accepted as significant.

**Results**

Figure 5 demonstrates that both MK-801 (0.1 mg/kg s.c.) and d-amphetamine (1.0 mg/kg s.c.) produced a conditioned place preference when paired against saline. Animals injected with saline on the least preferred side did not change their place preference. The administration of both MK-801 and d-amphetamine at these doses resulted in the stimulation of locomotor activity, expressed as total distance traveled (Figure 6). The locomotor activity was significantly higher on day 1 for both drugs; however, it was more variable on day 3 for the MK-801 animals which displayed a greater intensity of stereotyped behavior. No differences in locomotor activity were observed between any group on days 2 and 4 when all rats were given saline in the preferred side.

**Discussion**

These data demonstrate that MK-801, at a dose which stimulates locomotor activity, produces a conditioned place preference. The stimulation of locomotor
Figure 5. Place preference elicited by MK-801 and d-amphetamine. MK-801 (0.1 mg/kg s.c.) and d-amphetamine (1.0 mg/kg s.c.) were paired with the less preferred compartment. Data are expressed as the mean ± SEM of the difference in time spent in the drug paired side (CS+) and the vehicle paired side (CS-) for both pretest and test conditions.
Figure 6. Locomotor activity elicited during conditioning in the same rats by MK-801, d-amphetamine, or saline. Doses used were: MK-801 (0.1 mg/kg s.c., n=6), d-amphetamine (1.0 mg/kg s.c., n=5), or saline (n=7). Locomotor activity is expressed as the mean distance traveled (cm) ± SEM for the number of observations indicated. *p<0.05 with respect to saline control.
activity elicited by MK-801 is similar to that elicited by a dose of d-amphetamine which similarly elicits a place preference. Only two conditioning periods with the drugs were necessary to produce a conditioned place preference. This finding is consistent with the results of recent studies demonstrating that MK-801 facilitates lever pressing for brain stimulation reward and is self administered by rhesus monkeys (Beardsley et al., 1990; Corbett, 1989; Herberg and Rose, 1989). Other noncompetitive antagonists of the NMDA channel, such as ketamine and phencyclidine, are self administered by animals (Marquis and Moreton, 1987) and are abused by humans (Lerner and Burns, 1978). Furthermore, MK-801 has phencyclidine-like discriminative properties (Beardsley et al., 1990).

While d-amphetamine appears to produce its rewarding properties by causing the release of dopamine from nerve terminals in the nucleus accumbens, the site of action of MK-801 in producing conditioned place preference is unclear. In one recent study of male rats, MK-801 did not appear to increase dopamine turnover in the nucleus accumbens (Rao et al., 1990), while in a separate study using a higher dose of MK-801 in female rats an increase in dopamine turnover was observed (Löscher et al., 1991). MK-801 has been shown to increase the firing of dopaminergic neurons from the ventral tegmental area (French and Ceci, 1989) and increase dopamine turnover in the mesocortex (Löscher et al., 1991; Rao et al., 1990), which contains terminal fields of these neurons. It is therefore possible that these dopaminergic neurons, which have been proposed to play a significant role in the rewarding action of cocaine, may mediate the place preference
produced by MK-801.

Alternatively, it has also been shown that MK-801 elicits locomotor activity after direct injection into the nucleus accumbens (Raffa, 1989). This effect appears to be independent of dopamine as it is seen even after monoamine depletion (Carlsson and Svensson, 1990). Further studies will be required to resolve this issue.

In contrast to this finding, another noncompetitive NMDA antagonist which is abused by humans, PCP, has been shown to elicit a place aversion in rats at several doses (Acquas et al, 1989; Barr et al, 1985). While both MK-801 and PCP act as noncompetitive antagonists of the NMDA receptor and MK-801 produces many PCP-like effects, PCP is also a ligand at sigma receptors. It is therefore possible that the aversive effects of PCP as measured in conditioned place preference paradigms are not due to its ability to antagonize the NMDA receptor but may be related to its actions at the sigma receptor.

The conditioned place preference paradigm has been used as a means of evaluating the rewarding properties of drugs. A variety of drugs of abuse can produce conditioned place preferences. The finding that MK-801 can establish a conditioned place preference suggests that this drug may have abuse potential.
A ROLE FOR AMPA/KAINATE RECEPTORS IN THE NUCLEUS ACCUMBENS IN DRUG-INDUCED REWARD

Introduction

The nucleus accumbens, a region of the basal forebrain, has been implicated as a critical substrate in the expression of locomotor activity and drug reward (Koob and Bloom, 1988; Swerdlow et al, 1986). A role for dopaminergic neurotransmission in the nucleus accumbens in the expression of locomotor activity and drug reward is well established (Phillips and Fibiger, 1979; Wise 1978; Evenden and Ryan, 1990; Beninger, 1983; Dunnett et al, 1984; Fink and Smith, 1980; Mithani et al, 1986; Le Moal and Simon, 1991; Carr and White, 1983).

Recent studies have suggested an interaction between glutamatergic and dopaminergic systems within the nucleus accumbens. Several lines of evidence support this role. First, anatomical data suggest that the nucleus accumbens receives glutamatergic inputs from several cortical and thalamic structures, including the prefrontal and olfactory cortex, the subiculum, various nuclei of the amygdala (most prominent the lateral and basolateral nuclei), and the midline nucleus of the thalamus (Fuller et al, 1987). Furthermore, the three functionally defined glutamatergic, ionotropic receptors, AMPA, kainate, and NMDA, have been reported in the nucleus accumbens (Cotman et al 1987). Dopaminergic and glutamatergic neurons have been suggested to synapse on the same cell bodies in the striatum and nucleus accumbens (Freund et al, 1984; Kemp and Powell,
1971; Doucet, et al, 1986; Totterdell and Smith, 1989; see also Wilson and Groves, 1980; Smith and Bolum, 1990). In addition, glutamatergic interneurons have been demonstrated in the nucleus accumbens (Fonnum and Walaas, 1981). Second, electrophysiological data suggests that within the nucleus accumbens dopamine may modulate glutamatergic inputs from areas such as hippocampus and amygdala (Yang and Mogenson, 1984; Yang and Mogenson, 1986; Yim and Mogenson, 1982; Yim and Mogenson, 1986; Yim et al, 1991). Third, a reciprocal relationship between dopamine and glutamate seems to exist (Chesselet, 1984) such that dopamine inhibits glutamate release (Mitchell and Doggett, 1980; Rowlands and Roberts, 1980) and glutamate acting at either NMDA (Mount et al, 1990; Marien et al, 1983; Jhamandas and Marien, 1987; Jones et al, 1987) or AMPA/kainate receptors (Imperato et al, 1990; Desce et al, 1991) increases dopamine release (also see Roberts and Andersen, 1979; Chèramy et al, 1986; Romo et al, 1986; Kalivas et al, 1990). Fourth, dopamine may directly modulate the activity of excitatory amino acid-gated ion channels by increasing the open-time of the channel, thereby increasing the current transmitted through the channel (Knapp and Dowling, 1987; Knapp et al, 1990).

A role for glutamatergic NMDA and AMPA/kainate receptors in the nucleus accumbens in mediating locomotor activity is emerging. Injection of glutamatergic agonists into the nucleus accumbens stimulates coordinated locomotor activity (Donzanti and Uretsky, 1983; Boldry and Uretsky, 1988) that is inhibited by treatments which impair dopaminergic transmission, such as antagonism by
neuroleptics (Arnt, 1981) or inhibition of tyrosine hydroxylase with α-methyl-p-tyrosine (Boldry et al, 1991). Injection of glutamatergic antagonists into the accumbens inhibits locomotor activity elicited by either novelty (Mogenson and Nielsen, 1984), or by psychostimulants such as amphetamine or cocaine which require dopamine (Willins et al, 1992; Pulvirenti et al, 1989; Pulvirenti et al, 1991; Kaddis et al, 1991).

As many drugs abused by humans both activate locomotor activity in and are self-administered by animals, it has been proposed that a high degree of overlap exists between neural substrates mediating the locomotor activating and positively reinforcing effects of drugs (Swerdlow et al, 1986; Koob and Bloom, 1988). Furthermore, treatments which inhibit the increased locomotor activity elicited by these drugs similarly reduce their positively reinforcing effects. For example, intra-accumbens neuroleptic treatment or specific 6-hydroxydopamine lesions reduce both the locomotor activating and rewarding effects of a psychostimulant (amphetamine), and intra-accumbens methylhaloxonium administration reduces both the locomotor activating and rewarding effects of an opiate (heroin).

Thus, while a role for glutamatergic neurotransmission in the nucleus accumbens as a mediator of locomotor activity is emerging, it is unclear whether glutamatergic transmission plays a role in drug reward. In light of the recent findings that AMPA/kainate excitatory amino acid receptor antagonists block amphetamine elicited locomotion, the purpose of the present study was to determine whether the positively reinforcing effect elicited by d-amphetamine
similar requires activation of the AMPA/kainate receptor in the nucleus accumbens. Additionally, as it has been proposed that opiates may interact with a reward substrate in the nucleus accumbens which is distinct from that of amphetamine, the role of AMPA/kainate receptors in the nucleus accumbens in mediating the positively reinforcing effect elicited by morphine is assessed.

The conditioned place preference (CPP) paradigm, in which animals increase their contact with an environment (the conditioned stimulus) previously paired with rewarding stimuli (the unconditioned stimulus), is an effective means for assessing the effects of drugs on the neural substrates of motivation and reward (Hoffman, 1989). This paradigm is useful for assessing the effects of drugs on both the acquisition of reward, in which a drug is administered concurrently with the rewarding (unconditioned) stimulus during training, and the expression of reward, in which the drug is administered in the presence of the conditioned stimulus (the environment) on the test day. The purpose of the present study, therefore, was to determine if the AMPA/kainate receptor antagonist DNQX could inhibit the acquisition or expression of a place preference elicited by rewarding drugs, specifically either d-amphetamine or morphine.

Methods

Animals

Male rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 250 to 350 g, were housed 4 to a cage in a temperature controlled (23 ± 1°C) room with a 12
hour light-dark cycle.

**Surgery**

Each rat was anesthetized with ketamine (10 mg/kg i.p.) and xylazine (90 mg/kg i.p.) and placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA) with the toothbar set at -3.3 mm. The rats were then implanted with a 22 gauge stainless steel guide cannula (Plastic Products Co., Roanoke, VA) aimed at a 10° angle 2 mm above the nucleus accumbens at the coordinates (A 10.2; L -2.0; H 1.2; measured from the interaural line) using the stereotaxic guide of Paxinos and Watson (1982). Cannulae were secured with dental acrylic cement anchored to the skull by two stainless steel screws. Animals then received Chloramphenicol (100 mg/kg, i.m.) to protect against infection and were allowed at least five days to recover.

All drugs were administered via a 28 gauge cannula (Plastic Products) projecting 2 mm beyond the guide cannula. Drugs or vehicle were injected in a 0.5 µl volume at a rate of 0.5 µl/min.

**Drugs**

Morphine sulphate and d-amphetamine sulphate were purchased from Sigma Chemical Co. (St. Louis, MO). 6,7-dinitroquinoxaline-2,3-dione (DNQX) was obtained from Tocris Neuramin (Essex, England). 6-amino-7-fluoroquinoxaline-2,3-dione (AFQX) was generously provided by Dr. Duane D. Miller (The Ohio State
University, College of Pharmacy, Division of Medicinal Chemistry). DNQX and AFQX were dissolved in 0.1 N NaOH and adjusted to the appropriate volume with water, and the solutions were adjusted to pH 9.0 with 0.1 N HCl. The doses of DNQX and AFQX shown refer to the amount injected on each side of the nucleus accumbens; control animals received an equal volume (0.5 μl) of vehicle. Morphine sulphate and d-amphetamine sulphate were dissolved in normal saline.

**Conditioned Place Preference Procedure**

A 3-compartment conditioned place preference apparatus with a small central compartment (10 x 10 cm and 56 cm high) separating 2 larger compartments (38 x 76 cm and 56 cm high) was used. Guillotine doors separated the compartments. The small compartment was painted grey while the two larger compartments were painted either white or black. The white compartment had a floor composed of steel rods (1.5 cm apart), and the black compartment had a floor composed of wire mesh. Distance traveled and time spent in each compartment were monitored with a Videomex-V tracking system (Columbus Instruments, Columbus, OH). All training or test sessions were done between 8:00 am and 4:00 pm in an isolated environmental room maintained at a temperature of 23 ± 1 °C.

Place preference conditioning occurred over an 8 day period. On the day prior to training, animals were allowed to freely explore the apparatus for 15 minutes with the guillotine doors open, thus establishing a baseline preference, with the chamber they spent the least time in considered the non-preferred side. On days
1, 3, 5, and 7 of training, rats were injected with drugs (amphetamine, 1 mg/kg s.c.; or morphine, 10 mg/kg s.c.) or saline and confined to the non-preferred side for 35 minutes, while on days 2, 4, 6, and 8 all groups were injected with saline and confined to the preferred side for 35 minutes. Rats were tested for place preference on day 10. Rats were placed in the small grey compartment with the doors closed. The doors were then opened, and the amount of time spent in each compartment during a 15 minute test period was recorded.

Place preference data are expressed as the difference in minutes between time spent in the drug paired side (CS+; least preferred initially) and the time spent in the saline paired side (CS-). Values from both pretest baseline and test conditions are shown, with animals that acquire a place preference shifting their preference to the drug paired side. Locomotor data from the training days (the days in which the animals received the drug) are given as distance traveled (cm). All data are expressed as the mean and the standard error of the mean (SEM).

Catalepsy Test

Catalepsy was measured using a 9-cm cork test immediately after the training period during the morphine-DNQX acquisition experiment. Rats were placed with both forepaws on the cork, and the time of maintaining both forepaws on the cork was measured. The test was terminated at 180 s, even if rats had not moved the forepaws. The time in which rats remained in this position was recorded, and catalepsy was scored in a graded fashion: rats remaining cataleptic for up to 30
s were scored a 1; 30 to 60 s were scored a 2; 60 to 90 s were scored a 3; 90 to 120 s were scored a 4; 120 to 150 s were scored a 5; 150 to 180 s were scored a 6; and over 180 s were scored with a 7.

**Histology**

After each experiment, animals were decapitated, and their brains were removed and fixed in a 10% solution of formalin for 48 hours. Frozen sections (40 μm) were cut using a Cryo-Cut microtome (American Optical Corp., Buffalo, NY) to check the location of the tip of the injection needle track. When the tips of the needle tracks were found to lie outside the nucleus accumbens, the data for that animal were excluded from the study.

**Statistics**

The amphetamine place preference and locomotor data were evaluated statistically with an analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test for comparison of individual groups, with p<0.05 accepted as significant. Morphine place preference and locomotor data were evaluated with the students t test, and catalepsy data were evaluated using the Mann-Whitney U test.

**Results**

The first experiment was done to determine whether DNQX would affect amphetamine-induced place preference after two pairings between the least
preferred side and amphetamine. Under these conditions, d-amphetamine (1 mg/kg s.c.) elicited a place preference after only two pairings in non-cannulated animals. However, when injections of either DNQX or vehicle were administered simultaneously into the nucleus accumbens of cannulated animals, the expression of place preference was not seen (Figure 7). The administration of d-amphetamine resulted in the stimulation of locomotor activity, expressed as total distance traveled (Figure 8). Consistent with a previous report (Willins et al., 1992), the locomotor activity was significantly inhibited on day 1 by DNQX. However, DNQX did not inhibit locomotor activity on day 3 (Figure 8).

In the next experiment, due to the failure of cannulated controls to form a place preference after two amphetamine pairings (with the least preferred side), four amphetamine-least preferred side pairings were done. d-Amphetamine (1 mg/kg s.c.) did elicit a place preference in cannulated control animals after four pairings (Figure 9) even after vehicle injection. Simultaneous intra-nucleus accumbens administration of DNQX (1.0 µg/0.5 µl) significantly inhibited the acquisition of amphetamine-induced place preference (Figure 9). Similar to the previous experiment, DNQX inhibited locomotor activity on day 1, but not on days 3, 5, and 7 (Figure 10). In a separate experiment, administration of DNQX (1.0 µg/0.5 µl) after training, on the test day, significantly inhibited the expression of amphetamine-induced place preference (Figure 11). This dose of DNQX did not inhibit the basal locomotor activity of the animals during the test (Figure 12). In experiments to test the specificity of DNQX, administration of a structural analog
of DNQX which competes poorly or not at all for AMPA receptors, AFQX (Supko et al, 1990), did not inhibit the acquisition or expression of amphetamine-induced place preference.

In the next experiment, to test the specificity of this response, a different rewarding drug was used. Morphine has also been shown to elicit a place preference, but the anatomical substrates for this preference may be different than those of d-amphetamine. Morphine (10 mg/kg s.c.) elicited a place preference in cannulated control animals after four pairings (Figure 13). Administration of DNQX (1.0 μg/0.5 μl) after training, on the test day, significantly inhibited the expression of morphine-induced place preference (Figure 13). In contrast, pairing of intra-nucleus accumbens administration of DNQX (1.0 μg/0.5 μl) with systemic morphine during the training did not significantly inhibit the acquisition of morphine-induced place preference (Figure 14). Locomotor activity of rats during the acquisition experiment with the combination of morphine and DNQX was significantly lower than morphine alone controls (Figures 15). In addition, the dose of morphine used resulted in a mild catalepsy (Figure 17). Interestingly, the catalepsy elicited by morphine was potentiated by simultaneous intra-nucleus accumbens administration of DNQX (1.0 μg/0.5 μl) (Figure 17). However, while the DNQX-induced inhibition of d-amphetamine elicited locomotor activity was only seen on the first day, DNQX potentiated morphine catalepsy on all days tested. In both cases, while rats were cataleptic, the righting reflex was unaffected. The catalepsy assessment occurred immediately after the training period. Rats appeared cataleptic in the training
Figure 7. The effect of DNQX on place preference elicited by only two pairings with d-amphetamine. The place preference elicited by only two pairings with d-amphetamine (d-amph; 1.0 mg/kg s.c.) in the less preferred compartment (CS+) is inhibited by both DNQX (1.0 μg/0.5 μl) or vehicle simultaneously administered into nucleus accumbens. Data are expressed as the mean ± SEM of the difference in time spent in the d-amph paired side (CS+) and the saline paired side (CS-) for both pretest and test conditions. *p<0.05 with respect to saline control.
Figure 8. The effect of DNQX simultaneously administered into nucleus accum bens on the locomotor activity elicited by d-amphetamine. DNQX (1.0 μg/0.5 μl) simultaneously administered into nucleus accum bens inhibits the locomotor activity elicited by d-amphetamine (1.0 mg/kg s.c.) on day 1, but not day 3. Locomotor activity is expressed as the mean distance traveled (cm) ± SEM for the number of observations indicated. *p<0.05 with respect to saline control.
Figure 9. Effects of DNQX and AFQX on the acquisition of an amphetamine-induced conditioned place preference. DNQX (1.0 μg/0.5 μl), but not AFQX (1.0 μg/0.5 μl), simultaneously administered into nucleus accumbens inhibits the acquisition of a place preference elicited by d-amphetamine (d-amph; 1.0 mg/kg s.c.) after 4 pairings with the non-preferred compartment (CS+). Data are expressed as the mean ± SEM of the difference in time spent in the d-amph paired side (CS+) and the saline paired side (CS-) for both pretest and test conditions. *p<0.05 with respect to saline control.
Figure 10. Effects of DNQX and AFQX on the locomotor activity induced by amphetamine during conditioned place preference training. DNQX (1.0 μg/0.5 μl), but not AFQX (1.0 μg/0.5 μl), simultaneously administered into nucleus accumbens inhibits the locomotor activity elicited by d-amphetamine (1.0 mg/kg s.c.) on day 1. Locomotor activity was not inhibited by either drug on training days 3, 5, or 7. Locomotor activity is expressed as the mean distance traveled (cm) ± SEM for the number of observations indicated. *p<0.05 with respect to saline control.
Figure 11. Effects of DNQX and AFQX on the expression of an amphetamine-induced conditioned place preference. DNQX (1.0 μg/0.5 μl), but not AFQX (1.0 μg/0.5 μl), administered into nucleus accumbens immediately prior to the test inhibits the expression of place preference elicited by d-amphetamine (1.0 mg/kg s.c.). Data are expressed as the mean ± SEM of the difference in time spent in the d-amph paired side (CS+) and the saline paired side (CS-) for both pretest and test conditions. *p<0.05 with respect to saline control.
Figure 12. Effects of DNQX and AFQX on locomotor activity during the expression of an amphetamine-induced conditioned place preference. Neither DNQX (1.0 μg/0.5 μl) nor AFQX (1.0 μg/0.5 μl), administered into nucleus accumbens immediately prior to test inhibits normal locomotor activity in either the CS+ (white) or CS- (black) compartment. Locomotor activity is expressed as the mean distance traveled (cm) per time spent (min) in each chamber ± SEM for the number of observations indicated. *p<0.05 with respect to saline control.
Figure 13. Effect of DNQX on the expression of morphine-induced place preference. DNQX (1.0 μg/0.5 μl) administered into nucleus accumbens immediately prior to the test inhibits the expression of place preference elicited by morphine (MS; 10 mg/kg s.c.). Data are expressed as the mean ± SEM of the difference in time spent in the d-amph paired side (CS+) and the saline paired side (CS-) for both pretest and test conditions. *p<0.05 with respect to morphine control.
Figure 14. Lack of effect of DNQX on the acquisition of morphine-induced place preference. Pairing of intra-accumbens DNQX (1.0 μg/0.5 μl) with systemic morphine during the training did not inhibit the acquisition of a place preference elicited by morphine (MS; 10 mg/kg s.c.) after 4 pairings with the non-preferred compartment (CS+). Data are expressed as the mean ± SEM of the difference in time spent in the d-amph paired side (CS+) and the saline paired side (CS-) for both pretest and test conditions.
Figure 15. Effects of DNQX on the locomotor activity of rats given morphine during conditioned place preference training. DNQX (1.0 μg/0.5 μl), simultaneously administered into nucleus accumbens significantly reduces the already low level of locomotor activity of morphine treated (10 mg/kg s.c.) rats on day 1, 3, and 5, but not day 7. Locomotor activity is expressed as the mean distance traveled (cm) ± SEM. *p<0.05 with respect to morphine control.
Figure 16. Effects of DNQX on locomotor activity during the expression of a morphine-induced conditioned place preference. DNQX (1.0 μg/0.5 μl) was administered into nucleus accumbens immediately prior to test. Animals were free to interact with either the CS+ (white) or CS- (black) compartment. Locomotor activity is expressed as the mean distance traveled (cm) per time spent (min) in each chamber ± SEM for the number of observations indicated. Speeds for the two groups were not different in each compartment. However, speed for the DNQX group in the CS- was significantly lower than in the CS+.
Figure 17. Effects of DNQX on the catalepsy induced by morphine during conditioned place preference training. DNQX (1.0 μg/0.5 μl), simultaneously administered into nucleus accumbens potentiates the catalepsy elicited by morphine (10 mg/kg s.c.) on days 3, 5, and 7. Catalepsy is expressed as the mean score ± SEM. *p<0.05 with respect to morphine control.
Figure 18. Unilateral administration of AMPA into the nucleus accumbens does not elicit a conditioned place preference. Acquisition of a place preference was elicited by d-amphetamine (1.0 mg/kg s.c.) but not AMPA (0.5 μg/0.5 μl, administered unilaterally into the nucleus accumbens) after 4 pairings with the non-preferred compartment (CS+). Data are expressed as the mean ± SEM of the difference in time spent in the drug paired side (CS+) and the saline paired side (CS-) during the test. *p<0.05 with respect to saline controls.
chamber, however, they would try to elude the experimenter when he reached for them. Cataleptic appearance would subsequently resume while rats were being handled and transferred to the cork for catalepsy analysis.

DNQX did have an effect on locomotor activity in the test for expression of morphine-induced place preference. While speeds of DNQX treated rats were not different from saline treated rats in either compartment, the speed of DNQX treated rats in the CS- (black) compartment was lower then their speed in the CS+ (white) compartment (Figure 16).

Since the AMPA/kainate receptor antagonist DNQX appears to block some aspects of drug reward, the question arose as to whether an agonist would elicit reward. Although the glutamate agonist elicits pronounced locomotor activity when injected into the nucleus accumbens, unilateral administration of AMPA (0.5 μg/0.5 μl) into the nucleus accumbens did not result in a place preference (Figure 18). The AMPA was administered four times; the procedure was the same as above (data were collected manually, however).

Discussion

These data suggest that non-NMDA receptors within the nucleus accumbens may play a role in the mediation of reward elicited by conditioned stimuli. In addition, these data suggest that acquisition of place preference elicited by d-amphetamine and morphine may utilize different neural elements within the nucleus accumbens.
Effects of DNQX on Place Preference Acquisition

The acquisition of d-amphetamine induced conditioned place preference has been shown to depend on dopaminergic transmission in the nucleus accumbens (Hiroi and White, 1989; Carr and White, 1983). In the present study, DNQX administration into the nucleus accumbens inhibits the acquisition of d-amphetamine induced conditioned place preference. These results suggest that activation of both AMPA/kainate receptors and dopamine receptors in the nucleus accumbens is required for the acquisition of d-amphetamine induced conditioned place preference.

In contrast, the acquisition of a place preference elicited by morphine was not blocked by intra-accumbens DNQX. Morphine may elicit reward by acting on two substrates, the ventral tegmental area and the nucleus accumbens (see Koob and Bloom, 1988; Wise, 1990). While dopamine release in the nucleus accumbens (elicited by disinhibition of ventral tegmental dopaminergic neurons) probably does contribute to the rewarding effects of morphine (Phillips and LePiane, 1980; Phillips and LePiane, 1982; Bozarth and Wise, 1981; Bozarth, 1987; Britt and Wise, 1983; Vaccarino et al, 1985), a direct action of morphine at an opioid receptor, independent of dopamine, in the nucleus accumbens may also elicit reward (Olds, 1982; van der Kooy et al, 1982; Vaccarino et al, 1985; Mackey and van der Kooy, 1985). If dopamine release were the sole mediator of reward, it seems likely that DNQX administration would have blocked the acquisition of morphine-induced place preference as it blocked the acquisition of amphetamine-induced place preference.
preference. The present results are consistent with a model incorporating at least two substrates for morphine reward, one dependent on dopamine, the other independent. Alternatively, in the present study DNQX may have only affected a subpopulation of dopaminergic synapses, leaving dopaminergic transmission stimulated by morphine at the level of the ventral tegmental area undisturbed. In either case, it seems likely that the substrates of both amphetamine- and morphine-induced place preference converge, as lesions of the tegmental pedunculopontine nucleus, a brain structure located "down stream" in the proposed locomotor pathway, block the rewarding effects of both amphetamine and morphine in a conditioned place preference paradigm (Bechara and van der Kooy, 1989).

Effects of DNQX on Place Preference Expression

DNQX administration on the test day inhibits the expression of both d-amphetamine- and morphine-induced conditioned place preference. It has been demonstrated previously that dopaminergic transmission in the nucleus accumbens is stimulated in response to a conditioned reinforcing stimulus (Blackburn et al, 1987; Blackburn et al, 1989a; Blackburn et al, 1989b; Migler, 1975; Clody and Carlton, 1980; Phillips et al, 1991; Taylor and Robbins, 1984; Taylor and Robbins, 1986), that is, a neutral stimulus which gains rewarding properties by being paired to a rewarding stimulus. Stimuli such as those found in the drug paired side of the conditioned place preference chamber are conditioned stimuli. The expression of
amphetamine-induced place preference depends in part on dopaminergic transmission, as it is blocked by D1 and higher doses of D2 antagonists (Hiroi and White, 1991). The expression of d-amphetamine-induced place preference was similarly inhibited by DNQX in the present study. As the expression of morphine-induced place preference was also inhibited by DNQX, our results further suggest that the expression of a morphine-induced place preference may be mediated by the same dopaminergic mediated substrates as d-amphetamine, which are activated by a conditioned stimulus and possibly impaired by intra-accumbens DNQX. It is unlikely that DNQX resulted in an inhibition of the expression of place preference by impairing locomotion, as DNQX did not significantly inhibit locomotor activity during the test, and previous work in our laboratory has shown that DNQX does not inhibit spontaneous locomotion (Willins et al, 1992).

Interestingly, lesions of the lateral nucleus of the amygdala, (but not lesions of other amygdala nuclei, hippocampus, or cortex), similarly inhibit the acquisition and expression of amphetamine-induced place preference (Hiroi and White, 1991). It is therefore tempting to speculate that glutamatergic fibers projecting from the lateral nucleus of the amygdala to the nucleus accumbens and acting via AMPA/kainate receptors are required for amphetamine-induced place preference.

Effects of DNQX on Amphetamine Locomotor Activity

These data confirm the finding that intra-accumbens administration of the AMPA/kainate receptor antagonist DNQX inhibits the locomotor response to
systemic d-amphetamine (Willins et al, 1992). However, this inhibition is lost after one pairing with amphetamine. These data further suggest that the interaction between dopaminergic and glutamatergic neurons in the nucleus accumbens may change with exposure to amphetamine. Although this phenomena cannot be explained in the context of the present study, it may relate to the phenomenon of amphetamine sensitization, as sensitization has been demonstrated after one dose of amphetamine (Brown and Segal, 1977; Robinson, 1984). Furthermore, non-NMDA excitatory amino acid receptors have been implicated as mediators of the effect (Karler et al, 1991). Further studies will be required to resolve this issue.

The effects of DNQX in all of the above studies appear to be receptor mediated effects rather than non-specific effects. AFQX, a chemical analogue of DNQX, has been shown not to inhibit the binding of $[^3H]$-AMPA to its receptor in brain membranes (Supko et al, 1990). In the present study, the effects of intra-accumbens AFQX were not different from vehicle controls.

**Effects of DNQX on Morphine Motor Effects and Catalepsy**

Catalepsy, a condition characterized by immobility, elicited by morphine appears to be mediated by opiate receptors in the nucleus accumbens (Winkler et al, 1982; Havemann and Kuschinsky, 1985; Daugè et al, 1988). As morphine is reasonably selective for the $\mu$ opioid receptor (Chang, 1984), catalepsy is probably a $\mu$ mediated effect. Interestingly, the catalepsy elicited by morphine was potentiated by simultaneous intra-nucleus accumbens administration of DNQX.
While morphine catalepsy and akinesia are probably not due to effects on dopamine release and are probably related to post-synaptic actions (see Layer et al, 1991; Chapter III), inhibitory effects on glutamate release may also be involved. DAGO, an agonist of the μ opioid receptor, inhibits the release of glutamate in the striatum (Jiang and North, 1992), and high doses of AMPA, an excitatory amino acid agonist, appear to partially reverse morphine-induced akinesia. Thus, if morphine is preventing glutamate release from afferent glutamatergic projections in the nucleus accumbens, and this is related to catalepsy, the DNQX should potentiate this effect. Catalepsy has never been observed in our laboratory after a 1 μg dose of DNQX alone in the nucleus accumbens, which, in fact, does not seem to inhibit normal locomotor activity (Willins et al, 1992). Furthermore, unlike the DNQX-induced inhibition of d-amphetamine elicited locomotor activity, which was only seen on the first day, the potentiation of morphine catalepsy was observed on all days tested, and did not appear to be diminished in any way.

The data described above support the hypothesis that AMPA/kainate excitatory amino acid receptors within the nucleus accumbens are involved in the regulation of locomotor activity in the rat. These data further suggest that AMPA/kainate excitatory amino acid receptors in the nucleus accumbens are involved in the expression of the conditioned place preference reward elicited by amphetamine and morphine. However, while these data suggest a common substrate within the nucleus accumbens in mediating expression of both amphetamine and morphine-induced place preference, these data also suggest the presence of possibly two
distinct substrates within the nucleus accumbens which mediate the acquisition of place preference.
CHAPTER III
EFFECTS OF MORPHINE IN THE NUCLEUS ACCUMBENS
ON STIMULANT-INDUCED LOCOMOTION

Introduction

The nucleus accumbens is a brain region which has been described as a functional interface between limbic and motor systems (Mogenson and Yim, 1981). One of the procedures used to study the role of the nucleus accumbens in the regulation of motility has been to measure locomotor activity after micro-injection of compounds directly into the region. The results of such studies indicate that several different neurotransmitter systems in the nucleus accumbens are involved in the regulation of motor responses. The most extensively studied is the dopaminergic system. Activation of dopaminergic receptors either indirectly via amphetamine-induced release of dopamine or directly with injected dopamine or apomorphine produces a marked increase in locomotor activity (Fog, 1970; Kelly et al, 1975; Kuczenski, 1983; Pijnenburg et al, 1976). Agonists for glutamatergic excitatory amino acid receptors also stimulate an increase in locomotor activity (Arnt, 1981; Boldry et al, 1991; Donzanti and Uretsky, 1983). Among these, activation of the quisqualic acid or \(\alpha\)-amino-3-hydroxy-5-methylisoxazole-4-
propionic acid (AMPA) receptor elicits the largest response (Donzanti and Uretsky, 1983). There appears to be an interaction between the glutamatergic and dopaminergic systems as a low level of activation of dopaminergic receptors is required for the locomotor stimulating effects of the quisqualic receptor agonist AMPA (Boldry et al., 1991, Donzanti and Uretsky, 1983). Picrotoxin, which blocks the chloride channel associated with the GABA-A receptor, also stimulates a marked increase in locomotor activity (Morgenstern et al., 1984). However, this response appears to be independent of the dopaminergic system as it is not inhibited by dopaminergic blockers (Morgenstern et al., 1984).

In contrast to the stimulation of locomotor activity by dopaminergic and glutamatergic agonists, activation of some other neurotransmitter receptors in the nucleus accumbens results in a decreased level of motor activity. Thus, injection of morphine into this region results in akinesia and catalepsy (Costall et al., 1978; Winkler et al., 1982). This effect appears to be the result of a specific action on μ-receptors as the response is achieved by selective μ-agonists (Daugè et al., 1988). The specificity of this effect is further evidenced by observations that specific δ-agonists increase locomotor activity (Daugè et al., 1988; Havemann and Kuschinski, 1985) and specific kappa-agonists have little effect (Havemann and Kuschinski, 1985).

The fact that both μ-opiate receptor agonists and direct and indirect acting dopaminergic agonists act within the nucleus accumbens to produce opposite effects on motility suggests that dopaminergic and opioid systems have an
antagonistic interaction in the nucleus accumbens. The specific anatomy and biochemistry that contribute to this interaction have not been conclusively determined. Some studies suggest that morphine induced akinesia may be the result of a decrease in dopaminergic transmission in areas such as the striatum and nucleus accumbens (Carenzi et al, 1975; Carlson and Seeger, 1982; Kuschinsky and Hornykiewicz, 1973; Pollard et al, 1977; Pollard et al, 1978; Schwartz, 1979; Slater et al, 1980; Yonehara and Clouet, 1984). This hypothesis is supported by several observations. First, both morphine and drugs which impair dopaminergic transmission such as haloperidol can produce catalepsy and akinesia after systemic administration (Babbini and Davis, 1972; Kuschinsky and Hornykiewicz, 1973); second, morphine inhibits stereotypy induced by amphetamine but not apomorphine (Seeger and Carlson, 1980); third, opiate receptors are localized on both nucleus accumbens (Pollard et al, 1977) and nigrostriatal dopaminergic neurons (Pollard et al, 1978); fourth, an opiate degrading enzyme, enkephalinase, has been localized to nigrostriatal dopaminergic neurons (Malfroy et al, 1978); fifth, opiates can decrease dopamine release from striatal slices (Carenzi et al, 1975; Pollard et al, 1978; Yonehara and Clouet, 1984); and sixth, opiates can inhibit d-amphetamine-induced turning behavior of rats with unilateral 6-hydroxydopamine-induced lesions of nigrostriatal dopaminergic neurons (Slater et al, 1980). One direct test of dopaminergic-opiate interactions in the nucleus accumbens has been reported. Morphine coinjected into the nucleus accumbens with dopamine blocks the locomotor stimulating effect of the
catecholamine (Costall et al, 1978b). Thus, morphine may inhibit dopaminergic synapses in the nucleus accumbens by acting presynaptically at the dopaminergic nerve terminal as well as postsynaptically.

The observations reported above argue strongly for dopaminergic-opiate interactions within striatum and nucleus accumbens. However, the role of such interactions within the nucleus accumbens on the regulation of motility has not been well characterized. Furthermore, morphine interaction with non-dopaminergic stimulators of hypermotility has not been reported. The goal of the present work was to determine how morphine sensitive opiate mechanisms in the nucleus accumbens might interact with neuronal pathways that control locomotor stimulation. Towards this end, we have coinjected into the nucleus accumbens morphine along with compounds that elicit hypermotility and have measured the resultant locomotor activity. Using this procedure, the interaction of morphine with compounds that activate dopaminergic and glutamatergic neurotransmission and that inhibit GABAergic neurotransmission were compared. In addition, effects of intra-accumbens morphine on hypermotility elicited by systemic injection of caffeine and scopolamine were studied.

Methods

Measurement of Locomotor Activity

Male rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 250 to 350 g, were housed 4 to a cage in a temperature controlled (23 ± 1° C) room with a 12
hour light-dark cycle. For direct injection into the nucleus accumbens, the rats were anesthetized with a halothane/oxygen mixture and placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA) with the toothbar set at -3.3 mm. A midline incision was made in the scalp, and holes were drilled bilaterally into the skull at the coordinates: 10.2 mm anterior to the intraaural line and 1.2 mm lateral to the sagittal suture (Paxinos and Watson, 1982). The needle of a 10 μl Hamilton syringe (Hamilton Co., Reno, NE) was then inserted into the holes to a position 2.0 mm above the intraaural line. Drugs or vehicle were injected in a 0.5 μl volume at a rate of 0.5 μl/min. The needle was left in place for an additional minute to allow for diffusion of the solution. After removal of the needle, the incision was closed with wound clips and swabbed with 5% (w/v) lidocaine ointment.

After injections into the nucleus accumbens, anesthesia was discontinued, and the animals were removed from the stereotaxic frame. Animals recovered from the anesthesia within 5 minutes, after which they were placed in motor activity cages (Opto Varimex-Minor, Columbus Instruments, Columbus, OH) where they were allowed 10 minutes to adapt to the cages. The motor activity cages contained a grid (12 X 12) of infrared beams 3.5 cm apart and 5.0 cm from the bottom of the cage in a ventilated plexiglass box measuring 42 cm square and 20 cm high. Ambulatory activity was measured as the number of times 2 consecutive beams were interrupted. Measurement of locomotor activity was done between 8:00 am and 4:00 pm in an isolated environmental room maintained at a temperature of 23 ± 1° C.
**Histology**

After each experiment, animals were decapitated, and their brains were removed and fixed in a 4% solution of formalin for 48 hours. Frozen sections (40 µm) were cut using a Cryo-Cut microtome (American Optical Corp., Buffalo, NY) to check the location of the tip of the injection needle track. When the tips of the needle tracks were found to lie outside the nucleus accumbens, the data for that animal were excluded from the study. The histological examination showed that the majority of injections were localized to a relatively small subsection of the nucleus accumbens just medial and ventral to the anterior commissure at an anterior-ventral level corresponding to 10.2 mm anterior to Bregma according to the atlas of Paxinos and Watson (1982).

**Drugs**

The following compounds were purchased from Sigma Chemical Co. (St. Louis, MO): morphine sulphate, d-amphetamine sulphate, nialamide, dopamine, ascorbic acid, caffeine HCl, scopolamine, and [D-Thr²]-leucine enkephalin-Thr (DTLET). α-Amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) and (+)-MK-801 hydrogen maleate were purchased from Research Biochemicals Inc. (Natick, MA).

Morphine sulphate and AMPA were dissolved in distilled H₂O; all other drugs were dissolved in sterile saline. The doses of morphine refer to the free base; for all other drugs the doses refer to the weights of the salts. The doses shown refer to the amount injected on each side of the nucleus accumbens.
Statistics

Data were expressed as the mean and the standard error of the mean (SEM). The data were evaluated statistically using the one-tailed Mann-Whitney U-test with \( p<0.05 \) accepted as significant.

Results

Effects of Morphine and DTLET on Normal Locomotor Activity in the Rat

The bilateral injection of DTLET (1.0 \( \mu \text{g} \)), a specific \( \delta \) agonist, into the nucleus accumbens produced a significant increase in locomotor activity. Conversely, administration of morphine (5.0 \( \mu \text{g} \)) into the nucleus accumbens markedly inhibited locomotor activity (Figure 19). However this degree of akinesia did not impair performance on a rotorod. Animals placed thirty minutes after injection (time of maximum akinetic response) on a 45 mm diameter rod rotating at 1.25 rpm maintained themselves on the rod for the entire five minute testing period.

Effects of Morphine on Amphetamine- and AMPA-Stimulated Locomotor Activity in the Rat

d-Amphetamine, which induces hyperlocomotion in the rat primarily by releasing dopamine from dopaminergic nerve terminals and by preventing its reuptake, induced a pronounced locomotor effect in the present study (Figure 20). The locomotor effect was characterized by the animals running along the periphery of the cage. They would typically stop at the corner and sniff for a moment before
resuming ambulatory movement. Rats did not bump into the walls of the cage, and they would avoid obstacles placed in their path. However, when morphine (5.0 µg) was co-injected with d-amphetamine (10 µg) into the nucleus accumbens, the d-amphetamine-induced increase in locomotor activity was abolished (Figure 20). Rats given morphine along with amphetamine tended to stay in the middle of the activity cages, in contrast to animals receiving only saline which stayed in the corner of the cage.

AMPA, an excitatory amino acid and quisqualate receptor agonist which appears to require dopamine to induce locomotion, produced a large increase in locomotor activity after bilateral injection (0.5 µg) into the nucleus accumbens (Figure 21). The locomotion elicited by AMPA was qualitatively similar to that elicited by amphetamine; however, the amount of ambulatory movement after AMPA was greater. Co-administration of morphine (1.5 µg and 5.0 µg) into the nucleus accumbens inhibited AMPA-induced locomotor activity in a dose dependent manner, with the dose of 5 µg almost totally abolishing locomotor activity (Figure 21).

Data presented above demonstrate that 5 µg of morphine completely inhibits hypermotility elicited by either 10 µg d-amphetamine (Figure 20) or 0.5 µg of AMPA (Figure 21). To determine whether this interaction is competitive in nature, the ability of higher doses of d-amphetamine and AMPA to overcome morphine inhibition was studied. The dose of each stimulant was increased three-fold. While this resulted in a partial reversal of the morphine inhibitory effect when
AMPAs was the locomotor stimulant (Figure 22), the locomotor responses to higher doses of amphetamine (30 µg and 50 µg) were still completely blocked by morphine (Figure 22).

Effects of Morphine on other agents that stimulate Locomotor Activity in the Rat

The observation that morphine attenuated both normal locomotor activity and the hypermotility elicited by amphetamine or AMPA suggests that morphine injected into the nucleus accumbens can nonspecifically inhibit locomotor activity. Therefore, the effects of morphine injected into the nucleus accumbens were tested against several agents that stimulate hypermotility. Bilateral administration of MK-801 (5.0 µg), an antagonist of the NMDA receptor, into the nucleus accumbens stimulated locomotor behavior in the rat, and this effect was abolished by coadministration of morphine (5.0 µg) into the nucleus accumbens (Figure 23). Similarly, the increases in locomotor activity induced by systemic administration of both the methylxanthine caffeine (10 mg/kg s.c.) and the cholinergic antagonist scopolamine (0.5 mg/kg s.c.) were abolished by simultaneous administration of morphine (5.0 µg) into the nucleus accumbens (Figure 23). In addition, direct administration of dopamine (20 µg) into the nucleus accumbens two hours after pretreatment with nialamide (200 mg/kg i.p.) produced an increase in locomotor activity (Figure 24), which was abolished by coadministration of morphine (5 µg and 10 µg), (Figure 24). The dopamine hypermotility effect was long-lasting, as the rats were still hyperactive after 3 hours. The inhibition of this behavior by
morphine was long-lasting as well, as rats were akinetic as long as three hours after drug injection (data not shown). The dose of each of these agents that was tested was chosen to produce a submaximal response, and a reliable response would have been obtained from both the dose used and double that dose. Thus the morphine effect is not likely to result from a shift to the right of a compound with a bell-shaped dose response curve. No overt differences in the pattern of locomotion were observed for the ambulatory movement elicited by these agents.

In agreement with previous studies (Morgenstern et al, 1984), administration into the nucleus accumbens of picrotoxin (0.15 μg and 0.5 μg), a blocker of the chloride channel associated with the GABA-A receptor, also markedly stimulated locomotor activity. However, in sharp contrast to the other stimulants investigated in this study, the picrotoxin-induced stimulation of locomotor activity was not attenuated by coadministration of morphine (5 μg or 10 μg) into the nucleus accumbens (Figure 25).

Discussion

The results of the present study clearly demonstrate that morphine injected into the nucleus accumbens blocks the locomotor stimulation elicited by amphetamine, which acts indirectly by releasing endogenous dopamine, and dopamine (in the nialamide-pretreated rat), which directly activates dopaminergic receptors. This effect of morphine was not due to a general impairment in motor function, since morphine treatment did not interfere with performance of the animals on a rotorod,
Figure 19. Effects of morphine and DTLET on locomotor activity in the rat after bilateral injection into the nucleus accumbens. Animals received 5.0 μg of morphine (n=8), 1.0 μg of DTLET (n=5) or saline (n=4). After injection, the animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. *p<0.05 with respect to vehicle control.
Figure 20. Morphine inhibits d-amphetamine-induced locomotor activity in the rat after bilateral injection into the nucleus accumbens. Animals received 5.0 μg of morphine (n=8), 10 μg of amphetamine (n=6), or both morphine and amphetamine (n=7). The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. *p<0.05 with respect to stimulant alone control. The data for the morphine group are the same as shown in Figure 19.
Figure 21. Effect of morphine on AMPA-induced locomotor activity in the rat after bilateral injection into the nucleus accumbens. A solution of morphine (1.5 μg or 5.0 μg) or vehicle was co-injected with AMPA (0.5 μg) in a 0.5 μl volume. The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for 4 rats in the AMPA-treated groups or 5 rats in the control group; *p<0.05, **p<0.01 with respect to stimulant alone control.
Figure 22. Effect of morphine on locomotor activity induced by high concentrations of AMPA or d-amphetamine in the rat after bilateral injection into the nucleus accumbens. A solution of morphine (5.0 µg) was co-injected with AMPA (0.5 or 1.5 µg) or d-amphetamine (10, 30 or 50 µg) in a 0.5 µl volume. The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Data are expressed as a percentage of the stimulant control mean. Each point represents the mean ± S.E.M. of the percentage values for the number of rats shown in parenthesis. Control locomotor responses to AMPA; 0.5 µg (10930 ± 1781) and 1.5 µg (14053 ± 5737 counts), were not statistically different. Control responses to d-amphetamine; 10 µg (3202 ± 663 counts), 30 µg (4946 ± 586 counts), and 50 µg d-amphetamine (8214 ± 526); significantly increased with each successive dose (Mann-Whitney U-test). Asterisk indicates stimulant counteracted morphine effect (p<0.05 compared to stimulant alone control).
Figure 23. Effect of morphine on hypermotility elicited by bilateral injection of MK-801 into the nucleus accumbens or by systemic administration of caffeine or scopolamine. A solution of morphine (5.0 μg) or vehicle was co-injected with MK-801 (0.5 μg) in a 0.5 μl volume or administered simultaneously with caffeine (10 mg/kg s.c.) or scopolamine (0.5 mg/kg s.c.). The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for 4 rats in each group. *p<0.05 with respect to stimulant alone control.
Figure 24. Effect of morphine on dopamine-induced locomotor activity in the rat after bilateral injection into the nucleus accumbens. Rats were pretreated with nialamide (200 mg/kg IP), and two hours later a solution of morphine (5.0 μg, n=5, or 10.0 μg, n=6) or vehicle (n=6) was co-injected with dopamine (20 μg) in a 0.5 μl volume. The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for 5 or 6 rats in each group. *p<0.05 with respect to dopamine control.
Figure 25. Lack of effect of morphine on picrotoxin-induced locomotor activity in the rat after bilateral injection into the nucleus accumbens. A solution of morphine (5.0 μg or 10 μg) or vehicle was co-injected with picrotoxin (0.15 μg or 0.5 μg) in a 0.5 μl volume. The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for the number of observations in parenthesis.
Figure 26. Possible sites of action of morphine within the nucleus accumbens; the "downstream" model.
Figure 27. Possible sites of action of morphine within the nucleus accumbens; the "multiple pathways" model.
Figure 28. DNQX (1.0 μg/0.5 μl) inhibits the locomotor activity elicited by picrotxin (0.5 μg/0.5 μl) when both are administered simultaneously into the nucleus accumbens.
and morphine did not inhibit the locomotor stimulation produced by picrotoxin. The fact that morphine is equally effective against both the direct and the indirect dopaminergic stimulants strongly implies that the morphine inhibition is exerted at a locus at or "downstream" from the dopaminergic receptors in the nucleus accumbens (see Figure 26). It is unlikely that morphine is acting directly on dopaminergic receptors as morphine does not compete for dopaminergic receptors in binding assays (Carlson and Seeger, 1982), and important differences between morphine- and neuroleptic-induced catalepsy have been reported (De Ryck et al, 1980; Teitelbaum, 1982).

The inhibition of amphetamine-induced stimulation of locomotion is compatible with the results of several studies suggesting that morphine impairs motor function by inhibiting the depolarization-induced release of dopamine. (Carenzi et al, 1975; Carlson and Seeger, 1982; Kuschinsky and Hornykiewicz, 1973; Pollard et al, 1977; Pollard et al, 1978; Raffa et al, 1989; Seeger and Carlson, 1980; Slater et al, 1980; Yonehara and Clouet, 1984). However, several observations do not support this hypothesis. Thus, while opiate receptors have been localized on dopaminergic nerve terminals in the nucleus accumbens (Pollard et al, 1977), opiate receptors have been found in greater quantity on cell bodies and on terminals in the nucleus accumbens that are not dopaminergic (Esposito et al, 1987; Unterwald et al, 1989). In addition, while activation of opiate receptors by morphine has been shown to inhibit the depolarization-induced release of dopamine (Carenzi et al, 1975; Yonehara and Clouet, 1984), the amphetamine-
induced release of dopamine is produced by a different mechanism, involving the release of dopamine from a cytoplasmic pool and the inhibition of dopamine reuptake (Kuczenski, 1983). It is, therefore, unlikely that the inhibitory effect of morphine on the depolarization-induced release of dopamine is responsible for the antagonism of the amphetamine response. In our study the intraaccumbens injection of morphine was able to inhibit the hypermotility response to dopamine (Figure 23). These observations suggest that the ability of morphine to counteract the effects of amphetamine resides in a postsynaptic rather than a presynaptic action.

In addition to its ability to block locomotion elicited by activation of the dopaminergic system in the nucleus accumbens, morphine also attenuated locomotion elicited by the excitatory amino acid agonist AMPA. The hypermotility effects of AMPA are dependent upon intact dopaminergic neurotransmission within the nucleus accumbens (Arnt, 1981; Boldry et al, 1991; Donzanti and Uretsky, 1983). Thus, the ability of morphine to block effects of AMPA may result from its action which counteracts dopaminergic stimulation.

Although morphine inhibited the hypermotility induced by either AMPA or amphetamine, increasing the dose of AMPA overcame the morphine induced inhibition, while increasing the dose of amphetamine did not. There are several possible explanations for this difference. The ability of AMPA to counteract the inhibition by morphine could be related to the greater intensity of the locomotor response to this drug as compared to amphetamine. It is also possible that the
high dose of AMPA used (1.5 μg) may stimulate locomotion in a manner unrelated to the effects of morphine. Recently, we have compared the abilities of D1 and D2 dopaminergic receptor antagonists to attenuate hypermotility responses to amphetamine and AMPA. Higher doses of antagonists were needed to inhibit the effects of AMPA than were required to inhibit the response to amphetamine. Thus results with both dopaminergic antagonists and morphine suggests that AMPA is more powerful than amphetamine in stimulating nucleus accumbens locomotor circuits.

In contrast to the above findings, coadministration into the nucleus accumbens of morphine did not antagonize the locomotor response to picrotoxin, an inhibitor of the chloride channel associated with the GABA A receptor complex. This observation indicates that morphine does not inhibit the locomotor response to all drugs that stimulate locomotion by acting in the nucleus accumbens. One explanation for this observation is that picrotoxin acts at a site in the nucleus accumbens that is located "downstream" from the morphine site (Figure 26). Alternatively, it is possible that there are at least two divergent neuronal pathways in the nucleus accumbens involved in the regulation of motility (see Figure 27). Regardless of which hypothesis is correct, our results are consistent with the observation that picrotoxin-induced hypermotility does not require dopaminergic receptor activation, since haloperidol injected into the nucleus accumbens does not attenuate the locomotor activity elicited by picrotoxin (Morgenstern et al, 1984).

We observed that morphine in the nucleus accumbens blocked hypermotility
elicited by systemic administration of scopolamine and caffeine or by injection of MK-801 into the nucleus accumbens. We originally did these experiments to determine whether morphine exerts a non-specific locomotor depressant effect. It has been hypothesized that these compounds stimulate hypermotility by mechanisms other than dopaminergic activation in the nucleus accumbens. Thus, monoamine depletion or blockade of dopaminergic receptors do not abolish the locomotor effect of MK-801 (Carlson and Carlson, 1989; Raffa et al, 1989). Furthermore, destruction of dopamine neurons with 6-hydroxydopamine does not significantly inhibit hypermotility elicited by caffeine or scopolamine (Joyce and Koob, 1981), and the dopamine receptor antagonist, alpha-flupenthixol, does not block hypermotility elicited by caffeine (Swerdlow et al, 1986). However, our results suggest that all three compounds activate a morphine sensitive pathway in the nucleus accumbens, or that all three compounds activate a pathway elsewhere in the brain that is inhibited as a result of morphine action in the nucleus accumbens. If caffeine, scopolamine and MK-801 activate pathways in the nucleus accumbens, the focus of activity must be downstream from the dopaminergic receptor or be on a morphine-sensitive pathway that does not contain dopaminergic receptors:

The present results are consistent with the hypothesis that the \( \mu \)-opiate receptor subtype mediates akinesia in the nucleus accumbens (Daugè et al, 1988; Havemann and Kuschinski, 1985). In the striatum, \( \mu \) receptors mediate rigidity (see Havemann et al, 1980; Havemann et al, 1981). Administration of morphine,
which is about 125 times more active at \( \mu \)-sites then at \( \delta \)-sites (Chang, 1984), resulted in akinesia, while administration of DTLET, a highly selective \( \delta \)-receptor agonist, resulted in stimulation of locomotor activity. It has been demonstrated elsewhere that administration of other specific \( \mu \)-agonists such as DAGO into the nucleus accumbens results in akinesia (Daugè et al, 1988), whereas specific \( \delta \)-agonists into the nucleus accumbens result in hyperactivity (Daugè et al, 1988; Havemann and Kuschinski, 1985).

One limitation that must be considered in interpreting our results is that residual anesthetic and/or the stress from surgery might cause release of endorphins or other compounds that might affect behavior. Our experience is that injection of amphetamine, morphine, AMPA, and saline via implanted cannula into awake animals and injection into anesthetized animals (as used in the present study) produce identical locomotor responses. Furthermore, our results with MK-801, picrotoxin, and dopamine agree with results obtained by injecting these compounds via implanted cannula (Morgenstern et al, 1984; Pijnenburg, 1976; Raffa et al, 1989). Thus complications from the method of drug administration are thought to be minimal.

Based on our results and observations previously reported by others, we propose an explanation for the biphasic effect of systemic morphine and heroin on locomotion in rodents. After systemic administration, lower doses of the compounds exert locomotor stimulation by acting primarily on \( \mu \)-receptors in the ventral tegmental area (Holmes and Wise, 1985; Joyce and Iversen, 1979). This
produces a disinhibition of dopaminergic neurons, resulting in activation of
dopaminergic neurotransmission in the nucleus accumbens. Higher doses of the
compounds may act primarily on μ-receptors in the nucleus accumbens to produce
akinesia which overcomes the increased activity in the dopaminergic system. This
same argument would also explain why drug abusers sometimes use morphine or
heroin in combination with amphetamine or cocaine to decrease the hyperactivity
and agitation of the latter drugs.

Interestingly, while the locomotor activity elicited by picrotoxin was not blocked
by morphine, it was blocked by the AMPA/kainate excitatory amino acid antagonist
dNQX (Figure 28). Thus it appears that dNQX is able to block this morphine
insensitive locomotor pathway.
CHAPTER IV

EFFECT OF SEROTONERGIC AGONISTS IN THE NUCLEUS ACCUMBENS
ON D-AMPHETAMINE-STIMULATED LOCOMOTION

Introduction

Systemic administration of d-amphetamine elicits increases in locomotor activity in the rat. The mesolimbic dopaminergic projection from the ventral tegmental area to the nucleus accumbens, a possible interface between limbic and motor systems (Mogenson and Yim, 1981), is a critical substrate for this effect of amphetamine (Pijnenburg et al, 1976). Behavioral and biochemical studies indicate that d-amphetamine acts primarily by releasing dopamine from dopaminergic nerve terminals and by preventing its reuptake (Kuczenski, 1983). Serotonin (5-hydroxytryptamine, 5-HT) neuronal systems have been shown to modulate locomotor activity mediated by the dopaminergic system (for review see Gersen and Baldessarini, 1980; Soubrie et al, 1984). The nucleus accumbens receives serotonergic projections from the medial and dorsal raphe nuclei (Parent et al, 1981). Several lines of evidence indicate that these serotonergic neurons may exert an inhibitory tone over the mesolimbic dopaminergic neurons which innervate the nucleus accumbens and mediate d-amphetamine stimulated locomotor activity.
Amphetamine-stimulated locomotor activity is potentiated by a) the 5-HT antagonists cyproheptadine and methysergide (Hollister et al, 1976); b) treatments which reduce endogenous 5-HT concentrations such as pretreatment with p-chlorophenylalanine, an inhibitor of 5-HT synthesis (Hollister et al, 1976; Mabry and Campbell, 1973; Segal, 1976; Breese et al, 1974), or maintenance on a tryptophan free diet (Hollister et al, 1976); and c) electrolytic lesions of serotonergic cell bodies (Geyer et al, 1976; Lucki and Harvey, 1974; Green and Harvey, 1974; Carey, 1976; Neill et al, 1972; Costall et al, 1979) or lesions of serotonergic neurons with 5,7-dihydroxytryptamine (Hollister et al, 1976; Breese et al, 1974). Furthermore, prior administration of the 5-HT precursors tryptophan or 5-hydroxytryptophan inhibits d-amphetamine-stimulated locomotor activity in animals that are food deprived or maintained on a tryptophan deficient diet (Hollister et al, 1976); and d-amphetamine-stimulated locomotor activity is inhibited by infusions of 5-HT into the nucleus accumbens (Carter and Pycock, 1978; Costall et al, 1979) or cerebral ventricles (Warbritton et al, 1978).

Multiple 5-HT receptor subtypes have now been characterized, and specific agonists for these subtypes have been developed. Furthermore, various subtypes of 5-HT receptors are localized within the nucleus accumbens (Palacios and Dietl, 1988). Thus, the goal of the present work was to determine which 5-HT receptor subtypes in the nucleus accumbens are important in the modulation of d-amphetamine-stimulated locomotor activity. Towards this end, we have simultaneously administered systemic d-amphetamine and injected into the nucleus
accumbs various classes of 5-HT receptor agonists and have measured the resultant locomotor activity. Using this procedure, the effects of various 5-HT receptor subtype agonists on d-amphetamine-stimulated locomotor activity were evaluated.

Methods

Measurement of Locomotor Activity

Male rats (Harlan Sprague-Dawley, Indianapolis, IN), weighing 250 to 350 g, were housed 4 to a cage in a temperature controlled (23±1°C) room with a 12 hour light-dark cycle. For direct injection into the nucleus accumbs, the rats were anesthetized with a halothane/oxygen mixture and placed in a stereotaxic frame (David Kopf Instruments, Tajunga, CA) with the toothbar set at -3.3 mm. A midline incision was made in the scalp, and holes were drilled bilaterally into the skull at the coordinates: 10.2 mm anterior to the intraaural line and 1.2 mm lateral to the sagittal suture (Paxinos and Watson, 1982). The needle of a 10μl Hamilton syringe (Hamilton Co., Reno, NE) was then inserted into each of the holes to a position 2.0 mm above the intraaural line. Drugs or vehicle were injected in a 0.5μl or 1.0μl volume at a rate of 0.5μl/min. The needle was left in place for an additional minute to allow for diffusion of the solution. After removal of the needle, the incision was closed with wound clips and swabbed with 5% (w/v) lidocaine ointment. d-Amphetamine (0.5 mg/kg s.c.) was administered after removal of the Hamilton syringe, while the animal was still anesthetized.
After injections into the nucleus accumbens, anesthesia was discontinued, and the animals were removed from the stereotaxic frame and were placed in motor activity cages (Opto Varimex-Minor, Columbus Instruments, Columbus, OH). Animals recovered from the anesthesia and were upright and mobile within 5 minutes, after which they were allowed 10 minutes to adapt to the cages. At this point, collection of locomotor data began. The motor activity cages contained a grid (12 X 12) of infrared beams 3.5 cm apart and 5.0 cm from the bottom of the cage in a ventilated plexiglass box measuring 42 cm square and 20 cm high. Ambulatory activity was measured as the number of times 2 consecutive beams were interrupted. Measurement of locomotor activity was done between 8:00 am and 4:00 pm in an isolated environmental room.

**Histology**

After each experiment, animals were decapitated, and their brains were removed and fixed in a 10% solution of formalin for 48 hours. Frozen sections (40mm) were cut using a Cryo-Cut microtome (American Optical Corp., Buffalo, NY) to check the location of the tip of the injection needle track. When the tips of the needle tracks were found to lie outside the nucleus accumbens, the data for that animal were excluded from the study.
Drugs

Morphine sulphate, serotonin creatinine sulphate (5-HT), and d-amphetamine sulphate were purchased from Sigma Chemical Co. (St. Louis, MO). 1-(3-Chlorophenyl)piperazine HCl (mCPP), CGS-12066B, (±)-12,5-dimethoxy-4-iodo)-2-aminopropane (DOI), 8-hydroxy-2-(di-n-propylamino)-tetralin HBr (8-OH-DPAT), 1-phenylbiguanide, quipazine, 3-tropanyl-3,5-dichlorobenzoate (MDL-7222), and 1-(2-methoxyphenol)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide (NAN-190) were purchased from Research Biochemicals Inc. (Natick, MA). d-Amphetamine sulphate was dissolved in 0.9% saline. Morphine sulphate, DOI, 1-phenylbiguanide, and quipazine were dissolved in distilled H₂O. 5-HT and mCPP were dissolved in dilute (0.01 N) acetic acid. CGS-12066B was dissolved in 0.1 N HCl, and 8-OH-DPAT was dissolved in 0.1 N HCl with 0.1% w/v ascorbic acid. NAN-190 and MDL-7222 were dissolved in dilute (0.01 N) acetic acid with mild heating on a hot plate. Appropriate vehicle controls were run simultaneously. The doses shown refer to the amount injected on each side of the nucleus accumbens.

Statistics

Data were expressed as the mean and the standard error of the mean (SEM). The data were evaluated statistically with an analysis of variance (ANOVA) followed by the Newman-Keuls multiple range test for comparison of individual groups, with p<0.05 accepted as significant.
Results

Systemic d-amphetamine (0.5 mg/kg s.c.) induced a pronounced locomotor stimulation (Figure 29,30,31). In agreement with previous studies (Carter and Pycock, 1978; Costall et al, 1979), 5-HT (50 and 100 nmol) injected into the nucleus accumbens inhibited-amphetamine-stimulated locomotion (Figure 29).

Although 5-HT was able to inhibit d-amphetamine stimulated locomotion, the 5-HT agonist quipazine, the general 5-HT\textsubscript{1} agonist mCPP, and the 5-HT\textsubscript{1D}/5-HT\textsubscript{2} agonist DOI produced no inhibition at the highest dose tested, (100 nmol) (Figure 30). In contrast, the 5-HT\textsubscript{3} agonist 1-phenylbiguanide (100 nmol) potentiated d-amphetamine-stimulated locomotion, (Figure 30). The potentiation of d-amphetamine-stimulated locomotion by 1-phenylbiguanide was partially reversed by the 5-HT\textsubscript{3} antagonist MDL-7222 (Figure 30). Statistical analysis revealed that while the d-amphetamine- stimulated locomotor activity of animals receiving 1-phenylbiguanide and MDL-7222 was not significantly higher than those receiving vehicle, the d-amphetamine- stimulated locomotor activity of animals receiving 1-phenylbiguanide alone was not significantly different from animals receiving 1-phenylbiguanide and MDL-7222.

The specific 5-HT\textsubscript{1A} agonist 8-OH-DPAT (100 nmol) did not inhibit d-amphetamine-stimulated locomotion (Figure 31), but a pronounced lateral headweaving behavior was observed. The specific 5-HT\textsubscript{1B} agonist CGS-12066B (100 nmol) had no effect on d-amphetamine-stimulated locomotor activity (Figure 31). However, the combination of 8-OH-DPAT (50 nmol) and CGS-12066B (50
Figure 29. Effects of 5-HT bilaterally injected into the nucleus accumbens on systemic d-amphetamine stimulated locomotor activity in the rat. A solution of 5-HT or vehicle (V) was injected in a 0.5 µl volume, and d-amphetamine was given concurrently (0.5 mg/kg s.c.). The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for the number of observations in parentheses. *p<0.05 with respect to vehicle control.
Figure 30. Effects of several 5-HT agonists on systemic d-amphetamine-stimulated locomotor activity in the rat after bilateral injection into the nucleus accumbens. A solution of 1-(3-chlorophenyl)piperazine (mCPP), DOI, quipazine (QUIP), 1-phenylbiguanide (PBG), PBG + MDL-7222 (MDL), or vehicle (V) (each 100 nmol) was injected in a 1.0 µl volume, and d-amphetamine was given concurrently (0.5 mg/kg s.c.). The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for the number of observations in parentheses. *p<0.05 with respect to vehicle control.
Figure 31. Effects of 8-OH-DPAT (5-HT$_{1A}$ agonist) and CGS-12066B (5-HT$_{1B}$ agonist) on systemic d-amphetamine-stimulated locomotor activity in the rat after bilateral injection into the nucleus accumbens. A solution of vehicle (V), 8-OH-DPAT (100 nmol), CGS-12066B (100 nmol), or both (each 50 nmol) was injected in a 1.0 µl volume, and d-amphetamine was given concurrently (0.5 mg/kg s.c.). The animals were placed in motor activity cages, and locomotor activity was recorded for 1 hour. Each point represents the mean ± S.E.M. for the number of observations in parentheses. *p<0.05 with respect to vehicle control.
Figure 32. Lack of effect of serotonergic agonists in the nucleus accumbens on normal locomotor activity.
nmol) produced a significant inhibition of d-amphetamine-stimulated locomotor activity (Figure 31). Lateral headweaving was still observed with the combination of drugs. The inhibition of d-amphetamine-stimulated locomotor activity by intra-accumbens 5-HT (50 nmol), however, was not reversed by systemic (8 mg/kg s.c.) administration or coinjection into the nucleus accumbens (4 μg) of the 5-HT\textsubscript{1A} antagonist NAN-190.

Administration of all agonists alone (in the absence of d-amphetamine) into the nucleus accumbens (all 100 nmol) did not alter basal locomotor activity (Figure 32).

**Discussion**

After confirming earlier observations that 5-HT inhibits the locomotor response elicited by systemic amphetamine (Carter and Pycock, 1978; Costall et al, 1979), the present study presents information on the subtypes of 5-HT receptor in the nucleus accumbens that may regulate d-amphetamine-stimulated locomotor activity. The effects of agents with specificities for various subtypes of 5-HT receptor suggest that serotonergic modulation of dopaminergic activity within the nucleus accumbens is the result of a complex interaction of these receptor subtypes. Concurrent activation of both 5-HT\textsubscript{1A} and 5-HT\textsubscript{1B} receptors may be required to inhibit amphetamine stimulated hypermotility, while 5-HT\textsubscript{3} receptor activation appears to potentiate the amphetamine effect (Figure 33).

Although 5-HT injected into the nucleus accumbens attenuated the hypermotility
elicited by systemic administration of amphetamine, none of the compounds with selectivity for various receptor subtypes was able to reproduce this effect. However, the combination of 8-OH-DPAT, a 5-HT$_{1A}$ agonist, and CGS-12066B, a 5-HT$_{1B}$ agonist (see Neale et al., 1987; but also Schoeffter and Hoyer, 1989), mimicked the effects of 5-HT. The concept that this is an interaction between two distinct receptor mechanisms is derived from the observation that neither compound when used alone at double the dose used in the combination experiment was able to attenuate the hypermotility elicited by amphetamine. In support of this hypothesis, another interaction between 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors has been reported. Thus, a high dose of CGS-12066B potentiates 8-OH-DPAT stimulated hypothermia in mice (Berensen and Broekkamp, 1990). This suggests that such interactions might be important in the production of a variety of functional effects mediated by serotonin. On the other hand, the 5-HT$_{1A}$ antagonist NAN-190, in the present study, was unable to reverse the effects of 5-HT on d-amphetamine-stimulated locomotion. Either the dose of NAN-190 used in the present study was insufficient or 8-OH-DPAT may be acting at some site other than the 5-HT$_{1A}$ receptor. NAN-190, at the systemic dose used in this study, has previously been shown to block the behavioral syndrome, but not the hypothermia, elicited by 8-OH-DPAT (Przegalinski et al., 1990).

The 5-HT agonist mCPP, which has affinity for both 5-HT$_{1B}$ and 5-HT$_{1C}$ receptors, has been shown to inhibit spontaneous ambulatory behavior after systemic administration in the rat (Lucki et al., 1989) even though mCPP when
administered into the nucleus accumbens did not decrease hypermotility elicited by amphetamine in our experiments. The apparent discrepancy between these observations could be explained if mCPP after systemic administration exerts a hypomotility effect at a locus other than the nucleus accumbens. Another possibility is that the hypermotility effect of amphetamine is so powerful that it readily overcomes a weak hypomotility effect of mCPP.

PBG, a 5-HT\textsubscript{3} receptor agonist, had an effect opposite to that of 5-HT. This finding that activation of the 5-HT\textsubscript{3} receptor potentiates the effects of amphetamine is supported by the results of several recent studies. Direct injection of 2-methyl-5-HT, another 5-HT\textsubscript{3} receptor agonist, into the nucleus accumbens potentiates amphetamine stimulated locomotor activity (Costall et al, 1987). In addition, both 5-HT\textsubscript{3} receptor agonists, 2-methyl-5-HT and PBG, increase the release of endogenous dopamine from slices of the rat striatum (Blandina et al, 1989; Schmidt and Black, 1989), an area with many biochemical correlates to the nucleus accumbens. The dopamine release induced by 2-methyl-5-HT, but not PBG, was reversed by the selective 5-HT\textsubscript{3} antagonist ICS 205-930 (Blandina et al, 1989; Schmidt and Black, 1989). Thus while it is possible that activation of 5-HT\textsubscript{3} receptors in the nucleus accumbens may enhance the stimulant response to amphetamine by enhancing the release of dopamine by amphetamine at this site, PBG may be acting as an inhibitor of dopamine uptake (Schmidt and Black, 1989). In the present study, the potentiating effect of PBG on d-amphetamine-stimulated locomotor activity was partially blocked by the 5-HT\textsubscript{3} antagonist MDL-7222,
suggesting a role for both a direct action mediated by 5-HT₃ receptors and an inhibition of dopamine uptake.

Thus, it seems likely that 5-HT may have opposing effects on regulation of d-amphetamine-stimulated locomotor activity in the nucleus accumbens by enhancing the release of dopamine from nerve terminals (5-HT₃ receptor action) and by counteracting the effects of dopamine receptor activation (possibly 5-HT₁₇ + 5-HT₁₈ receptor action), see Figure 33. When 5-HT is infused into the nucleus accumbens it is likely that both effects occur, with the inhibitory effect predominating. Quipazine, unlike 5-HT, had no effect on d-amphetamine-stimulated locomotor activity in the present study. Although quipazine is generally considered a relatively nonselective 5-HT receptor agonist, there is evidence that quipazine acts as an agonist for the 5-HT₂ receptor (Lyon and Titeler, 1988) and as an antagonist for the 5-HT₁₇ and 5-HT₃ receptors (Schoeffter and Hoyer, 1989), resulting in the absence of a net change in amphetamine-stimulated locomotion. DOI, a selective agonist at 5-HT₁₅ and 5-HT₂ receptors, also had no effect on d-amphetamine stimulated locomotor activity. These data suggest that the 5-HT₂ receptor does not play an important role in modulation of locomotor activity elicited by amphetamine in the rat nucleus accumbens. This is supported by the results of several other recent studies which have implicated 5-HT₁ receptor subtypes, as opposed to the 5-HT₂ subtype, in the inhibition of locomotor activity (Lucki et al, 1989; Kennet and Curzon, 1988; Klodzinska et al, 1989), although to our knowledge none of these studies have
Figure 33. Possible sites of action of serotonin in the nucleus accumbens.
examined the role of these subtypes in the nucleus accumbens. Furthermore, 5-HT has a much higher affinity for 5-HT\(_1\) sites than for 5-HT\(_2\) sites (Nelson, 1988), and the nucleus accumbens contains a higher concentration of 5-HT\(_1\) specific binding sites, although 5-HT\(_{1A}\) receptors appear to comprise only 3% of 5-HT\(_1\) specific binding (Palacios and Dietl, 1988).

Interestingly, pronounced repetitive lateral head-weaving was elicited by injection of 8-OH-DPAT into the nucleus accumbens. This behavioral phenomenon is an important component of the serotonin motor syndrome (See Gersen and Baldessarini, 1980). Other components of the syndrome such as forepaw treading, hind limb abduction, tremor, or Straubs tail were not observed. Several recent studies suggest that activation of the 5-HT\(_{1A}\) receptor produces this complex behavioral syndrome (Middlemiss and Fozard, 1983; Sills et al, 1984; Lucki et al, 1984). Our data indicate that the 5-HT\(_{1A}\) receptors which mediate the head weaving component of the syndrome are located in the nucleus accumbens, while those mediating the other components are probably located elsewhere.

Serotonergic projections from the raphe nuclei have long been thought to regulate locomotor activity in rats (Gerfen and Baldessarini, 1980; Hollister et al, 1976; Jones et al, 1981). Furthermore, it has been proposed that some serotonin-mediated behaviors are modulated by multiple subtypes of 5-HT receptors (Glennon et al, 1991). The results of this study are consistent with this hypothesis and suggest that activation of no single 5-HT receptor subtype can mimic the effects of 5-HT. Furthermore, it appears that 5-HT neurons within the
nucleus accumbens can differentially modulate the locomotor activity elicited by d-amphetamine, perhaps through interactions between 5-HT₁₈, 5-HT₁₉, and 5-HT₃ receptors.
A reward system has evolved in vertebrates which reinforces biologically useful behaviors like approaching a goal object and subsequently eating, drinking, and reproducing. Thus it feels good to do these things. Additionally, an organism will engage in these rewarding behaviors again. Addicting drugs gain access to this system, and mimic natural reward by artificially stimulating it. By stimulating the reward system, addicting drugs elicit pleasure and result in a compulsion to maintain that state.

The mesolimbic dopamine system and especially its terminal fields within the nucleus accumbens comprise a critical substrate for both the locomotor-activating and rewarding properties of most abused drugs. A vast quantity of evidence suggests that an increase in dopamine release in the nucleus accumbens is required for the expression of reward and positive reinforcement. Several neural systems may interact with the dopaminergic system within the nucleus accumbens and thus play a role in the expression of reward. The studies described in this dissertation have demonstrated a role for excitatory amino acid receptors, opiate
receptors, and serotonin receptors in the nucleus accumbens in mediating the behaviors mediated by the dopamine system.

Recent studies suggest that glutamatergic afferents (inputs) from limbic structures such as amygdala and hippocampus interact with dopamine afferents within the nucleus accumbens. The purpose of the first set of experiments (Chapter 2) was to examine the role of NMDA and AMPA/kainate excitatory amino acid receptors in the expression of behavior associated with increased dopamine transmission in the nucleus accumbens. Thus, the results of the first set of experiments demonstrated that MK-801, a potent noncompetitive antagonist of central NMDA receptors, at a dose which produces behavioral activation, is positively reinforcing in a conditioned place preference paradigm (and therefore may have abuse potential). These findings are consistent with reports that MK-801 increases dopamine transmission in the nucleus accumbens. The results of the second set of experiments demonstrated that a non-NMDA glutamate antagonist (DNQX) within the nucleus accumbens could inhibit the expression of a drug-induced conditioned place preference. In addition it was found that DNQX inhibited the acquisition of a place preference elicited by amphetamine, while it did not significantly inhibit that of morphine. This finding is consistent with the hypothesis that multiple reward substrates may exist within the nucleus accumbens.

While a role for opiates in the nucleus accumbens in reward is well established, their effects on locomotor activity are less clear. The results of the next set of
experiments (Chapter 3) demonstrated an interaction between opiate, glutamatergic, and dopaminergic innervation of the nucleus accumbens, using locomotor activity as the measured response. The dopaminergic stimulants d-amphetamine or dopamine induced a large increase in locomotor activity when injected into the nucleus accumbens and were blocked by coadministration of morphine. The hypermotility response elicited by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), an excitatory amino acid agonist that requires dopaminergic receptor activation for the expression of its locomotor activating effect, was also abolished by coadministration with morphine. Intra-accumbens morphine also blocked the hypermotility elicited by systemic caffeine or scopolamine, compounds whose hypermotility responses do not involve the dopaminergic synapses within the nucleus accumbens. However, picrotoxin injected into the nucleus accumbens elicited a hypermotility that was not attenuated by coinjection of morphine. These data demonstrate that opiate and dopaminergic pathways have competing actions on regulation of locomotion in the nucleus accumbens, and that AMPA stimulated locomotor activity is sensitive to inhibition by morphine. The finding that morphine, acting at the μ receptor, can inhibit locomotor activity produced by some drugs, but not others, suggests that multiple locomotor substrates exist within the nucleus accumbens.

Serotonergic projections from the raphe nuclei to the nucleus accumbens modulate locomotor activity. In recent years, several serotonin receptor subtypes have been discovered and demonstrated in the CNS. Which receptors mediate
locomotor activity within the nucleus accumbens, however, is not clear. Thus, the purpose of the final set of experiments (Chapter 4) was to characterize the effects of various classes of serotonergic agonists administered into the nucleus accumbens on d-amphetamine-stimulated locomotor activity in order to determine which serotonin receptor subtypes are involved. Administration of the nonselective 5-HT agonist quipazine, the 5-HT₁ agonist mCPP, the 5-HT₁₅ agonist 8-OH-DPAT, the 5-HT₁₆ agonist CGS-12066B, and the 5-HT₁₅/5-HT₂ agonist DOI did not inhibit d-amphetamine-stimulated locomotor activity. The combination of the 5-HT₁₅ agonist 8-OH-DPAT and the 5-HT₁₆ agonist CGS-12066B, however, did inhibit d-amphetamine-stimulated locomotor activity. Inhibition of d-amphetamine-stimulated locomotor activity by intra-accumbens 5-HT was not reversed by the 5-HT₁₅ antagonist NAN-190. In contrast, the 5-HT₃ agonist 1-phenylbiguanide enhanced the locomotor effect of d-amphetamine. This effect was partially reversed by the 5-HT₃ antagonist MDL-7222. These studies suggest that serotonin has complex and multiple effects on the regulation of locomotor activity within the nucleus accumbens, depending on which receptor subtypes are involved.

A SIMPLE MODEL

On the basis of the behavioral pharmacology of dopaminergic agonists and antagonists, a general theory proposing a role for dopamine in the nucleus accumbens in reward and locomotion has been advanced. Indeed, reward and
locomotion may share a common neural substrate. Thus, increases in nucleus accumbens dopamine transmission both elicit reward and stimulate locomotion, and these behavioral phenomena are inhibited by dopamine antagonists. The κ agonist U50,488H both reduces dopaminergic transmission and locomotor activity, and is aversive. Clearly, amphetamine acts on a substrate within the nucleus accumbens which results in the expression of reward and locomotor activity. The findings described in this dissertation are consistent with this theory. However, the present findings further suggest a heterogeneity of function within the nucleus accumbens such that multiple substrates exist which mediate both locomotor activation and reward. For example, morphine in the nucleus accumbens, which is rewarding (van der Kooy et al, 1982), inhibits locomotor activity, while AMPA in the nucleus accumbens, which stimulates locomotion, is not rewarding (Figure 18). Furthermore, a D₁ antagonist inhibits the conditioned place aversion to systemic picrotoxin (Di Chiara et al, 1991), which suggests that an increase in dopamine transmission is necessary to associate internal cues signaling aversion and environmental cues. It thus appears that multiple functional substrates within the nucleus accumbens may exist.

If multiple substrates for reward and locomotor activity coexist within the nucleus accumbens, then this may explain the seeming paradox of the involvement of accumbens dopamine in stress. It is well established that stress increases dopamine transmission in the nucleus accumbens (Abercrombie et al, 1989), and that dopaminergic antagonists suppress responding to an aversive conditioned
stimulus, such as a tone which precedes a shock (Baldessarini, 1985). This paradox can be reconciled, however, if one entertains the possibility of multiple pathways through the nucleus accumbens. Dopamine may modulate distinct pathways subserving both stress and reward.

While dopamine is involved in reward and stress, it also seems to play an important role in motivation (Di Chiara et al, 1991) and the anticipation of reward (Phillips et al, 1991). In the next few paragraphs, I will propose a tentative explanation for these phenomena.

In Chapter 3, the effects of the AMPA/kainate antagonist DNQX on three events were measured; the acquisition of amphetamine reward, the acquisition of morphine reward, and the expression of reward elicited by either. The acquisition of a place preference indicates two things; 1) that the unconditioned stimulus was indeed rewarding, and 2) that the animal was able to associate the unconditioned stimulus (drug) with the appropriate conditioned stimulus (white side of the conditioned place preference apparatus). As DNQX did not inhibit the acquisition of morphine-induced place preference, it does not seem likely that it should have blocked the ability to associate a conditioned stimulus with an unconditioned stimulus, rather it seems more likely that the DNQX inhibited amphetamine-induced reward directly. This suggests a difference between the substrates of morphine and amphetamine induced reward. The expression of a place preference indicates the ability of the conditioned stimulus to elicit reward. That DNQX inhibited the expression of both morphine- and amphetamine-induced place preference
suggests that a similar substrate mediates the reward elicited by the conditioned stimulus in both cases.

I propose a simple model to account for these data (see Figure 34). This model, based on one proposed by Grace (1990), is greatly simplified, but may be of some heuristic value. In order to elicit reward, amphetamine must cause the release of dopamine within the nucleus accumbens, which comes from neuron DA and acts at receptor D (for dopamine). Simultaneously, glutamate must be released from neuron GLU2 and act at receptor A2 (for AMPA). Thus, dopamine antagonists (Mackey and van der Kooy, 1985) or DNQX will inhibit amphetamine reward. For morphine to elicit reward, it need only act at receptor \( \mu \). Neuron GLU2 does not have to be active, and DNQX does not inhibit morphine reward. In order for the conditioned stimulus to elicit reward, neuron GLU1 must be active, and glutamate must act at receptor A1, on neuron DA. This causes an increase in dopamine release (see Imperato et al, 1990; Desce et al, 1991), and therefore dopamine acts at receptor D. Thus both dopamine antagonists (Hiroi and White, 1989) and DNQX will block the expression of place preference. It is possible that neuron GLU1 comes from the amygdala, as the amygdala is required for the establishment of conditioned reinforcement (Cador et al, 1989). Neuron GLU2 may originate in the prefrontal cortex, as this area has been implicated in mediating cocaine reward (Goeders and Smith, 1983).

The phenomenon of motivation may interact with the proposed model in the following way. Phillips et al (1991) have shown that dopamine levels in the
Figure 34. A model of neuronal interactions in the nucleus accumbens, involving phasic and tonic dopamine release. Abbreviations: (VTA) ventral tegmental area; (AMY) amygdala; (PFC) prefrontal cortex.
nucleus accum bens rise in response to the anticipation of reward. If the recognition of a conditioned stimulus is mediated by the amygdala, and this causes activation of neuron GLU1 which releases dopamine through a presynaptic mechanism, then tonic levels of dopamine will rise in the accum bens, accounting for the data of Phillips et al (1991). This may act to "prime" the system. If the stimulus is rewarding enough, then phasic release will occur following some sort of positive feedback loop. Thus the difference between general motivation and reward may be the difference between tonic and phasic dopamine release. A similar but distinct system may function in parallel within the nucleus accum bens to account for conditioned avoidance responding and stress. It remains to be seen what effects DNQX in the nucleus accum bens might have in these paradigms.

IMPLICATIONS FOR CLINICAL TREATMENT OF HUMAN DRUG ADDICTION

Patterns of drug intake among addicted persons differ depending on the degree of addiction and drug which is abused. Use patterns range from stable levels of intake to periodic binges or "runs". The hallmark of compulsive, noncontrollable, drug use of any type is the intense craving for the drug, which is predictive of and usually is followed by relapse and repeated drug use.

The negative reinforcement theory of addiction holds that a condition of abnormal depression of the positive reinforcement pathway occurs after repeated administration, possibly by depletion of synaptic dopamine (Dackis and Gold, 1985). Thus while increased dopamine mediates reward, a condition of depleted
synaptic dopamine in the nucleus accumbens results in the intense craving which predicts relapse.

Drug craving, however, appears to be a highly motivated condition. The condition of drug craving has been likened to a powerful "lust" for the drug. Addicts become fixated on thinking about the drug and its procurement. Animal studies reveal that motivated behavior in response to an environmental cue involves increased dopamine transmission in the nucleus accumbens. This, then, is seemingly at odds with the dopamine depletion hypothesis.

The positive reinforcement theory, in line with incentive motivation theory, holds that it is the positively reinforcing effects of the drug which result in its reuse. Wise (1990) suggests that the addict remembers the last reinforcement, and the reactivation of that memory by associated stimuli is sufficient to elicit drug craving. Indeed, in one study of cocaine addicts (Gawin and Kleber, 1986), it was reported that, even after 28 weeks of abstinence and lack of craving, intense craving returned on seeing old cocaine-using friends and passing homes in which they had used cocaine. Intravenous users reported experiencing craving after having blood drawn for laboratory tests. In animal self-administration studies, "priming", the delivery of a noncontingent drug stimulus, is effective at starting the animal in responding at the beginning of a session. Thus, in humans, memories of past drug reinforcements associated with environmental cues may act as priming stimuli. Wise (1990) has further suggested that the biological correlate of craving may lie not in positive reinforcement substrates, but in substrates of memory.
Recently, the nucleus accumbens has been implicated in one form of memory, social memory (Ploeger et al, 1991).

The results contained in this dissertation suggest that functional heterogeneity exists within the nucleus accumbens, an important reward substrate. Furthermore, they suggest that modulation of the proposed reward pathway by other systems may occur. While it seems that the best therapy for the addicted person is removal of environmental cues associated with drug use, this may not be feasible, and pharmacological treatment (if possible in the future) may be desirable. The neural systems which modulate the nucleus accumbens may be possible substrates for a pharmacological treatment of drug addiction. In fact, 5-HT₃ receptor antagonists have recently been proposed as treatments for drug addiction (Trickelbank, 1989). While studies in this area are in their infancy, it is fervently hoped that further research will shed light on this important topic.

As a final word, this author feels that recent proposals suggesting the legalizing of drugs such as cocaine, amphetamine, and heroin, would be a mistake of epic proportions. Animal data suggests that these drugs, and especially cocaine and amphetamine, are the most addiction prone drugs known. Human data suggest that increased availability to psychoactive drugs results in increased consumption. In fact, the incidence of drug addiction among medical professionals who have easier access to psychoactive drugs was found to be much higher than in a matched control population. Furthermore, an epidemiological study done in the United States (U.S.) before opiates and cocaine were made illegal suggests that
the proportion of U.S. adult drug addicts was not different from the proportion of U.S. adult alcoholics currently (for review see Goldstein and Kalant, 1990). Recommendations for solving this immense problem are beyond the scope of this dissertation. It is hoped, however, that continued research and public education will help to reduce the magnitude of the current addiction problem.
APPENDIX

ABBREVIATIONS USED IN THIS DOCUMENT

5-HT- serotonin; 5-hydroxytryptamine
8-OH-DPAT- 8-hydroxy-2-(di-n-propylamino)-tetralin HBr
AbC- core of nucleus accumbens
AbS- shell of nucleus accumbens
ac- anterior commisure
ACPC- 1-aminocyclopropanecarboxylic acid
ACP- 1-amino-1,3-cyclopentane-dicarboxylic acid
ADCP- cis-1-amino-1,3-dicarboxycyclopentane
AFQX- 6-amino-7-fluoroquinoxaline-2,3-dione
AMPA- α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AP-3- 2-amino-3-phosphonopropionic acid
AP-4- 2-amino-4-phosphonobutyric acid
AP-5- 2-amino-5-phosphonovaleric acid
AP-7- 2-amino-7-phosphonoheptanoic acid
cc- corpus callosum
CGS-12066B- (±)-(1,2,5-dimethoxy-4-iodo)-2-aminopropane
CNQX - 6-cyano-7-nitroquinoxaline-2,3-dione
CP - caudate-putamen or dorsal striatum
CS+ - conditioned stimulus +
CS- - conditioned stimulus -
CPP - 3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonate
CTAP - D-Phe-Cys-Tyr-D-Trp-Arg-Thr-Pen-Thr-NH₂
CTX - cortex
DA - dopamine
DAGO - Tyr-D-Ala-Gly-MePhe-Glyol
DMT - dorsomedial thalamus
DNQX - 6,7-dinitroquinoxaline-2,3-dione
DOI - (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl
DPDPE - Tyr-D-Pen-Gly-Phe-D-Pen
DTLET - [D-Thr²]-leucine enkephalin-Thr
GAMS - D-glutamylamino-methyl sulfonate
GDEE - glutamic acid diethylester
GLU - glutamate
HIP - hippocampus
IC - islands of Calleja
ICI74864 N,N-bisallyl-tyr-Aib-Aib-Phe-Leu-OH {Aib: aminoisobutyrate}
LSD - lysergic acid diethylamide
mCPP- 1-(3-chlorophenyl)piperazine HCl
MDL-7222- 3-tropanyl-3,5-dichlorobenzoate
mfb- medial forebrain bundle
MK-801- [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate]
MS- morphine sulphate
NAN-190- 1-(2-methoxyphenol)-4-[4-(2-phthalimido)butyl]piperazine hydrobromide
NBQX- 2,3-dihydroxy-6-nitro-7-sulfamoyl benzo(f)quinoxaline
NDB- nucleus of the diagonal band
NMDA- N-methyl-D-aspartate
OT- olfactory tubercle
PFC- prefrontal cortex
PPN- pedunculopontine nucleus
QUIP- quipazine
RN- raphe nuclei
s.c.- subcutaneous injection
SEM- standard error of the mean
TFMPP- m-trifluoromethylphenylpiperazine
U50488H- (trans-(±)-3,4-dichloro-n-methyl-[2-(1-pyrrolidinyl)cyclohexyl]) benzeneacetamide methane sulphonate
VP- ventral pallidum
VTA- ventral tegmental area


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