DRUG-INDUCED PLACE CONDITIONING IN GOLDFISH: EVOLUTIONARY IMPLICATIONS FOR NEURAL MECHANISMS OF REWARD

VIRGINIA L. GRANT

DRUG-INDUCED PLACE CONDITIONING IN GOLDFISH: EVOLUTIONARY IMPLICATIONS FOR NEURAL MECHANISMS OF REWARD

BY

© VIRGINIA L. GRANT

A thesis submitted to the School of Graduate
Studies in partial fulfilment of the
requirements for the degree of
Doctor of Philosophy

Department of Psychology
Memorial University of Newfoundland
1992

St. John's Newfoundland

ISBN 0-315-78117-3

ABSTRACT

The underlying concern of this dissertation is with the evolutionary origins of a reward system extensively studied in rats. This system, called here the dopamine reward system, is characterized by three features: a dopamine mechanism, an opioid mechanism, and a functional dependence of the opioid mechanism on the dopamine mechanism. The same system appears to be present in all mammals, and possibly birds, suggesting that the system was also present in the common ancestor from which modern reptiles, birds, and mammals evolved. Thus, it seems possible that the dopamine reward system originated from an even earlier ancestor of vertebrates. To evaluate this possibility, goldfish were studied, because fish were the first modern vertebrates to evolve and, hence, are the most distantly related of all vertebrates to mammals.

The presence of dopamine and opioid reward mechanisms in goldfish was tested by determining whether drugs with known effects on dopamine and opioid mechanisms have rewarding effects in the place conditioning procedure. The indirect dopamine agonist amphetamine (3.6 and 5.0 mg/kg) reliably produced conditioned place preferences. The direct and relatively selective dopamine agonist, apomorphine, also had rewarding effects, but only at rather low doses (0.4-0.5

mg/kg; doses of 0.25, 1.0, and 2.0 mg/kg were ineffective). These findings provide evidence for a dopamine reward mechanism in goldfish. Two other findings, however, were not consistent with such an interpretation. (1) No rewarding effects were found with cocaine (10 and 20 mg/kg), a psychomotor stimulant similar to amphetamine. (2) The rewarding effects of amphetamine were not blocked by either of the dopamine antagonists haloperidol (0.15 mg/kg) or flupentixol (0.8 mg/kg). This second finding raises doubts about the role of dopamine in the rewarding effects of amphetamine.

In tests for the presence of an opioid reward mechanism in goldfish, morphine, whether administered intraperitoneally (5 to 30 mg/kg) or intracranially (0.3 μg), consistently failed to produce a rewarding effect. In view of these findings it was not feasible to test for the third feature of the dopamine reward system, namely, the functional dependence of the opioid mechanism on the dopamine mechanism.

The phylogenetic implications of these findings were discussed.

ACKNOWLEDGEMENTS

I would like to express my immeasurable gratitude to the following people for their contributions to the production of this dissertation:

To my supervisor, Dr. B. T. Lett for her inspiration of the topic of this dissertation, for her extraordinary support in every phase of the project, and for her invaluable logical analysis to keep me on track.

To Dr. S. Revusky, for his help in the development of this project and for his incredible patience, conscientiousness, and insights which helped me through the writing of the manuscript.

To Dr. D. Corbett, for his help in the development of this project and for his critical evaluation of neurophysiological aspects of this dissertation.

To my husband, Malcolm, for his help with all the technical aspects of the manuscript, with proof-reading and suggestions for making the manuscript more readable, and most of all for his understanding and unconditional support which helped me through the process without losing my sanity. And to our daughter, Jessica, for her moral support, for her effort in searching out and xeroxing material in the University of Toronto Library, as well as for her help with the care of the fish and with some of the

experiments during a summer holiday. And to my parents, sister, other members of my family and friends who all contributed in various ways to encouraging me in this endeavour.

To my external examiner, Dr. Derek van der Kooy, from the department of Anatomy, University of Toronto, and my internal examiners, Dr. Charles Malsbury and Dr. Gerard Martin, for their careful examination of this manuscript and their helpful comments and suggestions for revision, most of which I have incorporated in this last version of my dissertation.

I would also like to thank the following people for help with the care of the fish and with some of the experiments: Denise Hickey, Janet (Leslie) Kavanaugh, Maurice Kavanaugh, and Sandy Martland.

Thanks are due as well to the following people for help with some of the experiments: Grant Brown, Anne Dawe, Shelly Richardson.

I would also like to thank H. Lundbeck A/S for their donation of flupentixol to B. T. Lett.

... we cannot expect to understand ourselves or how nervous systems mediate behavior unless we gain some insight into the tremendous range of brains, from simple to complex -- by far the greatest spectrum of any organ system. To put the brain into perspective we will have to tap this variety, to learn how it evolved, to distinguish what is old from what is new in the most advanced nervous systems, and what the relevant differences between taxa are in their brains and behaviors.

(Bullock, 1984b, p. 510)

The advantages of comparative study lie in the ability to examine function within the brain, and the associated behaviour in the fully developed animal. The difficulties lie in the extrapolation of results to other vertebrates. Present-day vertebrates are not members of a linear phylogenetic scale, rather they represent the twigs on a tree whose trunk or trunks have long ceased to exist. Each species has undergone specialization during evolution and different parts of the brain may have gained or lost particular functions in the process. Extrapolation of results of neuroethological studies from one present-day species to another may be performed only with caution in the light of this evolutionary history. It is nevertheless unrealistic to neglect comparisons entirely, especially when they relate to basic brain functions like awareness, learning and appetitive behaviour.

It is from an understanding of the basic brain structure and function in vertebrates with apparently simple brains, that much can be learned about the phylogenetically older parts of the highly complex brains of mammals.

(Laming, 1981, pp. 7-8)

CONTENTS

TABLES

FIGURES

OVERVIEW

In rats and probably mammals in general, dopamine and opioid mechanisms play an important role in reward processes. There is evidence that this is also true of nonmammalian vertebrates, but relatively little attention has been paid to this possibility. The purpose of the present research is to determine whether or not there are similar dopamine and opioid reward mechanisms in the goldfish, a vertebrate phylogenetically distant from the mammal.

The ancestors of present-day fish branched off the phylogenetic tree over 360 million years ago during the Devonian period (Carroll, 1988; Schaeffer, 1969). This was long before amphibians, reptiles, and, lastly, mammals and birds became distinct classes. If there are dopamine and opioid reward mechanisms in fish, the first vertebrates to evolve, as there are in rats and other mammals, one of the last vertebrate classes to evolve, the same should be true of the classes that evolved in between. Thus, it would be reasonable to conclude that the dopamine and opioid mechanisms which mediate reward are evolutionarily old and originated in a common ancestor of all vertebrates. Conversely, substantial differences between fish and mammals would open the possibility of great variability in reward mechanisms among different classes of vertebrates and would

suggest that reward mechanisms have evolved independently or have undergone evolutionary divergence in different vertebrate classes.

In the research to be reported here, procedures similar to those used to identify dopamine and opioid reward mechanisms in rats were used with goldfish. These procedures used with rats involve the administration of drugs with known effects on dopamine or opioid transmission mechanisms and the assessment of the conditions under which they produce rewarding effects. In the present research, some of the drugs found to be rewarding to rats were administered to goldfish to determine if they produce a similar effect in goldfish.

Chapter 1 of this dissertation will review the evidence for the role of dopamine and opioid reward mechanisms in a major reward circuit of the rat brain. The chapter will begin with a brief description of this circuit and will conclude with an extensive review of the evidence concerning the main drugs that have been used to establish the role of dopamine and opioid mechanisms in reward processes. Chapter 2 of the dissertation will examine the generality of dopamine and opioid reward mechanisms across mammals and Chapter 3 will give the rationale for the present experiments with goldfish.

Chapter 4 of the dissertation will begin with a detailed explanation of the place conditioning procedure. Then a series of experiments using this procedure with goldfish will be reported. In these experiments, the rewarding effects of dopamine and opioid agonist drugs, and, when feasible, the effects of appropriate antagonists on these rewarding effects, were investigated. Finally, in Chapter 5 of the dissertation, the phylogenetic implications of the experimental findings will be discussed.

CHAPTER 1

DOPAMINE AND OPIOID REWARD MECHANISMS IN THE RAT

The study of the neural bases of reward began to flourish after the discovery by J. Olds and Milner of 'pleasure centers' in the brain (J. Olds, 1956; J. Olds & Milner, 1954). Electrical stimulation of certain areas in the brain was found to reinforce operant behavior in the rat. Since then, many sites in the brain have been found to mediate reward. For example, electrical stimulation of the frontal cortex, the medial and dorsal raphe, or central gray is rewarding and each region may activate different reward circuits. The most powerful rewarding effects are found with stimulation of a circuit associated with the medial forebrain bundle (Bozarth, 1988; Crow, 1972; Gallistel, 1983; Hand & Franklin, 1983; Stellar & Rice, 1989). In the following pages, this particular reward circuit will be referred to as "the reward circuit", although it is recognized that it may not be the only reward circuit. Both dopamine and opioid mechanisms are components of this circuit (Wise & Bozarth, 1984), as described in more detail below (see section 1.1).

1.1 A Reward Circuit in the Rat Brain

A major reward circuit in the rat brain, extending between the forebrain and midbrain, can be activated electrically or by means of certain drugs (see, for example, Bozarth, 1986; Fibiger & Phillips, 1988; Reid, 1987; Vaccarino, Schiff, & Glickman, 1989; Wise, 1978; 1982a, 1982b; 1983; 1989a). There is also evidence that natural rewards, like food, may be rewarding because they activate this same neural circuit (Di Chiara, Acquas, & Carboni, 1992; Evans & Vaccarino, 1990; Gratton & Wise, 1988; Hernandez & Hoebel, 1988a, 1988b; Hernandez, Lee, & Hoebel, 1988; Horvitz & Ettenberg, 1989; G.P. Smith & Schneider, 1988; Spyraki, Fibiger, & Phillips, 1982a; Stewart, de Wit, & Eikelboom, 1984; Wise, Jenck, & Raptis, 1986; but see Dworkin, Guerin, Goeders, & Smith, 1988; Phillips, Jakubovic, & Fibiger, 1987). Activation of this reward circuit also appears to produce psychomotor activity such as locomotion and approach behaviors associated with natural rewards (Burton, Mora, & Rolls, 1976; Christopher & Butter, 1968; Costall, Domeney, & Naylor, 1984; Delfs, Schreiber, & Kelley, 1990; Salamone, 1987, 1988; Stewart, 1992; Vaccarino & Corrigall, 1987).

1.1.1 Structures, Connections, and Neurotransmitters of the Reward Circuit

The medial forebrain bundle contains both ascending and descending fibers including those which connect parts of the reward circuit in the forebrain and midbrain (Domesick, 1988). Some of the major descending fibers of the reward circuit originate in the lateral hypothalamic area of the forebrain, join the medial forebrain bundle, and project to cells in the ventral tegmental area of the midbrain which feed into the ascending mesolimbic dopamine system. The neurons of this system have cell bodies located in the ventral tegmental area which send fibers, through the medial forebrain bundle, back to the limbic forebrain, where they synapse on cells in the nucleus accumbens, among other limbic structures. The mesolimbic dopamine neurons mediate reward by releasing dopamine into synapses on target cells in the nucleus accumbens (e.g., Fibiger & Phillips, 1988; Phillips & Fibiger, 1989). Opioid involvement in this system occurs primarily in the ventral tegmental area, where opioid peptides are believed to activate the ascending mesolimbic dopamine neurons which then release dopamine in the nucleus accumbens (Wise, 1989b; Wise & Bozarth, 1982). In addition, there is evidence for a second site of opioid action in the nucleus accumbens (M. E. Olds, 1982; Vaccarino, Bloom, & Koob, 1985). A more extensive

description of this system can be found elsewhere (e.g., Vaccarino, Schiff, & Glickman, 1989).

Various reward procedures have been used to reveal the role of dopamine and opioid mechanisms in reward. In a typical place conditioning procedure, exposures to one environment are paired with a drug while exposures to a second environment are not. The drug is considered rewarding if a preference is shown for the drug-associated environment (e.g., Hoffman, 1989; van der Kooy, 1987). In the self-administration procedure, drug administration is contingent on an operant response and the drug is considered rewarding if the operant response increases in frequency as a result of this contingency (e.g., Yokel, 1987). In the self-stimulation procedure, a drug is administered before giving the subject the opportunity to perform an operant response which delivers electrical stimulation to the brain. The drug is considered rewarding if it facilitates self-stimulation; for example, if the threshold for self-stimulation is reduced or some other rate-independent measure indicates an increase in the rewarding value of the stimulation (e.g., Reid, 1987). Presumably, rewarding drugs increase sensitivity to the electrical stimulation by facilitating transmission in the reward circuit (e. g., Wise, Bauco, Carlezon, & Trojniar, 1992).

Generally, drugs with known dopamine agonist properties have been shown to be rewarding by all three of the above procedures. Dopamine antagonist drugs, which interfere with dopamine transmission by blocking dopamine receptors, have been shown to attenuate the rewarding properties of the agonist drugs. Similarly, in all three procedures, opioid agonist drugs produce rewarding effects, and these effects are also attenuated by dopamine antagonist drugs, indicating, as will be explained later (see section 1.3.3), that the rewarding effects of opioids depend, at least in part, on normal activity in dopamine synapses.

A detailed examination of the evidence for the dopamine reward mechanism in the rat will be considered first, followed by similar evidence for the opioid reward mechanisms and the dependence of opioid reward on the dopamine mechanism.

1.2 The Dopamine Reward Mechanism

The evidence for a dopamine reward mechanism comes primarily from studies using drugs with either indirect or direct effects on dopamine receptors, as well as drugs with dopamine antagonist or dopamine-depleting properties.

1.2.1 Indirect Dopamine Agonists

Of the dopamine agonists, the indirect agonists, amphetamine and cocaine, have been the most extensively studied for their rewarding effects. Both amphetamine and cocaine are psychomotor stimulants which indirectly activate dopamine receptors by increasing synaptic levels of endogenous dopamine. Amphetamine does this by releasing endogenous dopamine from presynaptic terminals and by preventing its removal from the synapse; cocaine only prevents removal of endogenous dopamine from the synapse (Axelrod, 1970; Carlsson, 1970; Creese & Iversen, 1975; Heikkila, Orlansky, Cohen, 1975; Kuczenski, 1983; Moore, 1978; Scheel-Krüger, 1972).

1.2.1.1 Rewarding Effects of Amphetamine and Cocaine

Amphetamine and cocaine produce consistent rewarding effects in rats as measured by all three of the reward procedures, described earlier, as follows:

(1) Conditioned place preferences are produced by both amphetamine (Carr, Phillips, Fibiger, 1988; Gilbert & Cooper, 1983; Hoffman & Beninger, 1988; Kruszewska, Romandini, & Samanin, 1986; Lett, 1988; Nomikos & Spyraki, 1988b; Schenk, Hunt, Malovechko, Robertson, Klukowski, & Amit, 1986; Sherman, Roberts, Roskam, & Holman, 1980; Spyraki, Nomikos, Galanopoulou, & Daïfotis, 1988; Trujillo,

Belluzzi, & Stein, 1991) and cocaine (Houdi, Bardo, & Van Loon, 1989; Mackey & van der Kooy, 1985; Morency & Beninger, 1986; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Nomikos & Spyraki, 1988a; Spyraki, Fibiger, & Phillips, 1982b).

(2) Self-administration is obtained both with amphetamine (Davis, Smith, & Khalsa, 1975; Pickens, Meisch, & Dougherty, 1968; Wise, Yokel, & de Wit, 1976) and with cocaine (Dougherty & Pickens, 1973; Weeks & Collins, 1987; Yokel, 1987).

(3) Responding for electrical stimulation of structures in the reward circuit, such as the lateral hypothalamic and ventral tegmental areas, is facilitated both by amphetamine (Colle & Wise, 1988; Esposito, Perry, & Kornetsky, 1980; Hand & Franklin, 1983; Hunt & Atrens, 1992; Phillips & Fibiger, 1973) and by cocaine (Esposito, Motola, & Kornetsky, 1978; Frank, Manderscheid, Panicker, Williams, & Kokoris, 1992; Van Wolfswinkel, Seifert, & Van Ree, 1988; Wauquier, 1976).

1.2.1.2 Dopamine Involvement in Amphetamine and Cocaine Reward

In addition to increasing synaptic concentrations of endogenous dopamine, both amphetamine and cocaine have similar effects at norepinephrine and serotonin synapses

(Groves & Tepper, 1983; Holmes & Rutledge, 1976; Kuhar, Ritz, & Sharkey, 1988; Moore, Chiueh, & Zeldes, 1977; Parada, Hernandez, Schwartz, & Hoebel, 1988; Ritz, Lamb, Goldberg, & Kuhar, 1987; Scheel-Krüger, Braestrup, Nielson, Golembiowska, & Mogilnicka, 1977). Thus, to provide more conclusive evidence that it is the dopaminergic action of these drugs that is rewarding, additional procedures have been necessary. One strategy, as described below, has been to test whether selective interference with the dopaminergic actions of these drugs eliminates or attenuates their rewarding effects.

The development of amphetamine-induced conditioned place preferences is reduced or eliminated by dopamine antagonists like haloperidol, flupentixol, and metoclopramide (Hoffman & Beninger, 1989; Mackey & van der Kooy, 1985; Mithani, Martin-Iverson, Phillips, & Fibiger, 1986; Spyraki, Fibiger, & Phillips, 1982c) and by 6-hydroxydopamine-induced depletion of dopamine in the nucleus accumbens (Spyraki, Fibiger, & Phillips, 1982c). Comparable effects of such treatments are obtained in the analysis of amphetamine self-administration. Low doses of dopamine antagonists produce increased responding for amphetamine infusions, similar to the compensatory increase seen when the dose of amphetamine is reduced. High doses of dopamine antagonists produce an initial increase followed by

cessation of responding, similar to that produced by replacing amphetamine with saline in extinction procedures (Davis & Smith, 1975; Yokel & Wise, 1975; 1976). In addition, dopamine depletions by 6-hydroxydopamine infusions prevent the development and maintenance of amphetamine self-administration (Lyness, Friedle, & Moore, 1979). Support for dopamine involvement in amphetamine reward has also been found with the self-stimulation procedure. The facilitation of self-stimulation produced by amphetamine is reversed after administration of a dopamine antagonist (Gallistel & Freyd, 1987; Gallistel & Karras, 1984).

The rewarding effects of cocaine in the self-administration procedure are antagonized, in the same way as those of amphetamine, by dopamine antagonist drugs (de Wit & Wise, 1977; Ettenberg, Pettit, Bloom, & Koob, 1982; Gerber & Wise, 1989; Hubner & Moreton, 1991; Phillips, Broekkamp, & Fibiger, 1983) and dopamine-depleting lesions of the mesolimbic dopamine system (Dworkin & Smith, 1988; Pettit, Ettenberg, Bloom, Koob, 1984; D. C. S. Roberts, Corcoran, & Fibiger, 1977; D. C. S. Roberts, Koob, Klonoff, & Fibiger, 1980). However, in the place conditioning procedure, similar manipulations of cocaine-induced place preference have not yielded such clear findings as those just reported for amphetamine. If cocaine is administered intravenously or intracranially to either the lateral ventricles or the

nucleus accumbens, treatment with a dopamine antagonist does attenuate place preference conditioning (Aulisi & Hoebel, 1983; Morency & Beninger, 1986; Spyraki, Nomikos, & Varonos, 1987). Yet if cocaine is administered intraperitoneally, conditioned place preferences are not affected by blocking dopamine transmission with a dopamine antagonist, or by depleting the nucleus accumbens of dopamine with 6-hydroxydopamine lesions (Mackey & van der Kooy, 1985; Morency & Beninger, 1986; Spyraki, Fibiger, & Phillips, 1982b). These latter findings do not invalidate the proposed dopamine mechanism of reward, as it seems likely that cocaine produces its rewarding effects through a different, as yet not understood, mechanism when administered by the intraperitoneal route than when administered intravenously or into the ventricles (Morency & Beninger, 1986; Nomikos & Spyraki, 1988a; Spyraki, Nomikos, & Varonos, 1987).

1.2.2 Dopamine and the Direct Agonists

Apomorphine is widely used in dopamine receptor stimulation studies because it directly and selectively stimulates dopamine receptors (Andén, 1970; Andén, Rubenson, Fuxe, & Hökfelt, 1967; Creese, Hamblin, Leff, & Sibley, 1983; Neumeyer, Law, & Lamont, 1981; Niemegeers & Janssen, 1979; Ståhle & Ungerstedt, 1986) and hence appears to mimic

the actions of endogenous dopamine at dopamine receptors (Colpaert, Van Bever, Leysen, 1976). However, apomorphine also has effects on other neurotransmitter systems, although relatively high doses are needed for these effects (Gianutsos & Moore, 1980). When combined with the substantial evidence given earlier for amphetamine and cocaine, the following findings with apomorphine strongly indicate the presence of a dopamine mechanism in the reward system. Apomorphine produces place preferences (Papp, 1988; Parker, 1992; Spyraki, Fibiger, & Phillips, 1982c; Swerdlow, Swanson, & Koob, 1984; van der Kooy, Swerdlow, & Koob, 1983) and is self-administered (Baxter, Gluckman, & Scerni, 1976; Baxter, Gluckman, Stein, & Scerni, 1974; Davis & Smith, 1977; Dworkin, Guerin, Goeders, & Smith, 1988; D. C. S. Roberts, Corcoran, & Fibiger, 1977; D. C. S. Roberts & Koob, 1982; D. C. S. Roberts, Koob, Klonoff, & Fibiger, 1980; D. C. S. Roberts & Vickers, 1988; Wise, Yokel, & de Wit, 1976; Yokel & Wise, 1978; Zito, Vickers, & Roberts, 1985).

In addition to apomorphine, other direct dopamine agonists produce rewarding effects in rats. For example, bromocriptine (Morency & Beninger, 1986) and quinpirole (Hoffman & Beninger, 1988) produce place preferences and piribedil is self-administered (Yokel & Wise, 1978; Davis & Smith, 1977). There have been few investigations in which the rewarding effects of dopamine itself have been studied.

However, it has been reported that dopamine is self-administered to the nucleus accumbens (Dworkin, Goeders, & Smith, 1986; Goeders, 1988; Guerin, Goeders, Dworkin, & Smith, 1984).

Beside the findings noted above, there are reports that it can be difficult to obtain rewarding effects with apomorphine in both the place conditioning and self-administration procedures (Spyraki, Fibiger, & Phillips, 1982c; Wise, Yokel, & de Wit, 1976; Yokel & Wise, 1978; Zito, Vickers, & Roberts, 1985). One explanation for this difficulty is that direct dopamine agonists, such as apomorphine, are only rewarding over a narrow range of doses (Davis & Smith, 1977) because at higher doses apomorphine produces strong aversive effects which obscure any rewarding effect (see also, Weiss, Hurd, Ungerstedt, Markou, Plotsky, & Koob, 1992). Consistent with this explanation, a high dose of apomorphine produced a conditioned place aversion (Best, Best, & Mickley, 1973). Aversive effects of apomorphine are also evident in the conditioned taste aversion procedure (Garcia, Ervin, & Koelling, 1966; Garcia, Hankins, & Coil, 1977; van der Kooy, Swerdlow, & Koob, 1983), particularly at high doses (Revusky & Gorry, 1973).

Pretreatment with apomorphine has been reported to have rewarding effects as measured by facilitation of self-stimulation, but this is generally found only at very low

doses, while higher doses of apomorphine reduce the rewarding value of self-stimulation (Broekkamp & Van Rossum, 1974; Carey, 1982; Carey, Goodall, & Lorens, 1975; Leith, 1983; St. Laurent, Le Clerc, Mitchell, & Milliaressis, 1973; Strecker, Roberts, and Koob, 1982; Wauquier & Niemegeers, 1973b). Some of the findings of reduced self-stimulation at higher doses may be due in part to other effects of apomorphine which may mask its rewarding effects. Stellar & Rice (1989) suggest that the inhibition with high doses may be due to apomorphine-induced stereotypy, which interferes with performance of the self-stimulation response. Another possibility is that apomorphine may interfere with the perception of the contingency between the self-stimulation response and the rewarding brain stimulation; that is, apomorphine may produce effects equivalent to giving the animal noncontingent rewarding brain stimulation (but see Hall, Stellar, Rice, Meyers, & Coffey, 1988). It may also be that apomorphine produces aversive effects at higher doses which counteract its rewarding effects, as was suggested above for the place conditioning and self-administration procedures. Although the facilitating effect of high doses of apomorphine may be obscured by such side-effects, other findings indicate the importance of dopamine transmission in the self-stimulation procedure. For example, administration of dopamine antagonists attenuates

self-stimulation from electrodes in relevant reward sites, such as the medial forebrain bundle and the lateral hypothalamic area (Corbett, Stellar, Stinus, Kelley, & Fouriezos, 1983; Esposito, Faulkner, & Kornetsky, 1979; Ettenberg, 1989; Ettenberg & Duvauchelle, 1988; Fouriezos, Hansson, & Wise, 1978; Franklin, 1978; Gallistel, Boytim, Gomita, & Klebanoff, 1982; Lepore & Franklin, 1992; Lynch & Wise, 1985; Stellar, Kelley, & Corbett, 1983; Wauquier & Niemegeers, 1973a; but see Hunt & Atrens, 1992).

1.2.3 Site of Action of Dopamine Reward

At least four lines of evidence suggest that dopamine and dopamine agonists produce rewarding effects by their action at the nucleus accumbens, a terminal field of the mesolimbic dopamine system.

(1) As mentioned earlier (see section 1.2.1.2), dopamine-depleting lesions of the mesolimbic dopamine system interfere with the place preferences produced by amphetamine and cocaine, as well as with their self-administration. This suggests that the rewarding effect of amphetamine and cocaine is dependent on the release and/or maintenance of dopamine in synapses at the nucleus accumbens.

(2) Still more conclusive evidence is provided by studies in which dopamine or dopamine agonist action is restricted to the nucleus accumbens by intracranial

microinjections. Restricted infusions of amphetamine to the nucleus accumbens produce place preferences that can be blocked by administration of a dopamine receptor antagonist (Aulisi & Hoebel, 1983; Carr and White, 1983; 1986). Cocaine administered to the nucleus accumbens is similarly reported to produce a place preference (Aulisi & Hoebel, 1983). In the self-administration procedure, dopamine administered to the nucleus accumbens has rewarding effects (Dworkin, Goeders, & Smith, 1986; Goeders, 1988; Guerin, Goeders, Dworkin, & Smith, 1984). Amphetamine is also self-administered to the nucleus accumbens and such self-administration is prevented by pretreatment with a dopamine antagonist drug (Hoebel, Hernandez, McLean, Stanley, Aulissi, Glimcher, & Margolin, 1982; Monaco, Hernandez, & Hoebel, 1981). Amphetamine administered directly into the nucleus accumbens also facilitates intracranial self-stimulation from lateral hypothalamic or ventral tegmental electrodes (Broekkamp, 1976; Broekkamp, Pijnenburg, Cools, & Van Rossum, 1975). In a similar vein, administration of dopamine antagonist drugs directly to the dopamine terminal field in the nucleus accumbens interferes with self-stimulation from an electrode in the ventral tegmental area (Kurumiya & Nakajima, 1988).

(3) Kainic acid lesions of the nucleus accumbens interfere with apomorphine self-administration (Dworkin, Guerin, Goeders, & Smith, 1988). Such lesions selectively destroy cell bodies, with their post-synaptic dopamine receptors at which apomorphine is presumed to act, but the lesions leave the innervating fibers and fibers of passage intact.

(4) Dopamine metabolism in the nucleus accumbens is increased by electrical stimulation of the ventral tegmental area (Phillips, Blaha, & Fibiger, 1989) and by administering amphetamine either systemically or directly into the nucleus accumbens or lateral hypothalamus (Bozarth, 1987b; Di Chiara, Imperato, & Mulas, 1987; Hernandez, Lee, & Hoebel, 1988). Cocaine similarly increases dopamine metabolism in the nucleus accumbens (Di Chiara, Imperato, & Mulas, 1987; Hernandez, Guzman, & Hoebel, 1991; Hernandez & Hoebel, 1988b; Weiss, Hurd, Ungerstedt, Markou, Plotsky, & Koob, 1992). This identifies the nucleus accumbens as a site at which brain stimulation, amphetamine, and cocaine influence dopamine activity.

All these lines of evidence suggest that the dopamine synapses of the reward circuit are located in the nucleus accumbens.

1.3 Opioid Reward Mechanisms

Opioid agonist drugs produce strong rewarding effects. Morphine has been most frequently used to study the role of opioid neurotransmission in the reward system, but other opioid agonists, including heroin and a number of opioid peptides and their analogs, have also been tested. The rewarding effects are produced by the action of opioid agonist drugs at mu and possibly delta opioid receptors. Both of these types of opioid receptor are highly concentrated in areas, such as the ventral tegmental area, that are known to be implicated in reward (Goeders, Lane, & Smith, 1984; Iversen, 1983). Mu receptors are implicated because the powerfully rewarding agonist, morphine, binds with high affinity to mu receptors, although it also binds weakly to delta receptors (Akil, Bronstein, & Mansour, 1988; Atweh, 1983; Robson, Paterson, & Kosterlitz, 1983; Simantov, Childers, & Snyder, 1978). A role for delta receptors in reward is suggested by the rewarding effects of delta agonists, such as met- and leu-enkephalin and their analogs (Belluzzi & Stein, 1977; Hoebel, Hernandez, McLean, Stanley, Aulissi, Glimcher, & Margolin, 1982; Jenck, Gratton, & Wise, 1987; M. E. Olds & Williams, 1980; Phillips & LePiane, 1982). However, the evidence for a delta reward mechanism is not conclusive because enkephalins also bind to the mu receptor (J. L. Katz, 1989; but see Shippenberg, Bals-Kubik,

& Herz, 1987; Shippenberg, Herz, Spanagel, Bals-Kubik, & Stein, 1992).

1.3.1 Rewarding Effects of Opioid Agonists

Conditioned place preferences are produced by morphine (Advokat, 1985; Bardo, Miller, & Neisewander, 1984; Bardo & Neisewander, 1986; Barr, Paredes, Bridger, 1985; Bechara & van der Kooy, 1985, 1992a, 1992b; Blander, Hunt, Blair, & Amit, 1984; Mackey & van der Kooy, 1985; Mucha & Herz, 1985; 1986; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Nomikos & Spyraki, 1988b; Reid, Marglin, Mattie, & Hubbell, 1989; Rossi & Reid, 1976; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980; Spyraki, Nomikos, Galanopoulou, & Daïfotis, 1988) and other opioid agonists, such as heroin (Amalric, Cline, Martinez, Bloom, & Koob, 1987; Schenk, Hunt, Colle, & Amit, 1983; Spyraki, Fibiger, & Phillips, 1983) and a variety of opioid peptides and their analogs (Amalric, Cline, Martinez, Bloom, & Koob, 1987; Hoebel, Hernandez, McLean, Stanley, Aulissi, Glimcher, & Margolin, 1982; R. J. Katz & Gormezano, 1979; Phillips, LePiane, & Fibiger, 1983; Shippenberg, Bals-Kubik, & Herz, 1987; Stapleton, Lind, Merriman, Bozarth, & Reid, 1979; Stolerman, 1985). Cross-validation that opioid agonist drugs are rewarding is obtained from self-administration studies of morphine (Koob, Vaccarino, Amalric, & Bloom, 1987; Kumar,

1972; Weeks & Collins, 1987), heroin (Corrigall & Vaccarino, 1988; Koob, Pettit, Ettenberg, Bloom, 1984), and opioid peptides or their analogs (Belluzzi & Stein, 1977; Tortella & Moreton, 1980). Finally, opioid rewarding effects have also been shown in the self-stimulation procedure, because morphine (Adams, Lorens, & Mitchell, 1972; Esposito & Kornetsky, 1978; Kornetsky & Bain, 1983; Maroli, Tsang, & Stutz, 1978; Nazzaro, Seeger, & Gardner, 1981) and an enkephalin analog (Broekkamp, Phillips, & Cools, 1979) facilitate self-stimulation in sites such as the lateral hypothalamus and the ventral tegmental area.

1.3.2 Sites of Action of Opioid Reward

Wise (1989b) has proposed that opioids produce reward primarily by interacting with ventral tegmental opioid receptors which either directly or through interneuronal connections stimulate the mesolimbic dopamine neurons to release dopamine at their synapses in the nucleus accumbens. The ventral tegmental area is not the only site at which opioids produce rewarding effects, but it appears to be the most sensitive one. Direct administration of opioid agonists to other brain sites, such as the nucleus accumbens and the lateral hypothalamus, is also rewarding (M. E. Olds, 1982; J. E. Smith & Lane, 1983; Stinus, Cador, & Le Moal, 1992; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks,

1982), but the doses of opioid agonist drugs which produce reward in the ventral tegmental area are substantially lower than those reported to produce reward in other brain areas (Bozarth, 1983; Bozarth & Wise, 1982; Phillips, LePiane, & Fibiger, 1983; Wise, 1989b).

The following lines of evidence provide strong support for Wise's (1989b) theory that opioids produce reward through effects on the mesolimbic dopamine neurons in the ventral tegmental area.

(1) Opioid peptide-containing neurons and opioid receptors are located in close proximity to the cell bodies of the mesolimbic dopamine neurons in the ventral tegmental area (Dilts & Kalivas, 1988; Johnson, Sar, & Stumpf, 1980; North, 1992). This makes the proposed relationship anatomically feasible.

(2) Opioid agonists activate mesolimbic dopamine neurons following systemic administration (Finlay, Jakubovic, Phillips, & Fibiger, 1988; Kalivas, 1985; Matthews & German, 1984; Ostrowski, Hatfield, & Caggiula, 1982), as well as following direct administration to the ventral tegmental area (Hu & Wang, 1984; Spanagel, Herz, Bals-Kubik, & Shippenberg, 1991). In addition, increased dopamine activity in the nucleus accumbens has been observed following systemic administration of opioid agonists (Di Chiara & Imperato, 1988a, 1988b; Di Chiara, Imperato, &

Mulas, 1987; Leone, Pocock, & Wise, 1991; Wood, 1983). Thus, opioids produce effects on the mesolimbic dopamine neurons consistent with the proposed dependence of opioid reward on dopamine mechanisms.

(3) Opioid action restricted to the ventral tegmental area is rewarding, because administration of opioid agonists directly to the ventral tegmental area produces place preferences (Bozarth, 1987a; Bozarth & Wise, 1982; Hoebel, Hernandez, McLean, Stanley, Aulissi, Glimcher, & Margolin, 1982; Phillips & Lepiane, 1980, 1982; Phillips, LePiane, & Fibiger, 1983), supports self-administration (Bozarth & Wise, 1981b, 1982, 1984; Stewart, 1984), and facilitates self-stimulation (Broekkamp, Phillips, & Cools, 1979; Broekkamp, Van den Bogaard, Heijnen, Rops, Cools, & Van Rossum, 1976; Jenck, Gratton, & Wise, 1987; Phillips, Broekkamp, & Fibiger, 1983; Rompré & Wise, 1989a, 1989b; Van Wolfswinkel & Van Ree, 1985). In addition, Glimcher, Giovino, Margolin, & Hoebel (1984) found evidence that endogenous opioids in the ventral tegmental area mediate reward. They showed that the administration of thiorphan, an enkephalinase inhibitor, to the ventral tegmental area resulted in a conditioned place preference. This provides further confirmation that the ventral tegmental area is a site of opioid reward because thiorphan presumably produced reward by interfering with the destruction of endogenous opioids by enkephalinase in that area.

(4) The rewarding effect of systemic opioid agonists is reduced or eliminated by administration of opioid antagonist drugs directly to the ventral tegmental area (Koob, Vaccarino, Amalric, & Bloom, 1987; Britt & Wise, 1983). Thus, under normal conditions, opioid reward depends on the integrity of the ventral tegmental site.

1.3.3 Dependence of Opioid Reward on the Dopamine Mechanism

The evidence in the previous section shows that opioid mechanisms in the ventral tegmental area mediate reward and suggests that this rewarding effect is dependent on normally functioning mesolimbic dopamine neurons. More direct evidence for this dependency is that the rewarding effects of opioid agonists are reduced or eliminated following treatments which prevent normal dopaminergic transmission, such as antagonist blockade of dopamine receptors or 6-hydroxydopamine depletion of endogenous dopamine. The effects of such treatments on opioid reward have been studied in the place conditioning, self-adminstration, and self-stimulation procedures, as follows.

Opioid-induced place preferences are attenuated when rats are administered dopamine antagonists before conditioning (Acquas, Carboni, Leone, Di Chiara, 1989; Bozarth & Wise, 1981a, 1982; Hand, Stinus, & Le Moal, 1989; Le Moal, Stinus, & Hand, 1988; Leone & Di Chiara, 1987;

Phillips, Broekkamp, & Fibiger, 1983; Phillips, LePiane, & Fibiger, 1983; Phillips, Spyraki, & Fibiger, 1982; Shippenberg & Herz, 1987, 1988; Spyraki, Fibiger, & Phillips, 1983; but see Bechara, Harrington, Nader, & van der Kooy, in press; Mackey & van der Kooy, 1985) or when mesolimbic dopamine is depleted by means of 6-hydroxydopamine before conditioning (Phillips, Broekkamp, & Fibiger, 1983; Spyraki, Fibiger, & Phillips, 1983). Most strikingly, the conditioned place preference produced by an opioid agonist ((D-Ala^2),Met^5-enkephalinamide) administered directly to the ventral tegmental area is attenuated by pretreatment with haloperidol and is blocked by 6-hydroxydopamine lesions of the nucleus accumbens (Phillips, LePiane, & Fibiger, 1983). In the self-stimulation procedure, dopamine-depleting lesions also block the facilitating effect of morphine (Hand & Franklin, 1985).

Although slightly more ambiguous, the results obtained using the self-administration procedure are generally consistent with the aforementioned results from place conditioning and self-stimulation studies. Dopamine antagonists in sufficient doses reduce the self-administration of opiates (Ettenberg, Pettit, Bloom, & Koob, 1982; Gerber & Wise, 1989; Hanson & Cimini-Venema, 1972; Nakajima, 1989; Nakajima & Wise, 1987; Schwartz & Marchok, 1974; S. G. Smith & Davis, 1973), although not all authors

agree that this effect is due to a blockade of the reinforcing effects of opiates (S. G. Smith & Davis, 1973; Ettenberg, Pettit, Bloom, & Koob, 1982). This is because challenging opioid self-administration with dopamine antagonists has a different effect than a similar challenge of amphetamine self-administration (but see Nakajima, 1989). As mentioned earlier (see section 1.2.1.2), low doses of dopamine antagonists produce a compensatory increase in the self-administration of amphetamine with the result that more dopamine is released and maintained in the synapse to compete with the dopamine antagonist at the postsynaptic receptor. When opiate self-administration is challenged with the same low doses of dopamine antagonists, there is no evidence of a similar compensatory increase. Opiate self-administration is typically reduced or eliminated, and this effect has been attributed to motor debilitation or other non-reward related impairments caused by the dopamine antagonist (Ettenberg, Pettit, Bloom, & Koob, 1982).

However, the lack of a compensatory increase in opiate self-administration following administration of a low dose of dopamine blocker does not necessarily invalidate the theory that opioid reward is mediated by dopamine transmission. Wise and Rompré have argued that the lack of a compensatory increase is due to depolarization inactivation produced by the synergistic action of the

dopamine antagonists and opioid agonists on the dopamine neurons (Rompré & Wise, 1989a, 1989b; Wise & Rompré, 1989). Depolarization inactivation can be thought of as an extremely long absolute refractory phase during which the mesolimbic dopamine neurons are incapable of firing, and, thus, of releasing dopamine into their synapses. It occurs because both dopamine antagonists and opioid agonists are capable of depolarizing dopamine neurons. More specifically, dopamine antagonists, in addition to blocking the postsynaptic dopamine receptors which mediate the rewarding effect, also block dopamine autoreceptors on the mesolimbic dopamine neurons (Bannon, Freeman, Chiodo, Bunney, & Roth, 1987; Bunney, 1983). These dopamine autoreceptors are normally activated by endogenous dopamine to inhibit the firing of the mesolimbic dopamine neurons. Blocking of this inhibitory mechanism results in increased firing of the mesolimbic dopamine neurons. Opioid agonists cause firing of the mesolimbic dopamine neurons by stimulating opioid receptors. When both dopamine antagonists and opioid agonists are administered, the stimulation of the mesolimbic dopamine neurons is so intense and persistent that the neurons become completely depolarized and remain depolarized (depolarization inactivation). In this state, the mesolimbic dopamine neurons are not capable of firing and cannot release further

quantities of dopamine into the synapse to compete with the dopamine antagonist at the postsynaptic receptor. Hence, self-administered increases in the amount of opioid agonist would have no effect and could not counteract the blockade of the postsynaptic dopamine receptors by dopamine antagonists. In contrast, amphetamine produces reward by release and maintenance of dopamine in the synapse and, unlike opioid agonists, does not depolarize the dopamine neurons.

There are contradictory reports about the effect of 6-hydroxydopamine lesions of the mesolimbic dopamine system on opioid self-administration. Some researchers find that the opioid rewarding effect is attenuated (Bozarth & Wise, 1986; Dworkin, Guerin, Co, Goeders, & Smith, 1988; D. C. S. Roberts & Koob, 1982; J. E. Smith, Guerin, Co, Barr, & Lane, 1985) and others that it is not affected (Pettit, Ettenberg, Bloom, & Koob, 1984). It is possible to explain these discrepancies as follows. After either mesolimbic 6-hydroxydopamine lesions (Kalivas & Bronson, 1985; Stinus, Winnock, & Kelley, 1985) or chronic blockade of dopamine receptors with neuroleptics (Stinus, Nadaud, Jauregui, & Kelley, 1986), rats show a pronounced increase in motor activity in response to opioid agonists administered to the nucleus accumbens. Chronic treatment with dopamine antagonists also produces increased sensitivity to opioid

reward: the doses required to induce conditioned place preference and self-administration are below the usual threshold dose (Stinus, Nadaud, Deminière, Jauregui, Hand, & Le Moal, 1989). These findings suggest that prolonged interference with dopamine transmission increases the sensitivity of opioid mechanisms in the nucleus accumbens. Thus, while the lesions to the mesolimbic dopamine neurons may initially prevent opioid rewarding effects in the ventral tegmental area, after some time an increased sensitivity of opioid mechanisms in the nucleus accumbens may compensate for this.

If this interpretation is correct, interference with dopamine transmission does not attenuate the rewarding effects of opioid activity when opioid mechanisms in the nucleus accumbens are made supersensitive and are thus capable of compensating for the loss of the opioid mechanism in the ventral tegmental area. However, the evidence given earlier suggests that under normal conditions opioid reward is mediated primarily by opioid receptors in the ventral tegmental area and is dependent on the consequent activation of the mesolimbic dopamine mechanisms (but see Bechara, Harrington, Nader, & van der Kooy, in press; Bechara & van der Kooy, 1992a).

CHAPTER 2

GENERALITY OF DOPAMINE AND OPIOID REWARD MECHANISMS ACROSS MAMMALS

The evidence presented in Chapter 1 indicates that the reward circuit of the rat contains dopamine and opioid reward mechanisms which are interrelated such that opioid reward depends upon the normal functioning of the dopamine mechanism. In this section, evidence will be presented that a variety of mammals are rewarded by the same drugs that reward the rat (see also Griffiths, Bigelow, & Henningfield, 1980). Thus, the reward circuits in these animals also include dopamine and opioid mechanisms. First, evidence that dopamine agonists are rewarding will be reviewed, and this will be followed by evidence concerning opioid agonists.

2.1 The Dopamine Reward Mechanism

Most studies which have found rewarding effects of dopamine agonists in mammals other than the rat have used the self-administration procedure. Of the dopamine agonists studied in this procedure, the indirect dopamine agonists, amphetamine and cocaine, have been the drugs of choice. This is due to their powerful rewarding properties as well as to their relevance to problems of human addiction. As in rats, amphetamine is self-administered by baboons (Brady,

Griffiths, Hienz, Ator, Lukas, & Lamb, 1987; Griffiths, Winger, Brady, & Snell, 1976), rhesus monkeys (Balster & Schuster, 1973a; Hoffmeister & Goldberg, 1973; M. C. Wilson & Schuster, 1972), squirrel monkeys (Goldberg, 1973), dogs (Risner & Jones, 1980; Shannon & Risner, 1984), and cats (Balster, Kilbey, & Ellinwood, 1976). Similarly, cocaine is rewarding to baboons (Griffiths, Bradford, & Brady, 1979), rhesus monkeys (Balster & Schuster, 1973b; Johanson, 1976; M. C. Wilson & Schuster, 1972), squirrel monkeys (Goldberg & Kelleher, 1976; J. L. Katz, 1979; Stretch, 1977), dogs (Risner & Goldberg, 1983; Risner & Silcox, 1981), cats (Balster, Kilbey, & Ellinwood, 1976), and mice (Kuzmin, Zvartau, Gessa, Martellotta, & Fratta, 1992). Humans self-administer and report euphoric effects from amphetamine (Jasinski, Johnson, & Henningfield, 1984; Jönsson, 1972; Jönsson, Änggård, & Gunne, 1971) and cocaine (Fischman, 1984; Henningfield, Nemeth-Coslett, Katz, & Goldberg, 1987). One study has reported that low doses of amphetamine facilitate self-stimulation of the lateral hypothalamic area in the squirrel monkey (Spencer & Revzin, 1976).

As was pointed out earlier (see section 1.2.1.2), the finding of a rewarding effect induced by amphetamine or cocaine is consistent with, but does not in itself prove, a dopaminergic site of action. The reason is that both these drugs have effects on other neurotransmitter systems which

might conceivably mediate the rewarding effect. To establish that dopaminergic actions of amphetamine or cocaine are producing reward, it is necessary to show that the rewarding effect is attenuated or blocked by specific dopamine antagonist drugs or dopamine-depleting lesions. This has been shown in rats, as already explained (see section 1.2.1.2), and also in dogs and primates. The rewarding effects of amphetamine were reduced following administration of the dopamine antagonist pimozide to dogs (Risner & Jones, 1976) and humans (Gunne, Änggård, & Jönsson, 1972). Cocaine-induced reward was similarly attenuated by pimozide in rhesus monkeys (Woolverton, 1986; Woolverton & Kleven, 1988; Woolverton & Virus, 1989) and squirrel monkeys (Gill, Holz, Zirkle, & Hill, 1978; Winger, 1988). Both cocaine and amphetamine reward were also reduced by another dopamine antagonist, haloperidol, in rhesus monkeys (De La Garza, & Johanson, 1982; Woods, Herling, & Winger, 1978). Thus, in these mammals, the rewarding effects of amphetamine or cocaine are specifically due to their dopaminergic action.

The rewarding effect produced by the direct dopamine agonist, apomorphine, also provides strong evidence for a dopamine reward mechanism. As mentioned earlier (see section 1.2.2), apomorphine acts selectively at dopamine receptors (Andén, 1970), so any rewarding effect is not

readily attributable to an action on any other type of neurotransmitter system. Apomorphine has not been studied as extensively as amphetamine and cocaine. However, rats (see section 1.2.2), rhesus monkeys (Woolverton, Goldberg, & Ginos, 1984), and squirrel monkeys (Gill, Holz, Zirkle, & Hill, 1978) have been reported to self-administer apomorphine. It should be mentioned, however, that Woolverton, Goldberg and Ginos (1984) found apomorphine was self-administered by only three of five monkeys, even though a substitution procedure was used and the monkeys had been reliably self-administering either cocaine or amphetamine before the substitution of apomorphine. Similarly, Gill, Holz, Zirkle, and Hill (1978) found three of seven monkeys ceased responding for apomorphine when unlimited access to the drug was allowed. In addition, only one case of abuse of apomorphine taken orally by humans has been reported (Angrist & Gershon, 1978). Thus, for primates, as was reported above for rats, apomorphine produces a rewarding effect less consistently than does amphetamine or cocaine. It was suggested earlier (see section 1.2.2) that this may be a consequence of aversive side-effects of apomorphine counteracting the rewarding effects. The same suggestion was made by Woolverton, Goldberg, and Ginos (1984).

In short, the theory of a common mammalian dopamine reward mechanism is supported by: (1) the similar rewarding

effects of the indirect dopamine agonists, amphetamine and cocaine, across a wide variety of mammalian orders, (2) the similar antagonism of this rewarding effect by selective dopamine antagonists in rats, dogs, rhesus and squirrel monkeys, and humans, and (3) the similar rewarding effects of apomorphine, a direct and relatively selective dopamine agonist, in rats and monkeys.

2.2 The Opioid Reward Mechanism

Opioid agonists, like dopamine agonists, are rewarding to a wide range of mammals. Baboons (Lukas, Brady, & Griffiths, 1986), rhesus monkeys (Deneau, Yanagita, & Seevers, 1969; Harrigan & Downs, 1978), squirrel monkeys (Goldberg, Spealman, & Kelleher, 1979), dogs (B. E. Jones & Prada, 1973, 1981), cats (Kilbey & Ellinwood, 1980), and mice (Cazala, Darracq, & Saint-Marc, 1987; Criswell & Ridings, 1983; Kuzmin, Zvartau, Gessa, Martellotta, & Fratta, 1992) self-administer morphine and heroin. In mice, self-stimulation of the lateral hypothalamus is facilitated by morphine (Bendani & Cazala, 1988). Hamsters prefer a place associated with morphine (Schnur & Morrell, 1990). Humans self-administer and report subjective rewarding effects of heroin and morphine (Griffiths & Balster, 1979; Jasinski, Johnson, & Henningfield, 1984; Mello & Mendelson, 1987). Thus, these opioid agonists produce rewarding

effects in many different mammals. Few studies have tested opioid peptides or their analogs. However, rhesus monkeys self-administer enkephalin analogs (Mello & Mendelson, 1978; Young, Woods, Herling, & Hein, 1983).

An important question that remains to be answered is whether opioid-induced reward in mammals is dependent on dopamine neurotransmission as it appears to be in the rat (see section 1.3.3). Apart from the rat, the only other mammal that has been tested is the rhesus monkey. Pozuelo and Kerr (1972) found that the dopamine antagonist, haloperidol, interfered with morphine self-administration in this species. Because the effect has been shown in members of two divergent orders of mammals, rodents and primates, it seems likely that opioid reward is dependent on dopamine neurotransmission in other mammalian orders as well.

There are some findings, however, which indicate that opioid-induced reward may not be as prevalent, or at least as consistently potent, among mammals as the preceding summary would suggest. Although B. E. Jones and Prada (1973; 1981) found dogs would self-administer morphine, the effect was not easy to obtain. Most dogs had to be made dependent on morphine before they would self-administer it. In addition, Schnur and Morrell (1990) found that the dose of morphine required to induce place preferences in hamsters was much higher than the doses reported to induce similar

preferences in rats. Another study, with pigs as subjects, did not find a rewarding effect of morphine at all (Bedford, 1973). The significance of these discrepancies is not clear, but before much is made of them, factors which may mask a reward effect should be considered and investigated.

CHAPTER 3

INTRODUCTION TO THE STUDY OF DOPAMINE AND OPIOID REWARD IN GOLDFISH

As mentioned in the overview, the underlying concern of this dissertation is with the evolutionary origins of the reward circuit. The prevalence of reward processes across present-day vertebrate classes (Bitterman, 1975, 1984; MacPhail, 1982) and the obvious importance of such processes to the survival of these animals suggests that reward circuits probably existed in early, ancestral vertebrates. Additional evidence for such reward circuits is the similarity across vertebrate classes of the rewarding effects of electrical brain stimulation. For example, in addition to the rat (see Chapter 1), self-stimulation has been reported in a variety of mammals, such as cats, dogs, dolphins, mice, and primates, including humans (Bendani & Cazala, 1988; Bishop, Elder, & Heath, 1963; Bursten & Delgado, 1958; Delgado, 1976; Lilly & Miller, 1962; W. W. Roberts, 1958; Vandenbussche, 1976; Wauquier, Melis, Desmedt, & Sadowski, 1976). Rewarding effects of brain stimulation have also been found in some nonmammalian vertebrates, namely birds (Andrew, 1967; 1969; MacPhail, 1967) and even fish (Boyd & Gardner, 1962; Savage, 1971; Savage & Roberts, 1975). Such findings, of course, only indicate that neural circuits mediate reward in these

vertebrates; it does not imply that the activated circuits are the same.

The evidence presented in Chapter 2, however, suggests that at least part of the reward circuit is essentially the same in all mammals. This common part of the reward circuit, which shall be referred to hereafter as the dopamine reward system, has three known features: the dopamine mechanism, the opioid mechanism, and the functional dependence of the opioid mechanism on the dopamine mechanism. The statement of this last feature should not be taken to mean that all opioid reward is dependent on dopamine mechanisms. Other opioid reward mechanisms (e.g., in the nucleus accumbens) exist, but they are not part of the dopamine reward system, as defined here.

3.1 Selection of Fish as Subjects

Presumably, the dopamine reward system was derived from a common mammalian ancestor, as it is improbable that the same combination of features developed independently in so many different orders of mammals. It seems more likely that this system developed well before the evolution of mammals, because there is evidence for a dopamine reward mechanism in birds: apomorphine produces conditioned place preferences (Burg, Haase, Lindenblatt, and Delius, 1989) and cocaine supports self-administration (Winsauer & Thompson, 1991) in

the pigeon. Thus, the dopamine reward system found in mammals may also exist in pigeons and other birds. If this is so, the dopamine reward system must have been present in the reptilian common ancestor from which modern birds and mammals, as well as reptiles, evolved. Hence, it seems possible that the dopamine reward system originated in the earliest vertebrates. This is why the present experiments involved fish, the first vertebrates to evolve. Specifically, the goldfish (*Carassius auratus*) was selected for study as representative of the division of teleost fish, the most prolific division of the class of bony fish.

Fish, of all vertebrates, are the most distantly related to mammals. The common ancestor of present-day fish and mammals was one of the earliest vertebrates, and thus fish and mammals have had long lines of separate evolutionary history. Many modifications of reward circuitry have undoubtedly occurred during that period of separate evolution (Laming, 1981), but, if the common ancestor possessed a reward circuit (or circuits), some characteristics of that ancient circuit have likely been retained along the lines of descent of both fish and mammals. Study of the properties of reward mechanisms in fish, when compared to those of mammals, should thus reveal any characteristics of the circuit which have been retained, as well as any modifications that have occurred, over the

period of separate evolution of these vertebrates. Because vertebrates are a monophyletic taxon and all other vertebrate classes descended from the same early vertebrate ancestor of fish and mammals (Pough, Heiser, & McFarland, 1989), similarities found in fish and mammals are likely to be found in these other vertebrate classes as well. Any differences found in the reward circuits of fish and mammals should lead to a search for the point at which these differences emerged along the evolutionary lines leading to modern vertebrates.

In summary, the hypothesis which guided the present series of experiments was that the dopamine reward system identified in mammals constitutes part of a basic reward circuit which was possessed by the earliest vertebrates and has been retained along the various vertebrate lines of descent, including the line leading to present-day fish. To test this hypothesis, dopaminergic and opioidergic drugs were administered to goldfish using procedures similar to those described earlier for rats (see Chapter 1).

3.2 Dopaminergic and Opioidergic Drugs Selected for Administration to Fish

To test for the dopamine reward mechanism, the indirect dopamine agonists, amphetamine and cocaine, were selected because both are strongly rewarding and both have been

extensively studied in mammals. To establish that any observed rewarding effect was due to the action of amphetamine and cocaine on dopamine neurotransmission, the dopamine antagonists haloperidol and flupentixol were selected to block such effects. As an additional test for a dopamine reward mechanism, the direct dopamine agonist, apomorphine, was also used.

To test for the opioid reward mechanism, morphine was selected, because, like amphetamine and cocaine, it is a potently rewarding drug which has been extensively studied in mammals. Finally, if appropriate, the plan was to test whether morphine-induced reward is dependent on normally functioning dopamine neurotransmission in fish as in rats, and presumably all mammals, by blocking dopamine neurotransmission with haloperidol or flupentixol before morphine administration.

There are no reported studies of the rewarding or aversive effects of drugs on fish, with the exception of the publication of the first two experiments in the present series (Lett & Grant, 1988; see sections 4.2 and 4.3). However, other behavioral effects of some of the drugs proposed for use here have been studied in fish. Thus, before describing the present series of experiments which test the rewarding effects of dopaminergic and opioidergic drugs, these other behavioral effects on fish will be

considered. Of particular interest are drug effects on motor activity in fish, because the effects of dopaminergic and opioidergic drugs on motor activity often parallel, and may be related to, their rewarding effects in rats (e.g., Beninger, 1983; Di Chiara, Imperato, & Mulas, 1987; Spanagel, Herz, Bals-Kubik, & Shippenberg, 1991; Swerdlow, Vaccarino, Amalric, & Koob, 1986; Vaccarino & Corrigall, 1987; Wise, 1988; Wise & Bozarth, 1987).

3.2.1 Behavioral Effects of Dopamine Agonists in Fish

In rats, amphetamine and apomorphine produce increases in motor activity, for example, locomotion or stereotypy, which can be reversed by dopamine antagonists (Antoniou & Kafetzopoulos, 1991; Bechara & van der Kooy, 1992c; Carr & White, 1987; Fray, Sahakian, Robbins, Koob, & Iversen, 1980; Mazurski & Beninger, 1988; Offermeier & van Rooyen, 1986; Sanberg, Henault, Hagenmeyer-Houser, & Russell, 1987; Scheel-Krüger, 1972; Scheel-Krüger, Braestrup, Nielson, Golembiowska, & Mogilnicka, 1977; Ungerstedt, 1979; Winn, Williams, & Herberg, 1982). Similar effects of dopamine and dopamine agonists have been reported in other mammals, for example, cats (Motles, Martinez, Concha, Mejias, & Torres, 1989; W. J. Wilson & Soltysik, 1985), dogs (Di Chiara & Gessa, 1978), guinea pigs (Andrews & Holtzman, 1987), hamsters (Schnur & Martinez, 1989), mice (Gianutsos & Moore,

1980; Shannon, Bemis, & Peters, 1991; Ukai, Toyoshi, & Kameyama, 1991), marmosets (Barnes, Costall, Domeney, & Naylor, 1987), baboons (Hienz, Turkkan, Spear, Sannerud, Kaminski, & Allen, 1992) and java monkeys (Ellenbroek, van Aanholt, & Cools, 1990), as well as in birds (Burg, Haase, Lindenblatt, & Delius, 1989; Di Chiara & Gessa, 1978; Idemudia & McMillan, 1984; Lindenblatt & Delius, 1988; Sanberg, 1983).

There have been few investigations of the effects of amphetamine or apomorphine on activity in teleost fish. One study of a cichlid fish (blue acaras; *Aequidens pulcher*) found that both these drugs, when added to the aquarium water, increased swimming activity and produced a form of stereotypic foraging behavior (Munro, 1986). In another study (Tiersch and Griffith, 1988), large doses of apomorphine (20 to 400 mg/kg) were administered intraperitoneally to rainbow trout (*Salmo gairdneri*), a salmonid fish. With increasing doses of apomorphine, the trout were observed to remain stationary on the floor of the tank, to be unable to maintain an upright posture, and occasionally to arch the body in a tonic spasm.

Casual observation of the goldfish in the present experiments did not identify any obvious effect of amphetamine on the behavior of the fish. Following apomorphine administration, however, the goldfish appeared

to engage in short bursts of swimming alternating with long periods of resting, during which the fish remained stationary on the floor in a manner similar to that reported for trout (Tiersch & Griffith, 1988).

To investigate more systematically the behavioral effects of amphetamine and apomorphine on motor activity in goldfish, a series of pilot experiments was conducted. In all these experiments, a drug or saline was administered intraperitoneally, following which the fish were immediately placed in individual clear Plexiglas shuttleboxes, similar to those used in the place conditioning experiments (see section 4.1.2). For these pilot experiments, the floors of the shuttleboxes were covered with blue and white gravel.

Five minutes after the injection and placement in the shuttlebox, the amount of time the fish spent "resting" was assessed during a 10-min observation period. Resting was defined as some portion of the ventral surface of the fish touching the gravel on the floor of the shuttlebox, while the fish remained stationary and fin movement was minimal or non-existent. This measure was chosen because it occurred reliably and was easy to identify and quantify.

In the experiment in which amphetamine-induced resting was investigated, fish injected with 5.0 mg/kg of amphetamine spent significantly more time resting than control fish injected with saline ($t(37) = 5.56$, $p < .001$).

The mean time spent resting for the amphetamine-treated fish (n = 22) was 374.7 sec (SD = 191.3), compared to 83.6 sec (SD = 112.9) for the saline-treated fish (n = 17).

Another experiment similarly tested the effect of 0.5 mg/kg of apomorphine on resting. As with amphetamine, apomorphine produced significantly more resting than saline ($t(26) = 5.39$, $p < .001$). Apomorphine-treated fish (n = 14) rested for a mean of 407.7 sec (SD = 170.2) compared to 88.4 sec (SD = 142.0) for saline-treated fish (n = 14).

In both the preceding pilot experiments, the number of times the fish picked up gravel in their mouths was also recorded. This measure is similar to the foraging behavior exhibited by cichlid fish when exposed to amphetamine or apomorphine (Munro, 1986). However, in the present experiments with goldfish, neither drug affected such foraging activity.

Two further pilot experiments were conducted to determine if apomorphine-induced resting could be reduced by the dopamine antagonist pimozide. Pimozide or distilled water was administered intraperitoneally 4 hr before the apomorphine injection. In the first experiment, pretreatment with 1.0 mg/kg of pimozide did not significantly alter the resting induced by 0.5 mg/kg of apomorphine ($t(28) = 0.96$). The goldfish treated with pimozide before apomorphine (n = 15) spent a mean of 293.3

sec (SD = 163.8) resting; those pretreated with distilled water (n = 15) spent a mean of 353.1 sec (SD = 175.5). However, in the second experiment, when the dose of pimozide was reduced to 0.5 mg/kg and the dose of apomorphine was increased to 2.0 mg/kg, apomorphine-induced resting in goldfish was significantly attenuated by pimozide ($t(18) = 2.67$, $p < .02$). The mean time spent resting by the fish given pimozide before apomorphine (n = 10) was 211.1 sec (SD = 157.0), whereas the mean time spent resting by the fish given distilled water before apomorphine (n = 10) was 398.4 sec (SD = 156.8).

Dopamine antagonists themselves affect motor behavior in rats. Pimozide, for example, produces catatonia with doses greater than about 0.2 mg/kg (Worms, Broekkamp, & Lloyd, 1983). No controlled test was made of the ability of pimozide alone to affect motor behavior in fish, however in the first of the two pilot experiments on the antagonist effects of pimozide, an additional group of fish (n = 15) was given 1.0 mg/kg of pimozide followed by a saline injection instead of the apomorphine injection. This pimozide-alone group spent 284.9 sec (SD = 221.6) resting, a score which did not differ significantly from the mean of 353.1 sec of the fish given apomorphine alone ($t(28) = 0.94$). This suggests that pimozide may produce resting behavior itself, because in three other pilot experiments

which included saline control groups, the mean resting scores associated with saline treatment were all less than 90 sec, well below the 284.9 sec of resting induced by pimozide.

It is not clear whether the present resting measure reflects a locomotor or stereotypy effect in fish comparable to that in rats. However, the findings yield several important pieces of information. First, amphetamine and apomorphine produced behavioral effects in the fish within 15 min of administration. This time frame is similar to that for the effects of amphetamine and apomorphine on motor activity in mammals (Costall & Naylor, 1973; Fink & Smith, 1980; Fray, Sahakian, Robbins, Koob, & Iversen, 1980; Motles, Martinez, Concha, Mejias, & Torres, 1989; Offermeier & van Rooyen, 1986; Schnur & Martinez, 1989). Second, the effect of apomorphine and the interference with that effect by pimozide strongly suggest a role for dopamine-receptor mediation in apomorphine-induced resting. Third, the similarity of the effect of amphetamine to that of apomorphine suggests that the effect of amphetamine may also be mediated by dopamine receptors. Thus it seems likely that goldfish possess dopamine receptors that function to some degree like those of mammals.

3.2.2 Behavioral Effects of Opioid Agonists in Fish

Morphine typically has a biphasic effect on motor activity in rats (Vasko & Domino, 1978); an inhibitory or sedative effect initially masks a longer lasting excitatory or activating effect (Bozarth, 1985; Broekkamp, Van Den Bogaard, Heijnen, Rops, Cools, & Van Rossum, 1976; Wise & Bozarth, 1987). The motor activating effect of morphine, like that of the dopamine agonists, parallels its rewarding effect, and appears to be mediated, at least in part, by the mesolimbic dopamine system (Druhan & Stewart, 1990; Swerdlow, Vaccarino, Amalric, & Koob, 1986; Vezina & Stewart, 1984; Wise, 1988). Infusion of opioid agonists into the ventral tegmental area increases motor activity, an effect which is blocked by administration of a dopamine antagonist to the nucleus accumbens (Kalivas, Widerlöv, Stanley, Breese, & Prange, 1983). The effects of morphine on motor activity have not been as extensively studied in other vertebrates. However both sedative and motor activating effects, dependent on dosage, have been observed in hamsters (Schnur, Bravo, & Trujillo, 1983), mice (Marcais, Bonnet, & Constentin, 1981) and cats (Dhasmana, Dixit, Jaju, & Gupta, 1972). There are species differences, however. With rats and hamsters, morphine in low doses produces excitation and in high doses produces inhibition of

motor activity, whereas with mice and cats the reverse is true (Domino, Vasko, & Wilson, 1976).

Some studies have examined the effects of morphine on activity in teleost fish. Avis and Peeke (1975) found that morphine added to the aquarium water of convict cichlids (*Cichlasoma nigrofasciatum*) decreased territorial aggression. However, they argued that this was not a sedative effect because the same treatment did not affect predatory aggression. Csányi, Doka, Castellano, Puglisi-Allegra, and Oliverio (1984) found intracranial injections of morphine produced increased swimming, erratic behavior, and circling activity in paradise fish (*Macropodus operculatis*), although the pattern of these effects depended on the strain of fish (Doka, Csányi, Castellano, & Oliverio, 1985).

There have been no studies of the effects of morphine on motor activity in goldfish, although a high intracranial dose (30 mg/kg) was shown to have an analgesic effect (Ehrensing, Michell, & Kastin, 1982). However, other opioid agonist drugs have been shown to have an effect on motor activity in goldfish. Satake (1979) reported that met-enkephalin, administered intracranially, produced a sedative effect, shown by an increase in the amount of time the fish spent at the surface of the water when confined in a narrow space. Olson, Kastin, Montalbano-Smith, Olson, Coy, and

Michell (1978) found that two enkephalin analogs also significantly reduced motor activity in goldfish, although they found no effect of met-enkephalin itself.

Although casual observation of the goldfish in the present experiments revealed no obvious reaction to morphine, a pilot experiment, similar to those just described for amphetamine and apomorphine (see section 3.2.1), was conducted. The resting behavior of goldfish given intraperitoneal injections of either 5.0 or 15.0 mg/kg of morphine was compared to that of goldfish given saline. Five minutes after the injection the fish were observed for 10 min and the amount of time spent resting was assessed. Unlike the increases in resting produced by amphetamine and apomorphine (see section 3.2.1), the 5.0 and 15.0 mg/kg doses of morphine did not produce significantly more resting than saline, $t(41) = 0.47$ and $t(41) = 1.2$, respectively. The fish receiving saline ($n = 22$) spent, on average, 74.1 sec ($SD = 129.5$) resting compared to 95.9 sec ($SD = 168.2$) for the fish receiving 5.0 mg/kg of morphine ($n = 21$) and 125.2 sec ($SD = 143.7$) for the fish receiving 15.0 mg/kg of morphine ($n = 21$). The number of times the fish picked up gravel in their mouths was assessed as well, but this foraging measure also revealed no differences between the saline and morphine treated fish.

The present findings that morphine had no effect on the resting or foraging measures in goldfish suggest that either morphine is ineffective in fish in these regards, or that the effect had a delayed onset which was not detected within the time frame of the procedure used here.

3.3 The Place Conditioning Procedure as a Measure of Reward in Fish

The place conditioning procedure was used to measure the rewarding effects of drugs in the following experiments. Place conditioning, which will be described in more detail in the next section, is basically a classical conditioning procedure. The conditioned stimulus is a place which is paired with an unconditioned stimulus, such as a drug or some natural reward, such as food. After several pairings of the place conditioned stimulus with the unconditioned stimulus, the rewarding nature of the unconditioned stimulus is shown by a preference for the place with which that stimulus was paired. A variety of drugs (Carr, Fibiger, & Phillips, 1989; Hoffman, 1989; Swerdlow, Gilbert, & Koob, 1989) and natural stimuli, such as food (Bechara & van der Kooy, 1992a; Tombaugh, Grandmaison, & Zito, 1982; Spyraki, Fibiger, & Phillips, 1982a), water (Crowder & Hutto, 1992),

and sex (Ågmo & Berenfeld, 1990; Miller & Baum, 1987), are rewarding to mammals when assessed by the place conditioning procedure.

Fish, like other vertebrates, are capable of learning about the rewarding properties of natural stimuli, such as food, in various operant and classical conditioning paradigms (see MacPhail, 1982, for a review). Although goldfish have not previously been studied in the place conditioning procedure, their behavior has been appropriately modified in a conceptually similar classical conditioning procedure, autoshaping. In the autoshaping procedure a target conditioned stimulus, such as a light, is paired with an unconditioned stimulus, such as food. As a result of these pairings, animals learn to approach and contact the target stimulus associated with food (Brown & Jenkins, 1968), just as they learn to approach and stay in the place associated with food in the place conditioning procedure. In the autoshaping procedure, goldfish also learn to approach and contact a target stimulus previously paired with food presentation (Woodard & Bitterman, 1974). Thus, it seems likely that goldfish should be capable of learning in the place conditioning procedure, and should show, like rats, preferences for places associated with rewarding stimuli.

CHAPTER 4

STUDIES ON DOPAMINE AND OPIOID REWARD IN GOLDFISH

As was just mentioned (see section 3.3), the place conditioning procedure was selected to study the rewarding properties of dopaminergic and opioidergic drugs in goldfish. In this procedure, a drug that is likely to have rewarding or aversive properties is administered to experimental animals in a novel place. A different novel place is usually associated with the absence of the drug. This alternative place may differ along one or more dimensions (e.g., color, floor texture, odor, etc.) from the place associated with the drug. After a number of exposures to the two places with their associated drug and no-drug experiences, the animals are given a place preference test in which they are allowed free access to both places. Typically, the amount of time the experimental animals spend in the drug-associated place is compared to the amount of time spent in that place by no-drug control animals that experienced both places drug-free. The drug is said to produce a conditioned place preference if the experimental animals spend more time in the drug-associated environment than do the no-drug control animals. If the reverse is true, the drug is said to produce a conditioned place aversion. An additional within-subjects comparison can be used to further increase the sensitivity of the test; the

preferences of the animals after drug-place pairings can be compared to their predrug preferences established by place preference tests before conditioning (for more extensive discussions of the use of the place conditioning procedure, see, for example: Beninger, 1989; Carr, Fibiger, & Phillips, 1989; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Phillips & Fibiger, 1987; Stewart & Eikelboom, 1987; Swerdlow, Gilbert, & Koob, 1989; van der Kooy, 1987).

A different between-subjects comparison can also improve the sensitivity of the test. The preference scores of animals that had a drug paired with a place can be compared to those of animals that experienced the drug associated with the alternative place. For example, compartment A could be associated with the drug and compartment B associated with no drug for one group of animals, designated Group A. The reverse associations could be established for a second group of animals, designated Group B. Both groups could then be tested for the amount of time they spend in, say, compartment A. If the drug is neutral, the two groups of animals should spend equivalent periods of time in compartment A. If, however, the drug is rewarding, Group A, which had the drug paired with compartment A, should prefer A and spend more time in A, than should Group B, which had the drug paired with compartment B. This design should maximize the probability

of finding a difference between Group A and B in the time spent in compartment A, because the conditioned drug effect should pull Group A into compartment A and pull Group B in the opposite direction, toward compartment B and hence away from compartment A. For the converse reason, this design should also be very sensitive in detecting conditioned place aversions. This was the design used in the present series of experiments.

4.1 General Method

The procedures common to all the following experiments will be described here, with specific procedural details given in the descriptions of the individual experiments.

4.1.1 Subjects

Goldfish were obtained from local suppliers or from Straits Aquariums in Toronto, Ontario. Unless otherwise specified, the comet (or common) variety of goldfish was used; however, in some experiments additional varieties (fantails, shubunkins, black moors) were included. All fish were maintained on a 12 hr light-dark cycle and were fed once a day in the late afternoon. Except as noted below, the fish were maintained in home tanks which were either standard, commercial 38-liter glass aquaria or clear plastic tanks (47 cm x 24 cm x 20 cm; 23 liters capacity) filled

with constantly filtered water. Ten fish were housed in each of the glass aquaria, and two or three fish in each of the clear plastic tanks. Water temperature varied between 19°C and 25°C. This water was treated with 15 ml of non-iodized salt and 5 ml of Stresscoat per 38 l of water. The fish were acclimatized to their home tanks for at least three weeks before the start of any experiment. In each experiment, the groups were equated for mean body weight.

4.1.2 Apparatus

There were 30 clear Plexiglas shuttleboxes (30 x 11 x 20 cm) placed on three shelves of each of two adjacent racks. These were in the same room as the home tanks. Each shuttlebox was covered with white posterboard over one end and halfway along the two sides adjacent to the end. This formed two adjacent compartments (15 x 11 x 20 cm), one with three clear sides, the Clear compartment, and the other with three white sides, the White compartment. A clear Plexiglas barrier could be inserted to confine fish in one compartment or the other. The shuttleboxes were arranged on the racks with the white end of one shuttlebox adjacent to the clear end of the next, so the fish in the shuttleboxes were not visible to each other. On conditioning and test days the shuttleboxes were half-filled with water, treated, like the home tank water, with salt and Stresscoat. A preliminary

experiment, using the procedures described below, showed that goldfish did not have a noticeable preference for one compartment of the shuttlebox over the other in the absence of conditioning.

4.1.3 Drugs

Apomorphine (apomorphine HCl; Sigma Chemical Co.), flupentixol (cis-z-flupentixol, 2HCl; H. Lundbeck A/S), cocaine (cocaine HCl; BDH Chemicals), amphetamine (d-amphetamine sulphate; Smith, Kline and French), and morphine (morphine sulphate; BDH Chemicals), were dissolved in 0.6% saline. Haloperidol in the form of Haldol (McNeil Pharmaceuticals) was diluted with distilled water. Each 1.0 ml ampoule of Haldol contained 5.0 mg of haloperidol, with 1.8 mg methylparaben and 0.2 mg propylparaben as preservatives, and lactic acid for pH adjustment. Sodium pentobarbital was in solution as Somnotol (MTC Pharmaceutical). Dosages are given in terms of the salt. Haloperidol and apomorphine solutions were prepared immediately before their injection. All other drugs were prepared the day before the start of an experiment and kept in a refrigerator when not in use. In all experiments, drugs were injected intraperitoneally, except in Experiment 7e when morphine was injected intracranially. The volume of intraperitoneal injections was 0.05 ml when the dose was

based on the average weight of a group of fish. When doses were calculated for individual fish, the volume of injection was 10.0 ml/kg.

4.1.4 Procedure

On drug-conditioning trials, each fish was injected with the appropriate drug and immediately confined for 30 min in either the white or clear compartment of one of the Plexiglas shuttleboxes. On nondrug-conditioning trials the fish usually were simply confined to the other compartment of the shuttlebox for 30 min, but in a few experiments, to be noted below, the fish were injected with saline beforehand. The fish were confined to the appropriate compartment by a clear Plexiglas barrier inserted between the clear and white compartments. In all experiments, the groups of fish that had the conditioning drug paired with the clear compartment of the shuttlebox were designated Group Clear, and those which had the conditioning drug paired with the white compartment were designated Group White. There were 10 fish in each group except when noted otherwise.

There were six drug- and six nondrug-conditioning trials, unless indicated differently. The fish always had equal exposure to the compartment paired with the drug (either clear or white) and the compartment paired with no

drug (either white or clear). Drug- and nondrug-conditioning trials occurred at the same time each day, starting in the early afternoon, unless stated otherwise. Only one trial was administered on any one day. Days on which drug-conditioning trials occurred were always followed by at least 2 days in which no drugs were administered; on these intervening days either there was a nondrug-conditioning trial or the fish remained in their home tanks.

At least 2 days intervened between the day of the last conditioning trial and the test day. On test days, the Plexiglas barrier was removed, allowing access to both the white and clear compartments of the shuttleboxes. At the start of the test, each fish was placed along the dividing line between the two compartments, and an observer recorded with a stopwatch the amount of time the fish spent in the clear compartment of the shuttlebox during a 10 min period. The observer was positioned approximately 1.5 meters away from the shuttlebox, on a line with the line dividing the clear from the white compartment. When a fish was totally in the clear compartment it was thus visible to the observer; when it was totally in the white compartment it was not visible. When the fish was positioned across the midline separating the two compartments, it was considered to be in the clear compartment if the observer could see one of the eyes of the fish.

4.1.5 Analyses

In all experiments, the time spent in the clear compartment was compared as a function of whether the clear compartment or the white compartment had been paired with drug administration by means of analysis of variance and/or *t*-tests. Although the probability levels for *t*-tests were given as two-tailed, in cases when a drug that was expected to produce a place preference actually had effects in the opposite direction (i.e., toward an aversion) the *t*-value was given as negative.

4.2 Experiment 1: The Pentobarbital Experiment

The purpose of the first experiment was to test the effectiveness of the present procedures with goldfish. Pentobarbital was used because it had a strong effect on the behavior of the fish. Fish treated with a large dose of pentobarbital appeared unable to maintain an upright posture and lay, with their bodies in an arched position, on the bottom of the tank. On the other hand, the doses of amphetamine, apomorphine, cocaine, and morphine to be used had either a less dramatic effect or no noticeable effect at all (see section 3.2).

The place conditioning procedure was used to determine if goldfish, like rats (Mucha & Iversen, 1984), would learn an aversion to a place associated with pentobarbital. Mucha and Iversen (1984) found conditioned place aversions in rats with doses of 10 and 20 mg/kg of pentobarbital delivered subcutaneously. In the present experiment, 15 mg/kg of pentobarbital was paired with the clear compartment of a shuttlebox for half the fish and with the white compartment for the rest of the fish.

4.2.1 Method

4.2.1.1 Subjects

Group Clear weighed an average of 3.9 g (SD = 0.99); Group White weighed an average of 4.1 g (SD = 1.38). Each group was maintained in a separate home tank.

4.2.1.2 Procedure

In unspecified details, the procedures were as indicated in the General Method. On drug-conditioning trials, each goldfish was given an intraperitoneal injection of 0.06 mg of pentobarbital (about 15.0 mg/kg) and then confined for 30 min in the compartment to be paired with the drug. This was the clear compartment for Group Clear and

the white compartment for Group White. On nondrug-conditioning trials, the fish were confined to the other compartment of the shuttlebox for 30 min.

Half the fish from each group were tested 2 days after the last drug-conditioning trial and the remainder of the fish were tested on the following day. During the 10 min test, the amount of time the fish spent in the clear compartment of the shuttlebox was recorded.

4.2.2 Results

Pentobarbital produced an aversion for the place with which it was paired, $t(18) = 2.30$, $p < .05$. Group Clear, which had pentobarbital associated with the clear compartment, spent less time on average, 227.1 s (SD = 137.4), in that compartment than did Group White, 349.4 s (SD = 97.2).

4.3 Experiments 2a, 2b, and 2c: The Amphetamine Experiments

The next issue was whether goldfish could acquire a conditioned place preference when the conditioning drug was amphetamine. Amphetamine was selected because of its consistently strong rewarding properties in mammals and the evidence that these rewarding properties involve the

activation of dopamine neurotransmission (see sections 1.2.1.1 and 2.1). If the dopaminergic mechanisms mediating the rewarding effect of amphetamine in mammals are present in fish, then pairing an injection of amphetamine with a distinctive place should produce a preference for that place over another, equally familiar place that has not been paired with amphetamine. That is, fish that had amphetamine paired with the clear compartment should show a greater preference for that compartment than fish that had amphetamine paired with the white compartment.

Place preferences have been found in rats with doses up to 5.0 mg/kg (Gilbert & Cooper, 1983; Lett, 1988; Reicher & Holman, 1977; Spyraki, Fibiger, & Phillips, 1982c; Trujillo, Belluzzi, & Stein, 1991). The largest, 5.0 mg/kg dose was chosen for the first amphetamine experiment, Experiment 2a. This turned out to be the first demonstration of a conditioned place preference in goldfish and, hence, its reliability was assessed by two replications of Experiment 2a, with the following modifications. In Experiment 2b, the dose of amphetamine was reduced from 5.0 mg/kg to 3.6 mg/kg and the number of drug- and nondrug-conditioning trials was reduced from six of each to three of each. In addition, the fish were given a saline injection before being confined in the shuttleboxes on nondrug-conditioning trials. In

Experiment 2c, the replication was carried out with different strains of goldfish. Because the differences between the fish were easier to detect, drug doses were tailored to each fish's individual weight. Doses based on the average weight of all the fish in an experiment had been used earlier.

4.3.1 Method

4.3.1.1 Subjects

In Experiments 2a and 2b, the fish were maintained as described in the General Method with Group Clear in one tank and Group White in another. In Experiment 2c, different strains of goldfish were used: comets (n = 6), fantails (n = 7), black moors (n = 2), and shubunkins (n = 5). These fish were assigned to Group Clear and Group White so that strain of fish was counterbalanced as closely as possible. The fish were maintained in the same way as those in Experiments 2a and 2b, except that half the fish in Group Clear and half the fish in Group White were housed together in one tank, with the rest of each group housed together in another tank. Individual fish could be reliably identified. The mean weights and standard deviations of each group in each experiment are given in Table 4.1.

Table 4.1

Weights of Fish and Drug Dosage Data for Amphetamine in Experiments 2a, 2b, and 2c.

EXP	Group	*n*	Weight		Amphetamine		
			Mean	*SD*	Amount mg[b]	Dose (mg/kg)	Volume
2a	Clear	10	4.0	—[a]	0.02	5.0	0.05 ml
	White	10	4.0	—[a]	0.02	5.0	0.05 ml
2b	Clear	10	5.6	1.9	0.02	3.6	0.05 ml
	White	10	5.6	1.6	0.02	3.6	0.05 ml
2c	Clear	10	7.6	4.4		5.0	10.0 ml/kg
	White	10	7.6	4.5		5.0	10.0 mg/kg

Note. EXP = Experiment number

[a]Records for the weights of individual fish in this experiment were lost. Thus, the standard deviations are not available.

[b]per fish

4.3.1.2 Procedure

The procedure for each experiment was the same as that outlined in the General Method. On drug-conditioning trials the fish in each group were injected with amphetamine and confined to the appropriate compartment of the shuttlebox. On nondrug-conditioning trials, the compartments in which the fish in each group were confined were reversed. Prior to nondrug trials, the fish in Experiment 2b were given a saline injection, but the fish in Experiment 2a and 2c were not injected. After equal numbers of drug- and nondrug-conditioning trials (six of each in Experiments 2a and 2c; three of each in Experiment 2b), the place preferences of the fish were tested in the 10-min place preference test.

The experiments differed in the dose of amphetamine administered. The dose and the injection volume for each group in each experiment are given in Table 4.1. In Experiment 2a and 2b, the doses were based on the average weight of the fish. Thus, Table 4.1 also gives the absolute amount (and volume) of the amphetamine injection given to each fish in each group in these two experiments. In Experiment 2c, the doses were based on the weights of individual fish.

4.3.2 Results and Discussion

Amphetamine was an effective reward. Figure 4.1 shows the means and 95% confidence intervals of the time spent in the clear compartment by each group in each experiment. In all three experiments, Group Clear, which had amphetamine paired with the clear compartment, spent more time in that compartment than did Group White, which had amphetamine paired with the white compartment. Specifically, Experiment 2a showed that a significant conditioned place preference could be induced with a 5.0 mg/kg dose of amphetamine and six pairings of the drug with the place, $t(18) = 4.21$, $p < .001$. Experiment 2b yielded a conditioned place preference with a lower dose of amphetamine (3.6 mg/kg) and with just three place-drug pairings, $t(18) = 2.1$, $p < .05$. Experiment 2c showed that amphetamine-induced place preference can be found with mixed strains of goldfish, $t(18) = 3.05$, $p < .05$.

These findings with amphetamine are consistent with the proposal that goldfish, like rats, possess dopamine mechanisms which mediate reward. However, as explained below, such findings with amphetamine are not conclusive evidence that the effects are mediated by dopamine. The next experiment specifically attempted to determine the role of dopamine in mediating amphetamine-induced conditioned place preference in goldfish.

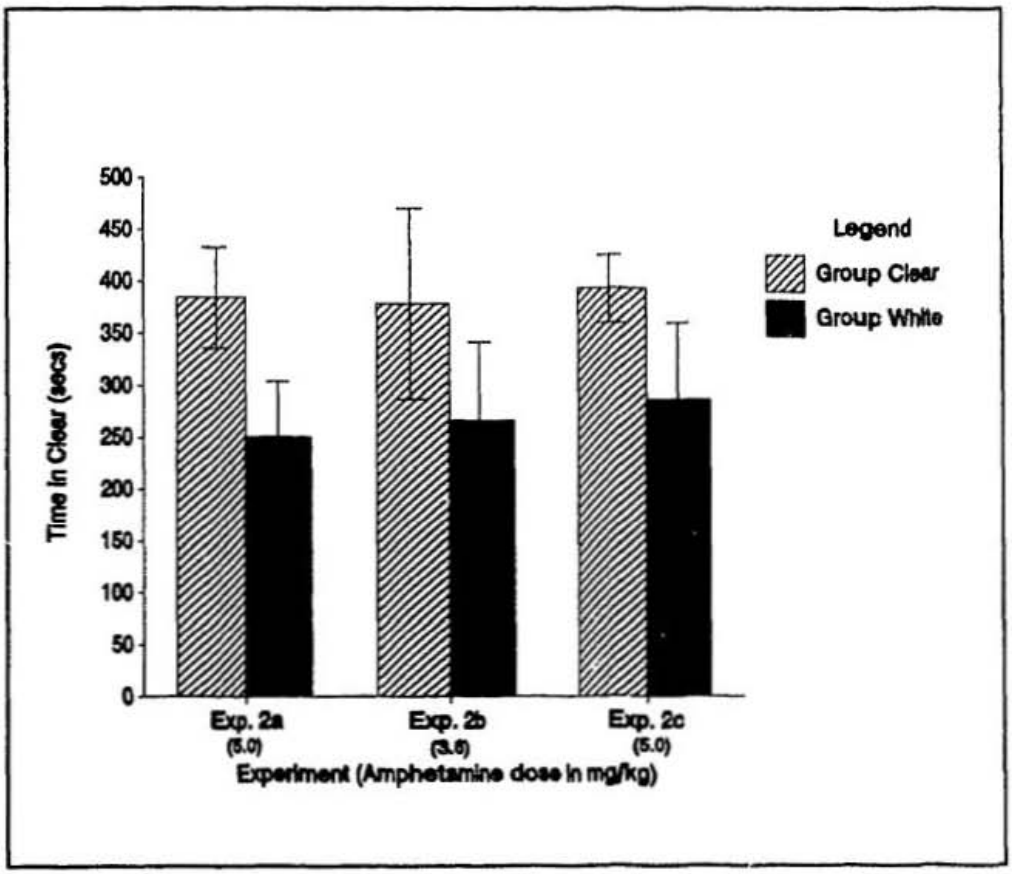

Figure 4.1. Number of seconds spent in clear compartment by Group Clear and Group White after place conditioning with amphetamine in Experiments 2a, 2b, and 2c. Brackets represent 95% confidence limits.

4.4 Experiment 3: The Haloperidol-Amphetamine Experiment

Amphetamine increases levels of synaptic dopamine, and it is through the consequent increase in stimulation of postsynaptic dopamine receptors that its rewarding effect is produced in rats (Bozarth, 1986; Wise & Bozarth, 1981). But amphetamine also increases levels of synaptic norepinephrine and serotonin (Groves & Tepper, 1983; Holmes & Rutledge, 1976; Parada, Hernandez, Schwartz, & Hoebel, 1988). The purpose of Experiment 3 was to determine whether amphetamine reward in goldfish is mediated by an increase in synaptic concentrations of dopamine, rather than norepinephrine or serotonin, by finding out if the rewarding effect of amphetamine would be blocked by a dopamine-blocking agent.

Haloperidol, a neuroleptic of the butyrophenone type, has been used as a dopamine-blocking agent in place conditioning experiments with rats (e.g., Mackey & van der Kooy, 1985; Mithani, Martin-Iverson, Phillips, & Fibiger, 1986; Phillips, Spyraki, & Fibiger, 1982; Spyraki, Fibiger, & Phillips, 1982a, 1982b, 1982c). At low doses, for example, less than 0.2 mg/kg, haloperidol selectively blocks dopamine receptors (Andén, Butcher, Corrodi, Fuxe, & Ungerstedt, 1970; Bunney, 1983; Niemegeers & Janssen, 1979; Phillips, Spyraki, and Fibiger, 1982). For the present experiment, a dose of 0.15 mg/kg was selected to antagonize the increase in dopamine activity produced by 5.0 mg/kg of

amphetamine. In rats, this dose of haloperidol interferes with rewarding intracranial self-stimulation as well as a variety of apomorphine- and amphetamine-induced behaviors, such as stereotypy and agitation, that have been attributed to the dopaminergic activity of these drugs (Niemegeers & Janssen, 1979; Worms, Broekkamp, & Lloyd, 1983). In addition, 0.15 mg/kg of haloperidol blocks place preferences produced by 1.5 mg/kg of amphetamine (Phillips, Spyraki, & Fibiger, 1982; Spyraki, Fibiger, & Phillips, 1982c).

In the present experiment, the amphetamine-induced place preferences of fish pretreated with haloperidol were compared with the amphetamine-induced preferences of control fish not given haloperidol.

4.4.1 Method

4.4.1.1 Subjects

Forty goldfish, weighing an average of 5.2 g (SD = 1.5), were maintained in 20 clear plastic tanks, two fish to each tank. The tanks (47 cm x 24 cm x 20 cm; 23 liters capacity) were filled with treated water and filtered for 24 hr every other day. Ten fish were assigned to each of four groups equated for mean weights. The groups differed in whether they received haloperidol or distilled water (Water) before their amphetamine injections, and whether they had the Clear or the White compartment of the shuttlebox paired

with the amphetamine injection. One fish in the group which received water prior to amphetamine paired with the white compartment died before the test day.

4.4.1.2 Drugs

Half an ampoule of Haldol (2.5 mg haloperidol in 0.5 ml vehicle) was diluted with 166 ml distilled water, to give a dose of 0.15 mg/kg of haloperidol injected in a volume of 10 ml/kg. The haloperidol solution was freshly prepared just before each set of injections. Amphetamine was prepared, as described in the General Method (see section 4.1.3), to give a dose of 5.0 mg/kg in a volume of 10 ml/kg. Doses were adjusted to each fish's individual body weight.

4.4.1.3 Procedure

Before drug-conditioning trials, the goldfish were given an intraperitoneal injection of haloperidol or an equivalent volume of distilled water. Seventy-five minutes after the haloperidol or distilled water injection, the fish were injected with amphetamine and immediately confined in either the clear or the white compartment of a shuttlebox for 30 min, as in earlier experiments. On nondrug-conditioning trials, the fish were confined in the other compartment of the shuttlebox for 30 min. No injections were given on nondrug-conditioning trials.

Half the fish were tested for place preferences 2 days after the last (drug) conditioning trial. The rest of the fish were tested on the following day.

4.4.2 Results and Discussion

Amphetamine produced the same strong conditioned place preference as found previously, but there were no detectable effects of haloperidol. The means and 95% confidence intervals of the amount of time each group spent in the clear compartment can be seen in Figure 4.2. An analysis of variance was carried out on the time the fish spent in the clear compartment with the following independent factors: the amphetamine compartment (Clear versus White) and the preconditioning treatment (Haloperidol versus Water). Only the main effect of compartment was significant, $F(1, 35) = 107.5$, $p < .0001$. Thus, amphetamine produced a strong conditioned place preference. The absence of a significant interaction effect, $F(1, 35) = 1.86$, indicates that the effect of the amphetamine was not altered by the administration of haloperidol. Nor was there any overall effect of haloperidol, $F(1, 35) = 1.86$. Individual t-tests confirmed that conditioned place preferences were obtained with amphetamine following pretreatment with both haloperidol, $t(18) = 7.5$, $p < .001$, and distilled water, $t(17) = 7.3$, $p < .001$.

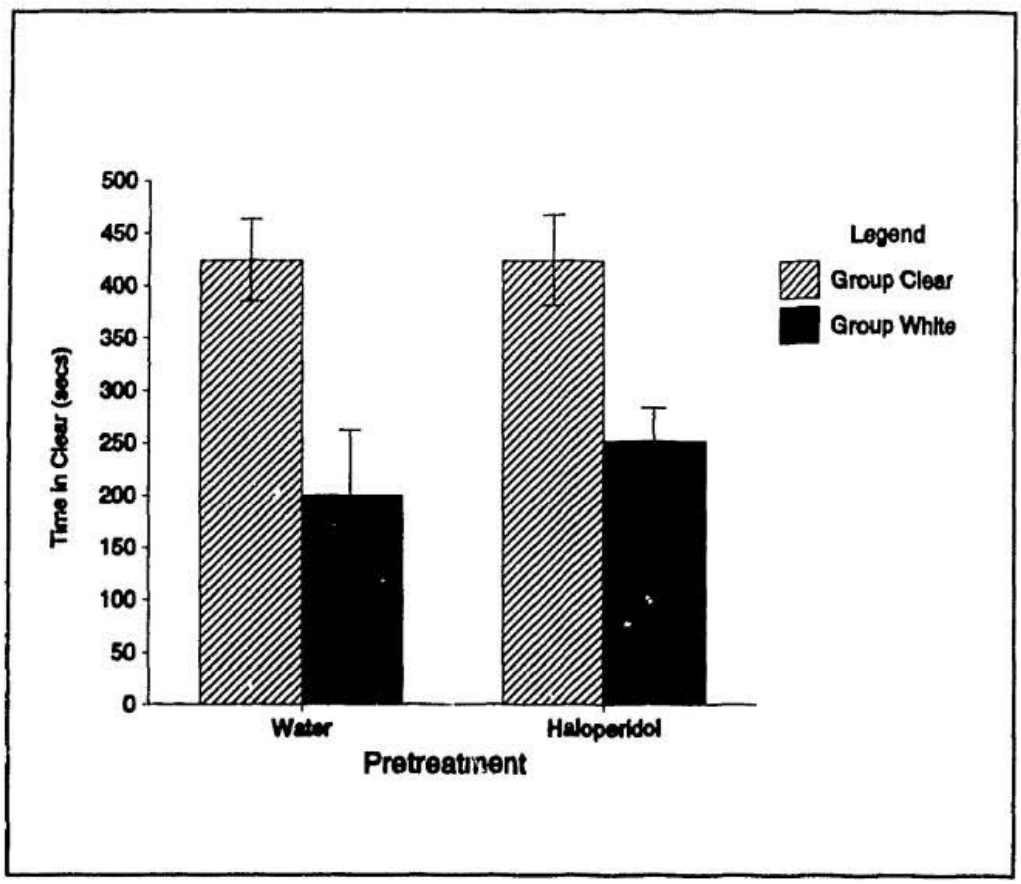

Figure 4.2. Number of seconds spent in clear compartment by Group Clear and Group White as a function of treatment with either water or haloperidol prior to place conditioning with amphetamine in Experiment 3. Brackets represent 95% confidence limits.

That haloperidol did not affect the amphetamine-induced conditioned place preference suggests that dopamine does not play a role in this rewarding effect. It is conceivable, however, that other factors, such as the low dose and type of dopamine blocker used, may have contributed to the observed lack of effect. Haloperidol blocks both postsynaptic dopamine receptors and dopamine autoreceptors (Ståhle & Ungerstedt, 1986). Blockade of the postsynaptic receptors prevents activation of the postsynaptic neuron, whereas blockade of the autoreceptors on the presynaptic neuron stimulates dopamine release (Bunney, 1988). The increase in dopamine release caused by the action of haloperidol at the autoreceptors, in combination with the increase in dopamine release produced by the relatively high dose of amphetamine, would raise the synaptic level of dopamine appreciably. This high concentration of synaptic dopamine might have been sufficient to overcome the effects of the postsynaptic receptor blockade produced by the low dose of haloperidol. If so, a higher dose of the dopamine antagonist drug would provide more competition for the postsynaptic dopamine receptor, and thus would be more likely to interfere with the rewarding action of any dopamine released by amphetamine and by the antagonist interacting with the dopamine autoreceptors. However, while this possibility has not been definitely excluded, it seems unlikely due to the results of the next experiment.

4.5 Experiment 4: The Flupentixol-Amphetamine Experiment

As there was no suggestion of any blocking effect for haloperidol in Experiment 3, a dopamine antagonist of the thioxanthene type, flupentixol, was used in this next experiment. Although flupentixol and haloperidol have similar effects, there are important differences between them (Ståhle & Ungerstedt, 1986). For example, flupentixol interacts more strongly with D-1 dopamine receptors than does haloperidol (Arnt, 1982; Creese, Hamblin, Leff, & Sibley, 1983; Titeler, 1983; Ungerstedt, Herrera-Marschitz, & Brugue, 1981). Both D-1 and D-2 dopamine receptors have been implicated in the mediation of reward (e.g., Hoffman & Beninger, 1989; Beninger, Hoffman, & Mazurski, 1989; Hubner & Moreton, 1991; Koob & Hubner, 1988; Robledo, Maldonado-Lopez, & Koob, 1992), thus a stronger blockade of D-1 receptors might facilitate interference with the dopamine activity produced by amphetamine.

Because a low dose of haloperidol failed to block amphetamine-induced conditioned place preference, a relatively large, 0.8 mg/kg dose of flupentixol was selected. This dose was successfully used by Mackey and van der Kooy (1985) to block amphetamine-induced place preference in rats. The disadvantage of such a high dose, however, is that in addition to antagonizing dopaminergic activity, it may also antagonize norepinephrine and

serotonin neurotransmission (Bunney, 1983, 1988; Leysen, Niemegeers, Tollenaere, & Laduron, 1978; Møller-Nielson, Pedersen, Nymark, Franck, Boeck, Fjalland, & Christensen, 1973; Niemegeers & Janssen, 1979; Peroutka & Snyder, 1980; Ståhle & Ungerstedt, 1986).

4.5.1 Method

4.5.1.1 Subjects

Sixty goldfish (25 comets, 16 shubunkins, and 19 fantails), weighing an average of 13.0 g (SD = 5.2), were maintained in groups of three in the same clear plastic home tanks as those used in Experiment 3. These tanks were filled with treated, continuously filtered water. Each tank contained three fish. The fish were assigned to one of four groups of 15 fish each. No two fish in the same home tank were in the same group and all groups had approximately equal mean weights and roughly equal numbers of the different varieties of goldfish.

4.5.1.2 Procedure

The procedure was similar to that of Experiment 3. To ensure that the fish were injected with amphetamine when the flupentixol was maximally effective (Corbett, Stellar, Stinus, Kelley, & Fouriezos, 1983), the fish were given the preconditioning injection of 0.8 mg/kg flupentixol 4 hr

before each drug-conditioning trial. As in the previous experiment, on each drug-conditioning trial the fish were administered 5.0 mg/kg of amphetamine immediately before being confined in either the clear (Group Clear) or the white (Group White) compartment of the shuttleboxes for 30 min. On nondrug-conditioning trials the fish were confined in the other compartment. No preconditioning or conditioning injections were given on nondrug-conditioning trials. The remaining two groups of fish received the same treatment except that they were administered saline (0.6%) instead of flupentixol before amphetamine was paired with either the clear (Group Clear) or the white (Group White) compartment of the shuttleboxes. As in the previous experiment there were six drug- and six nondrug-conditioning trials. All the fish were tested for place preference 2 days after the last (drug) conditioning trial.

4.5.2 Results and Discussion

Despite the relatively large dose used, flupentixol, like haloperidol in the previous experiment, had no effect upon the amphetamine-induced conditioned place preference. Figure 4.3 shows, for each group, the means and 95% confidence intervals of the time spent in the clear compartment during the preference test. Amphetamine produced place preferences in the fish pretreated with

flupentixol; Group Clear spent more time in the clear compartment than Group White. A similar preference is evident for the groups pretreated with saline. Thus, the amphetamine-induced place preference was not disrupted by pretreatment with flupentixol.

A 2 x 2 analysis of variance, with amphetamine compartment (clear or white) and preconditioning drug (flupentixol or saline) as the independent factors, confirmed these conclusions. That amphetamine was rewarding was shown by the main effect for amphetamine compartment, $F(1, 56) = 24.5$, $p < .01$. That the rewarding effect of amphetamine was not affected by flupentixol was shown by the nonsignificant interaction, $F(1, 56) = 1.0$, $p > .05$. Unexpectedly, there was a significant main effect of preconditioning drug, $F(1, 56) = 4.75$, $p < .05$, indicating a greater preference for the clear compartment among the groups pretreated with saline, as can be seen in Figure 4.3. No explanation for this finding, other than sampling error, can be offered at this time. Individual t-tests confirmed that pretreatment with flupentixol did not prevent an amphetamine-induced place preference, $t(28) = 3.8$, $p < .001$. The fish pretreated with saline also showed the expected place preference, $t(28) = 3.1$, $p < .01$.

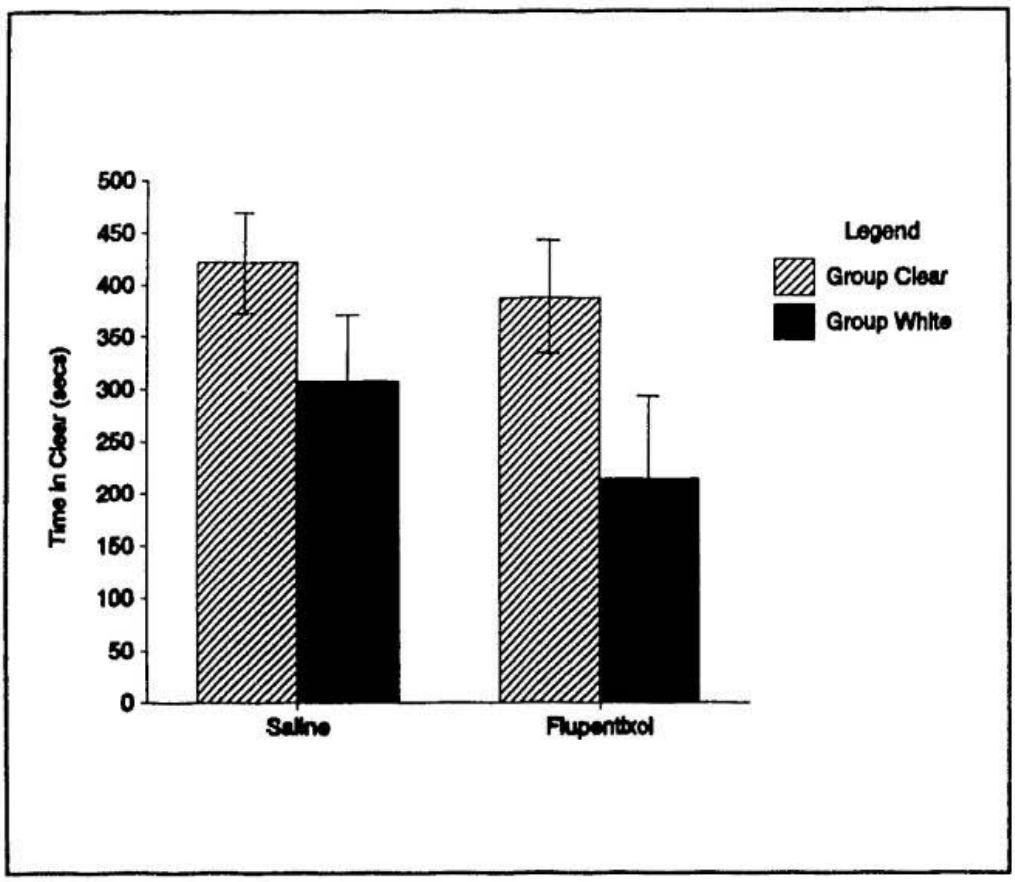

Figure 4.3. Number of seconds spent in clear compartment by Group Clear and Group White as a function of treatment with either saline or flupentixol prior to place conditioning with amphetamine in Experiment 4. Brackets represent 95% confidence limits.

In summary, although the amphetamine-induced conditioned place preference was replicated in both this and the previous experiment, neither flupentixol nor haloperidol had the expected blocking effect. Because both flupentixol and haloperidol block dopamine activity, the logic of the experimental design leads to the conclusion that amphetamine does not produce conditioned place preferences in goldfish through its dopaminergic effects.

There are, however, alternative explanations for the failure of the dopamine blocking agents, haloperidol and flupentixol, to block or attenuate amphetamine-induced conditioned place preference. The amphetamine dose may have been too large, so that the amount of dopamine available at the postsynaptic dopamine receptor was adequate to compete successfully with the dopamine antagonist drugs. Although this might explain the lack of effect of the low dose of haloperidol used in Experiment 3, it seems an unlikely explanation for the lack of effect in Experiment 4, when the flupentixol dose used was large: over eight times the ED50 to prevent apomorphine-induced agitation in the rat (Niemegeers & Janssen, 1979).

A more likely explanation is that these antagonist drugs did not reach the relevant receptors, or that they reached them at a time too early or late to have a blocking effect on the action of amphetamine (Garattini, 1978). This

contention is supported by casual observations of the fish, in their home tanks, during the period between injection of the dopamine antagonist and injection of amphetamine. No obvious effect on the behavior of the fish was detected following the administration of either haloperidol or flupentixol. It should be noted, however, that more systematic study might reveal an effect of these dopamine antagonist drugs on the behavior of fish, because behavioral effects of amphetamine and pimozide were also not readily detectable by casual observation, yet were obtained in the pilot experiments reported earlier (see section 3.2.1).

The lack of a behavioral effect of haloperidol is, perhaps, not surprising because the present dose (0.15 mg/kg) is below the ED50 (0.2-0.5 mg/kg) for inducing catalepsy in rats (Fielding & Lal, 1978; Worms, Broekkamp, & Lloyd, 1983), although not, apparently, in mice (Fujiwara, 1992). However, the present 0.8 mg/kg dose of flupentixol produces a pronounced cataleptic effect in rats (Ettenberg, Koob, & Bloom, 1981) and is well above the ED50 of 0.1 mg/kg (Møller-Nielson, Pedersen, Nymark, Franck, Boeck, Fjalland, & Christensen, 1973). The lack of any obvious effect of this dose of flupentixol on the behavior of fish suggests that the drug was not reaching the relevant receptors.

Moreover, if the flupentixol had reached the relevant receptors at the relevant time, it should have had <u>some</u>

effect on the amphetamine-induced place preference, based on studies of rats. The same (0.8 mg/kg) dose in rats would have been large enough to interfere with not only dopamine, but also norepinephrine, and serotonin transmitter systems (Bunney, 1983; Møller-Nielson, Pedersen, Nymark, Franck, Boeck, Fjalland, & Christensen, 1973; Leysen, Niemegeers, Tollenaere, & Laduron, 1978; Niemegeers & Janssen, 1979; Peroutka & Snyder, 1980; Ståhle & Ungerstedt, 1986), the same transmitter systems which are affected by amphetamine in rats (Groves & Tepper, 1983; Parada, Hernandez, Schwartz, & Hoebel, 1988). Thus the lack of any antagonist effect of this high dose of flupentixol against amphetamine in the present experiment suggests that flupentixol was not reaching the relevant receptors in the fish. Otherwise, the explanation would have to be that amphetamine produces reward through a totally different transmitter system from the dopamine, norepinephrine, or serotonin systems that it affects in rats. This possibility seems most unlikely.

4.6 Experiments 5a, 5b, 5c, 5d, and 5e: The Apomorphine Experiments

An additional way of testing the reinforcing effectiveness of dopaminergic activity is to use a direct agonist of dopamine receptors. If amphetamine induces a place preference in fish by stimulating dopamine receptors

through increasing synaptic levels of endogenous dopamine, then a dopamine agonist which directly stimulates dopamine receptors should also produce conditioned place preference.

Apomorphine is a relatively selective dopamine agonist which acts directly at dopamine receptors (Andén, 1970; Colpaert, Van Bever, & Leysen, 1976; Neumeyer, Law, & Lamont, 1981; Leysen, Niemegeers, Tollenaere, & Laduron, 1978; Niemegeers & Janssen, 1979; Ståhle & Ungerstedt, 1986). This selective agonistic property of apomorphine is frequently used in tests of the efficacy of putative dopamine receptor antagonists (Enna & Coyle, 1983; Niemegeers & Janssen, 1979). Apomorphine is rewarding in rats (see section 1.2.2), with conditioned place preferences produced by subcutaneous doses of apomorphine from 0.3 mg/kg up to 10 mg/kg (Papp, 1988; Spyraki, Fibiger, & Phillips, 1982c; van der Kooy, Swerdlow, & Koob, 1983). Pigeons also show a conditioned preference for a place associated with 1.0 mg/kg of apomorphine injected intramuscularly (Burg, Haase, Lindenblatt, & Delius, 1989). The following series of experiments was conducted to determine if such direct activation of dopamine receptors by apomorphine is also rewarding in fish.

A 1.0 mg/kg dose of apomorphine, intraperitoneally injected, was administered in Experiment 5a. This relatively low dose was selected because apomorphine has aversive properties, in addition to its rewarding property, and these aversive properties may obscure the rewarding effect at higher doses (see sections 1.2.2 and 2.1). There are two lines of evidence for this aversive component: (1) Best, Best, and Mickley (1973), using the intraperitoneal route of administration, found that a high, 15 mg/kg dose of apomorphine induced conditioned place aversions in rats. (2) Taste aversion studies (Garcia, Ervin, & Koelling, 1966) show apomorphine to have aversive effects which are most pronounced at high doses (Revusky & Gorry, 1973). Apomorphine is also aversive by another criterion; it produces a reaction, indicative of disgust, to a taste with which it has been paired (R. J. Smith & Parker, 1985). That goldfish might experience similar aversive effects of apomorphine is likely because they are capable of learning taste aversions to food paired with lithium chloride (Gordon, 1979) and because high doses of apomorphine (60 mg/kg or more) produce a related effect, vomiting, in another teleost fish, the rainbow trout (Tiersch & Griffith, 1988).

Both of the preceding lines of evidence suggest that high doses of apomorphine should be avoided, but there are also disadvantages to low doses. In the rat, very low doses (e.g., 0.05 mg/kg) of apomorphine stimulate the more sensitive dopamine autoreceptors, rather than the postsynaptic receptors (Roth, 1983; Skirboll, Grace, & Bunney, 1979), and thus reduce the level of synaptic dopamine (Grace, 1988; Neumeyer, Law, & Lamont, 1981; Ståhle & Ungerstedt, 1986). In addition, the apomorphine was to be injected intraperitoneally to the fish. In rats, apomorphine injected intraperitoneally is less effective than when administered subcutaneously, apparently because there is greater first pass metabolism of the drug when it is administered intraperitoneally (Baldessarini, Arana, Kula, Campbell, & Harding, 1981; Riffee & Wilcox, 1985; R. V. Smith, Wilcox, Soine, Riffee, Baldessarini, & Kula, 1979). The lowest dose of apomorphine found to produce place preference in rats was 0.3 mg/kg when injected subcutaneously (Papp, 1988). This dose when administered by the less effective intraperitoneal route might be sufficiently low to preferentially stimulate the autoreceptors rather than the reward-related postsynaptic dopamine receptors. To avoid this possibility a dose of 1.0 mg/kg of apomorphine, triple the lowest dose found to produce a place preference subcutaneously (Papp, 1988), was

selected. This dose produced conditioned place preferences in rats when administered intraperitoneally (Parker, 1992) and is one-fifteenth of the dose found to produce a place aversion intraperitoneally (Best, Best, & Mickley, 1973).

It has been suggested that there is a narrow range of doses in which direct dopamine agonists have rewarding effects (Davis & Smith, 1977). Because it happened that the 1.0 mg/kg dose of apomorphine did not produce a place preference in Experiment 5a, other doses were tested in Experiments 5b, 5c, 5d, and 5e.

4.6.1 Method

4.6.1.1 Subjects

Table 4.2 identifies the groups for each experiment in this series. For each group, the number of fish in the group and the mean weight and standard deviation of the group are indicated. The fish in all experiments were comets, except in Experiment 5d where a number of strains of goldfish were used. In this latter experiment, with eight comets, six fantails, five shubunkins, and one black moor, the strain of fish was balanced as closely as possible across the two groups. In Experiment 5d, one fish in Group Clear (the black moor) died before the test day.

Table 4.2

Weights of Fish and Drug Dosage Data for Apomorphine in Experiments 5a, 5b, 5c, 5d, and 5e.

EXP	Group	n	Weight		Apomorphine		
			Mean	SD	Amount (mg)*	Dose (mg/kg)	Volume
5a	Clear	10	6.6	0.9	0.0066	1.00	0.05 ml
	White	10	6.6	0.9	0.0066	1.00	0.05 ml
5b	Clear	10	6.7	0.8	0.0028	0.42	0.05 ml
	White	10	6.4	0.5	0.0028	0.42	0.05 ml
5c	Clear	10	7.3	0.5	0.0030	0.43	0.05 ml
	White	10	7.1	0.5	0.0030	0.43	0.05 ml
5d	Clear	9	8.2	4.3		0.40	10 ml/kg
	White	10	8.1	3.7		0.40	10 ml/kg
5e	Clear	10	6.1	0.5	0.0118	2.00	0.05 ml
	White	10	6.2	0.5	0.0118	2.00	0.05 ml
	Clear	10	6.2	0.5	0.0030	0.50	0.05 ml
	White	10	6.3	0.5	0.0030	0.50	0.05 ml
	Clear	10	6.2	0.5	0.0015	0.25	0.05 ml
	White	10	6.1	0.7	0.0015	0.25	0.05 ml
	Clear	5	5.9	0.8	saline	0.00	0.05 ml
	White	5	6.0	0.9	saline	0.00	0.05 ml

Note. EXP = Experiment number

*per fish

In all experiments, each group was housed together in a 38-liter tank, with the following exceptions: in Experiment 5d, the tanks contained 10 fish of which 5 were from Group Clear and 5 from Group White; in Experiment 5e, each (n = 5) of the two groups which received saline was housed in a separate 19-litre tank.

4.6.1.2 Procedure

In Experiments 5a to 5d, six apomorphine-conditioning trials and six nondrug-conditioning trials were conducted. In Experiment 5e, there were three of each. The doses of apomorphine varied among the five experiments. The dose and the volume of the injection given to the fish in each group in each experiment are given in Table 4.2. Single doses were used in each of Experiments 5a, 5b, 5c, and 5d. Experiment 5e was a dose-response experiment. With the exception of Experiment 5d, in which the administered dose was based on the weight of the individual fish, the doses in all the other experiments were based on the average weight of the fish in Groups Clear and White. For these experiments, the actual amount of apomorphine administered, as well as the approximate dose, are indicated in the table.

One minor difference between the experiments was the time of day when the conditioning trials were conducted. Trials were conducted during the late afternoon in Experiments 5a and 5c, and during the morning for the rest of the experiments. Another difference was that the preference test in the dose-response experiment, 5e, was conducted over two consecutive days. Those fish which had been conditioned with saline and with the 2.0 mg/kg dose of apomorphine were tested on the first of these days; the rest of the fish were tested on the second day.

4.6.2 Results and Discussion

Apomorphine produced place preferences, although the effect was obtained only at intermediate values of the doses tested. Figure 4.4 presents the means and 95% confidence intervals for each group in Experiments 5a, 5b, 5c, and 5d, and Figure 4.5 presents the same information for each group in the dose-response experiment, Experiment 5e. For Experiments 5b, 5c, and 5d, in Figure 4.4, and for the 0.5 mg/kg group in Figure 4.5, it can be seen that intermediate doses, between 0.4 and 0.5 mg/kg, produced differences between Group Clear and Group White which suggest a place preference; Group Clear, which had apomorphine paired with

the clear compartment, spent more time there than Group White, which had apomorphine paired with the white compartment.

The doses of 1.0 and 2.0 mg/kg, in Experiments 5a (Figure 4.4) and 5e (Figure 4.5), respectively, did not produce place preferences. Although the 1.0 and 2.0 mg/kg doses produced differences which suggest a place aversion, the effects were not statistically reliable: $t(17) = -1.68$ in Experiment 5a and $t(18) = -0.5$ in Experiment 5e.

In Experiment 5e (Figure 4.5), the lowest, 0.25 mg/kg, dose did not produce a significant difference between Group Clear and Group White, $t(18) = -0.73$. As expected, in the same experiment (Figure 4.5), the saline vehicle also did not produce a reliable effect, $t(8) = 0.21$.

There were four attempts (in Experiments 5b, 5c, 5d, and 5e) to produce place preferences with doses of apomorphine around 0.45 mg/kg. All of these intermediate doses produced differences in the direction of a place preference. However, statistical analyses showed two of these yielded nonsignificant t-values, (Experiment 5c: $t(18) = 1.5$; Experiment 5e: $t(18) = 1.5$), one yielded significance at the .05 level (Experiment 5d; $t(18) = 2.25$), and one at the .001 level (Experiment 5b; $t(18) = 4.23$).

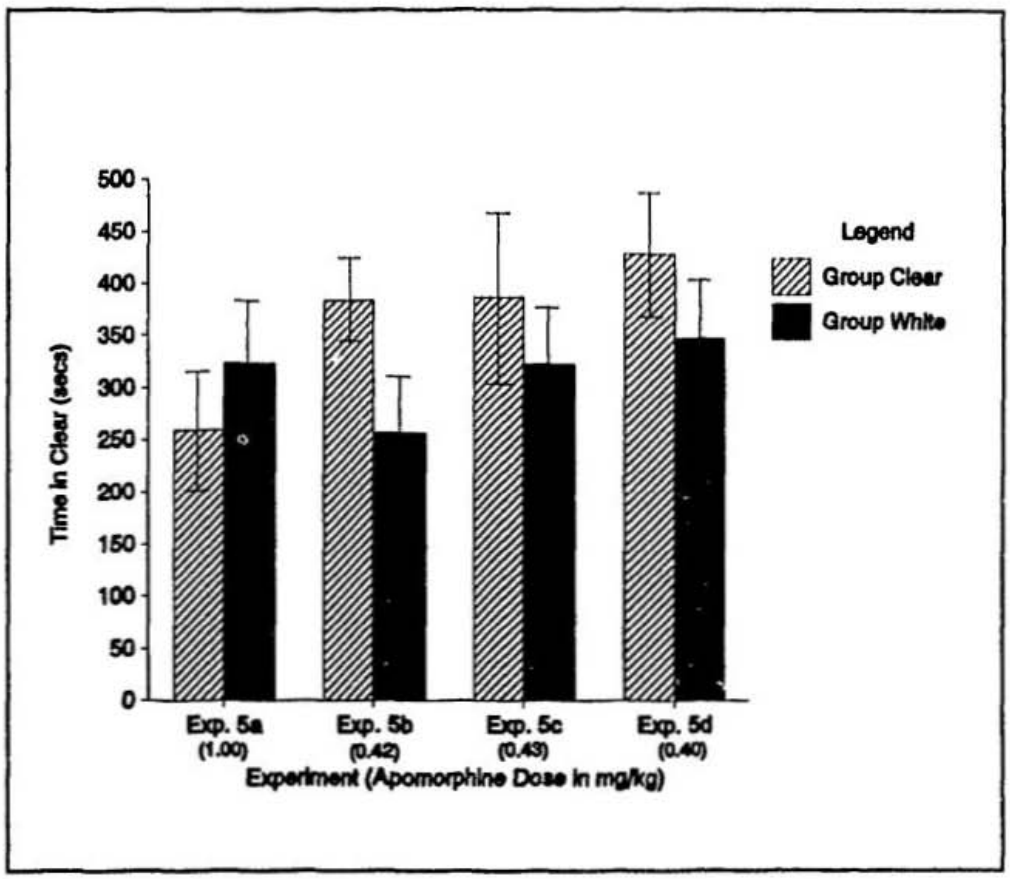

Figure 4.4. Number of seconds spent in clear compartment by Group Clear and Group White after place conditioning with different doses of apomorphine in Experiments 5a, 5b, 5c, and 5d. Brackets represent 95% confidence limits.

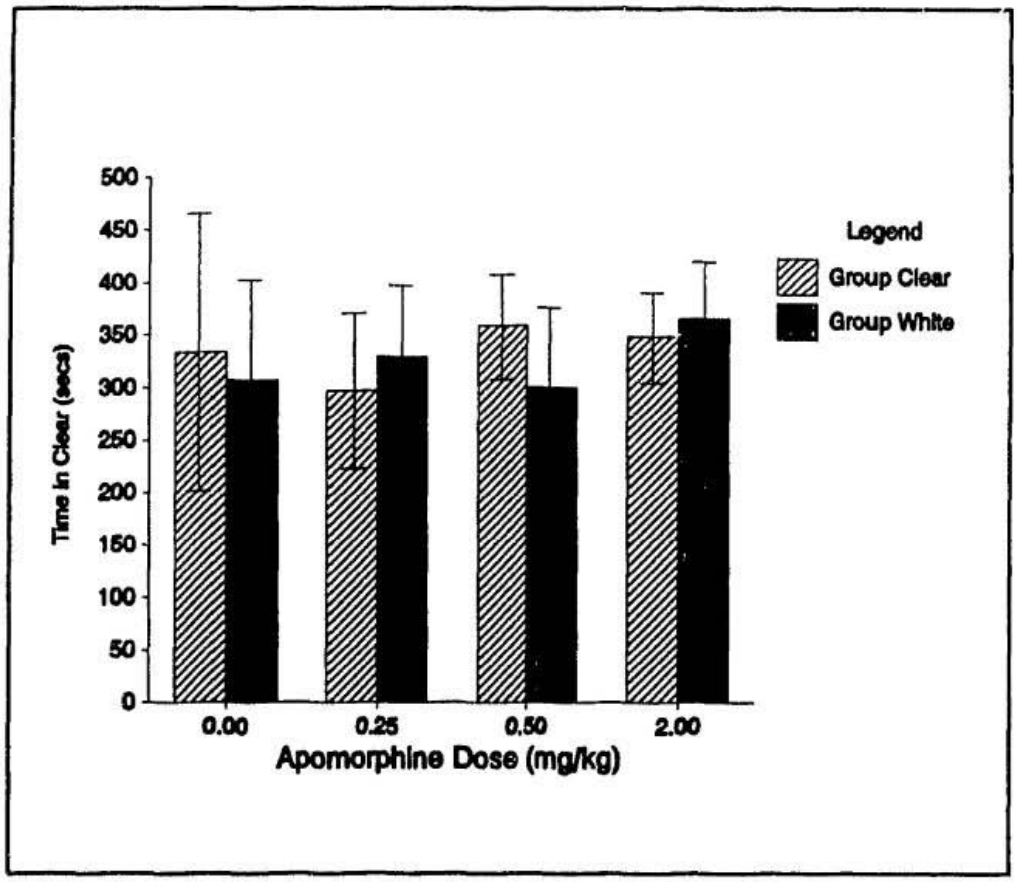

Figure 4.5. Number of seconds spent in clear compartment by Group Clear and Group White as a function of different doses of apomorphine in Experiment 5e. Brackets represent 95% confidence limits.

Because the present results seemed somewhat inconsistent, the overall effect of all doses of apomorphine (from 0.25 to 2.0 mg/kg) was assessed by combining the results of all the experiments using the meta-analytic method of adding z scores, suggested by Rosenthal (1984). The analysis yielded a z value of 2.12, $p < .05$. The results at the 0.25, 1.0, and 2.0 mg/kg doses were deliberately included, despite being in the direction of an aversion, to make any conclusion of an overall rewarding effect more convincing. Thus, even though the doses which produced effects in the direction of a place aversion were included in the meta-analysis, the overall result indicates that apomorphine produces a place preference. The doses which were effective in producing reward were, however, fairly low (around 0.45 mg/kg), much lower than those typically used to reward rats. The lowest dose tested (0.25 mg/kg) may have been ineffective because it was below the threshold for the rewarding effect. The higher doses tested (1.0 and 2.0 mg/kg) may have been ineffective because they had aversive effects which counteracted the rewarding effect.

The possibility of an aversive effect at high doses of apomorphine was considered in the introduction to the

present experiments, although it was not expected that the 1.0 and 2.0 mg/kg doses would be high enough to produce detectable aversive effects. When apomorphine is administered subcutaneously to rats, place preferences are induced with doses as high as 10 mg/kg (van der Kooy, Swerdlow, & Koob, 1983). It may be that fish are more susceptible than rats to the aversive effects of apomorphine. Alternatively, intraperitoneal administration may produce more serious aversive effects than does subcutaneous administration. An early study in which apomorphine was administered intraperitoneally to rats in the place conditioning procedure reported an aversive effect, but at a very high, 15 mg/kg, dose (Best, Best, & Mickley, 1973). More recently, a dose of 1.0 mg/kg of apomorphine, given intraperitoneally, was found to produce conditioned place preference, whereas higher doses of 15 and 25 mg/kg, although not aversive, were ineffective (Parker, 1992). Thus, the pattern of results obtained here was similar to that obtained with rats in that higher doses of apomorphine produced less consistent rewarding effects than lower doses (see also section 1.2.2), although the inconsistencies would not be expected at doses as low as 1.0 and 2.0 mg/kg, based on the rat data.

4.7 Experiments 6a and 6b: The Cocaine Experiments

The present experiments assessed the rewarding effects of cocaine, a psychomotor stimulant which is reliably rewarding to mammals (see sections 1.2.1 and 2.1) and which, like amphetamine, increases levels of synaptic dopamine (Kuczenski, 1983). Spyraki, Fibiger, and Phillips (1982b) found that 5, 10, and 20 mg/kg of cocaine produced equivalent place preferences in rats. The dose of cocaine selected for Experiment 6a was 10 mg/kg. In Experiment 6b, the dose was raised to 20 mg/kg because the dose of 10 mg/kg in Experiment 6a turned out to be ineffective.

4.7.1 Method

4.7.1.1 Subjects

In Experiment 6a, the fish in Group Clear were housed in one 38-liter tank, and the fish in Group White in another. In Experiment 6b, different strains of goldfish were used (four shubunkins, seven fantails, and nine comets). Because each fish could be identified, five fish assigned to Group Clear and five assigned to Group White were housed together in one tank, with the rest of the fish housed in another. During the course of Experiment 6b, one

shubunkin from Group Clear died. Table 4.3 gives the number of fish, as well as the mean and standard deviation of the weights of all the fish in each group.

4.7.1.2 Procedure

For both experiments the procedure was the same as that given in the General Method; on the drug-conditioning trials, cocaine was paired with the clear compartment for Group Clear and with the white compartment for Group White. On the nondrug-conditioning trials, no injections were given, and the groups were exposed to the other compartment. Following six drug- and six nondrug-conditioning trials, the fish were tested for their place preference.

The main difference between the two experiments was the dose of cocaine administered. The dose of cocaine and the volume of the injection are given for each group in each experiment in Table 4.3. Because each fish in Experiment 6a received a dose based on the average weight of the fish in the group, the absolute amount of cocaine administered to each fish in that experiment is included in the table. A minor difference was that conditioning trials in Experiment 6a occurred in the midmorning, whereas in Experiment 6b, they occurred in the early afternoon.

Table 4.3

Weights of Fish and Drug Dosage Data for Cocaine in Experiments 6a and 6b.

EXP	Group	n	Weight		Cocaine		
			Mean	SD	Amount (mg)[a]	Dose (mg/kg)	Volume
6a	Clear	10	10.3	1.6	0.1	10.0	0.05 ml
	White	10	10.3	1.9	0.1	10.0	0.05 ml
6b	Clear	9	17.7	11.5		20.0	10 ml/kg
	White	10	17.9	12.1		20.0	10 ml/kg

Note. EXP = Experiment number

[a]per fish

4.7.2 Results and Discussion

Neither the 10 nor the 20 mg/kg (Experiment 6a and 6b, respectively) dose of cocaine produced a place preference. This is evident from inspection of the means and 95% confidence intervals shown in Figure 4.6 and was confirmed statistically: $\underline{t}(18) = 1.03$ and $\underline{t}(17) = 0.66$, in Experiments 6a and 6b, respectively.

Considering the similar mode of action of cocaine and amphetamine and the effectiveness of amphetamine in producing a place preference in goldfish, this negative finding with cocaine is surprising. However, there are differences between amphetamine and cocaine in their pharmacokinetic properties and mechanisms of action (R. T. Jones, 1984; Kuhar, Ritz, & Sharkey, 1988; McMillen, 1983; Scheel-Krüger, Braestrup, Nielson, Golembiowska, & Mogilnicka, 1977; Witkin, Goldberg, & Katz, 1989). Additionally, it has been suggested that the site at which cocaine and amphetamine induce rewarding effects may be different, at least in rats (Goeders, 1988; Goeders & Smith, 1983, 1984; Isaac, Neisewander, Landers, Alcala, Bardo & Nonneman, 1984).

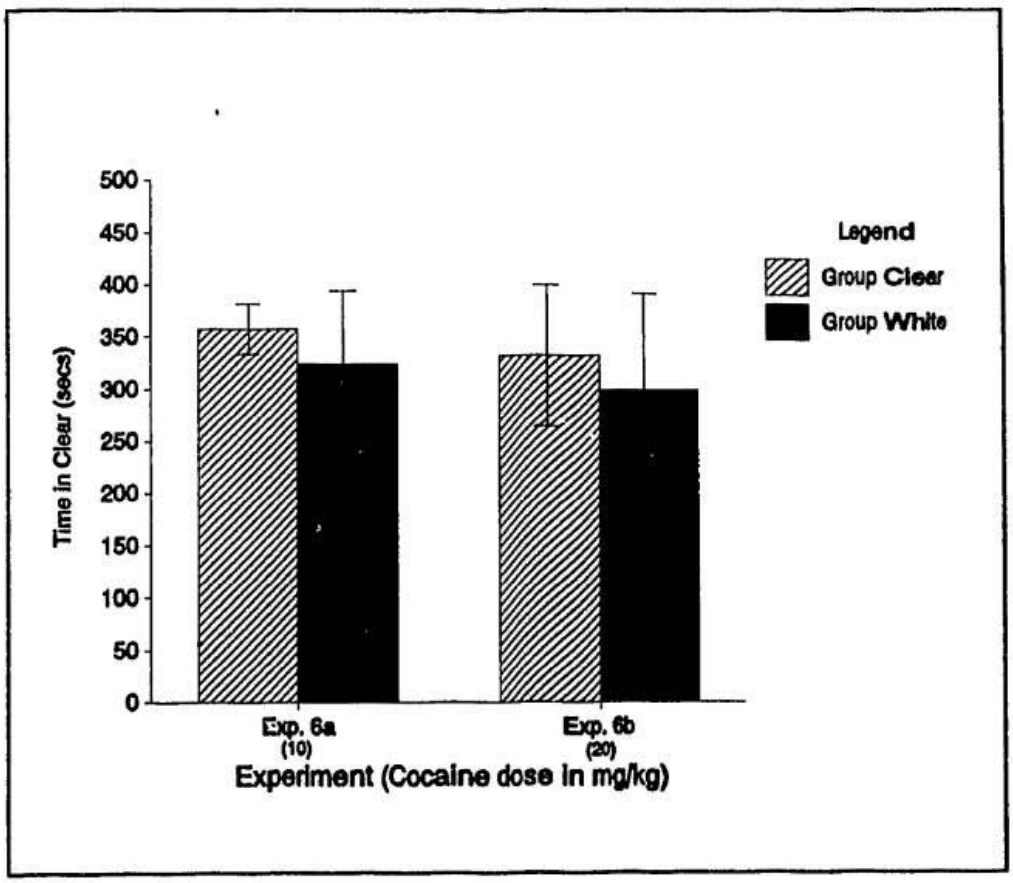

Figure 4.6. Number of seconds spent in clear compartment by Group Clear and Group White after place conditioning with different doses of cocaine in Experiments 6a and 6b. Brackets represent 95% confidence limits.

Nomikos and Spyraki (1988a) claim that there are differences in place conditioning with cocaine in rats depending upon whether an unbiased or biased procedure is used to assign animals to the compartment of the apparatus to be associated with the drug. In the unbiased procedure, each animal is randomly assigned to one or the other compartment of the apparatus to be associated with the drug. In the biased procedure, each animal's preconditioning preference for the two compartments is assessed, and then the drug is paired with the less-preferred compartment. When cocaine is administered intraperitoneally, place preferences are found when a biased procedure is used and the drug is paired with the less-preferred compartment; conditioned place preferences are usually not found when cocaine is associated with the preferred compartment, nor when an unbiased procedure is used (Nomikos & Spyraki, 1988a; but see Mackey & van der Kooy, 1985). It is not clear why intraperitoneally administered cocaine produces less consistent place preferences in rats when an unbiased procedure is used. Interestingly, in the present experiments, this same unbiased procedure resulted in a similar failure of cocaine to produce place preference in goldfish.

4.8 Experiments 7a, 7b, 7c, 7d, and 7e: The Morphine Experiments

Opioid mechanisms are involved in reward in rats and other mammals. In earlier sections (1.3 and 2.2) on opioid reward, the evidence for this conclusion was that opioid agonist drugs have rewarding effects in a variety of mammals as demonstrated in place conditioning, as well as other reward procedures. However, there appear to be species differences in sensitivity to morphine's rewarding effects. Hamsters (Schnur & Morrell, 1990) and dogs (B. E. Jones & Prada, 1973, 1981; Schuster & Johanson, 1981) are reportedly less sensitive to this effect of morphine than rats, and for pigs morphine may not be rewarding at all (Bedford, 1973).

For Experiment 7a, a moderately low dose of 5 mg/kg was selected, based on the doses used in various studies that found a conditioned place preference in rats with morphine injected intraperitoneally (Bechara & van der Kooy, 1985; Blander, Hunt, Blair, & Amit, 1984; Nomikos & Spyraki, 1988b; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980; Vezina & Stewart, 1987). These doses ranged from a low of 1.25 mg/kg in the study by Bechara and van der Kooy (1985) to a high of 20 mg/kg in the study by Blander, Hunt, Blair, and Amit (1984).

Because of negative results in Experiment 7a, higher doses of morphine, administered intraperitoneally, were used in Experiments 7b, 7c, and 7d. Following consistently negative results with the intraperitoneal route, an intracranial route of administration was used in Experiment 7e. The dose administered intracranially was 0.3 μg per fish.

4.8.1 Method

4.8.1.1 Subjects

In each experiment there were two groups of goldfish: Group Clear and Group White. In Experiments 7a, 7b, 7c, and 7e, each group was housed in a separate 38-liter tank. In Experiment 7d, the fish (36 comets and 4 fantails) were housed in 20 clear plastic tanks with one fish from Group Clear and one from Group White in each tank. The number of fish and the means and standard deviations of the weights for each group in each experiment are given in Table 4.4. In Experiment 7d, one fish in Group Clear died during the experiment and another fish from the same group was eliminated because of a procedural error. In Experiment 7e, two fish from Group Clear and one from Group White were eliminated because of injuries suffered during the injection procedure.

Table 4.4

Weights of Fish and Drug Dosage Data for Morphine in Experiments 7a, 7b, 7c, 7d, and 7e.

EXP	Group	n	Weight		Morphine		
			Mean	SD	Amount (mg)[a]	Dose (mg/kg)	Volume
7a	Clear	10	6.5	0.9	0.033	5.0	0.05 ml
	White	10	6.5	0.8	0.033	5.0	0.05 ml
7b	Clear	10	9.9	3.1	0.15	15.0	0.05 ml
	White	10	10.0	2.8	0.15	15.0	0.05 ml
7c	Clear	10	4.5	1.3	0.0675	15.0	0.05 ml
	White	10	4.5	1.1	0.0675	15.0	0.05 ml
7d	Clear	18	14.7	4.5		30.0	10 ml/kg
	White	20	14.7	5.9		30.0	10 ml/kg
7e[b]	Clear	8	4.0	1.0	0.3 µg	75 µg/kg	5.0 µl
	White	9	3.9	0.9	0.3 µg	75 µg/kg	5.0 µl

Note. EXP = Experiment number

[a]per fish

[b]In Experiment 7e, the morphine was administered intracranially instead of intraperitoneally; thus, the amount, dose, and volume of the morphine injection is given in µg, µg/kg, and µl, respectively.

4.8.1.2 Procedure

The basic procedure in all experiments was the same as those described in the General Method. On drug-conditioning trials, fish in Group Clear and Group White were given morphine before confinement in the clear and white compartments, respectively. On nondrug-conditioning trials, the fish were given no injections before confinement in the other compartment.

The experiments differed in the dose of morphine administered; the doses, as well as the injection volume, for each experiment are given in Table 4.4. In addition, in those experiments in which the dose was based on the mean weight of all the fish in the experiment (Experiments 7a, 7b, 7c, and 7e), rather than the weight of the individual fish as in Experiment 7d, the absolute amount of morphine injected is listed.

Experiment 7e differed from all the others in that intracranial injections (as described by Agranoff and Klinger, 1964) were given, instead of the intraperitoneal injections given in the other experiments. Each goldfish was wrapped in a damp paper towel and held firmly by hand. A Hamilton microliter syringe (701-N) was modified with a

polyethylene flange slipped over the needle to expose 2 mm of the needle tip. The needle, directed posteriorly at an angle of approximately 45°, was inserted to a depth of 2 mm through the skull at a well-defined point where the medial suture intersected a line between the orbits. Then, 5.0 μl of morphine solution (Olson, Kastin, Montalbano-Smith, Olson, Coy, & Michell, 1978) was slowly infused over a period of 30 sec into the cranial cavity overlying the brain.

There were only three drug-conditioning and three nondrug-conditioning trials in Experiment 7e, compared to six of each in the other experiments. Conditioning trials started early in the morning in Experiment 7a, in midmorning in Experiment 7c, 7d, and 7e, and in midafternoon in Experiment 7b. The usual 10 min place preference test was conducted following the completion of all the conditioning trials.

4.8.2 Results and Discussion

No evidence for place conditioning induced by morphine was found in any of Experiments 7a, 7b, 7c, or 7d. The data are summarized in Figure 4.7, which shows the mean and 95% confidence intervals for the time spent in the clear compartment by each group in each experiment.

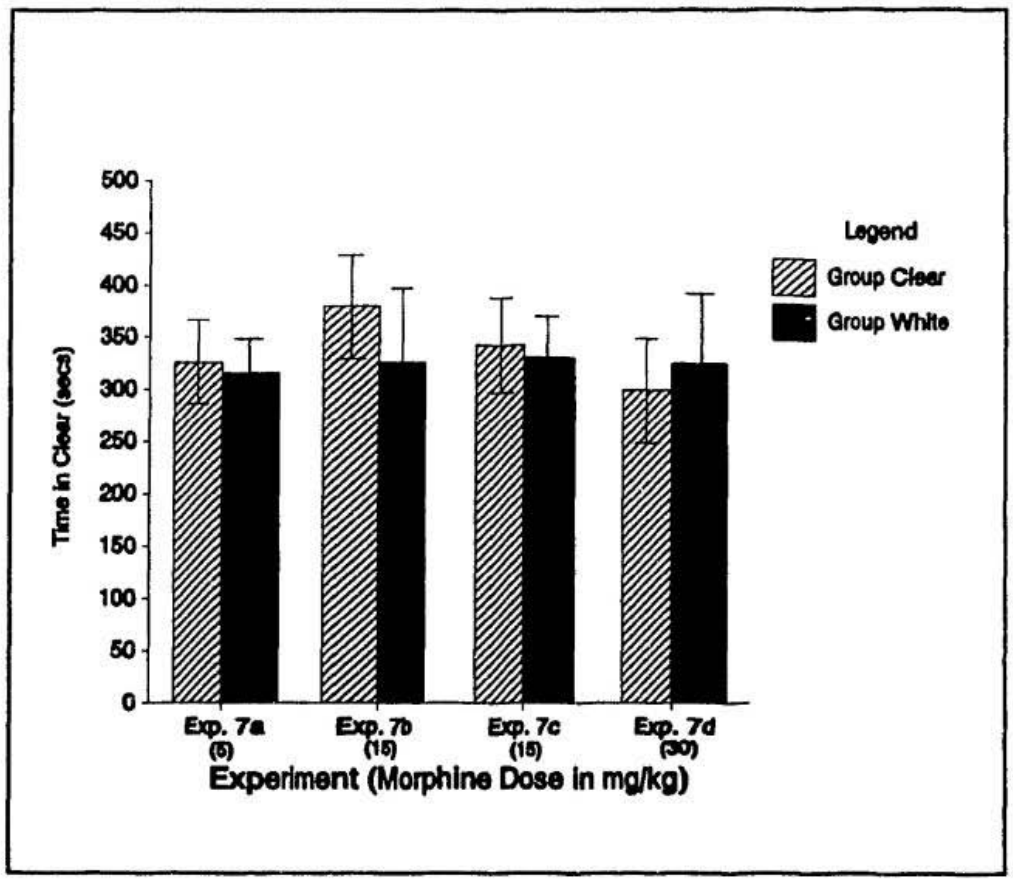

Figure 4.7. Number of seconds spent in clear compartment by Group Clear and Group White after place conditioning with different doses of morphine in Experiments 7a, 7b, 7c, and 7d. Brackets represent 95% confidence limits.

Although the differences between the groups in four of the five experiments are in the direction of a place preference, with Group Clear spending more time in the clear compartment than Group White, none of the differences is very large and a meta-analysis of all the experiments (except Experiment 7e, in which a different route of administration was used) was at a chance level ($z = 0.81$, $p > .05$). Each of the individual t-scores between groups yielded $p > .05$: $t(18) = 0.44$; $t(18) = 1.4$; $t(18) = 0.45$, and $t(36) = -0.63$; for Experiments 7a to 7d, respectively.

The lack of a morphine-induced place preference in Experiments 7a to 7d provides strong evidence that intraperitoneal morphine administrations are not rewarding to fish, but it remains possible to conjecture that this was due to some aversive peripheral effects that counteracted any positive effect. This possibility is suggested by the place aversions produced by peripheral actions of morphine in rats (Bechara, Zito, & van der Kooy, 1987). On this basis, avoiding peripheral activity by administering morphine centrally would be expected to produce conditioned place preferences. However, a test of this possibility in Experiment 7e, when morphine was injected into the intracranial cavity overlying the brain, yielded

nonsignificant results, $t(15) = 0.10$. $p > .05$. Group Clear spent a mean of 373.8 sec (SD = 152.9) in the clear compartment compared to 370.0 sec (SD = 59.28) for Group White.

The failure to find morphine-induced conditioned place preferences in the present experiments suggest that goldfish do not possess an opioid reward mechanism. However, other explanations are possible. For example, it is possible that morphine did not reach the reward-related opioid receptors during the 30-min postinjection interval when they were in the morphine-associated compartment (see also, section 3.2.2). In other words, the effect of morphine occurred too late to be associated with the compartment and produce a conditioned preference for it.

There is evidence, however, that opioid mechanisms in goldfish do, in fact, differ from those in other vertebrates, and thus reason to suspect that goldfish may be insensitive to morphine. Most vertebrates possess both low and high affinity opioid binding sites, but goldfish apparently possess only the low affinity opioid binding sites (Buatti & Pasternak, 1981; Moon Edley, Hall, Herkenham, & Pert, 1982). Low affinity opioid binding sites have been suggested to correspond to delta opioid receptors;

high affinity opioid binding sites have been suggested to correspond to mu receptors (Atweh, 1983; Goodman, Snyder, Kuhar, & Young, 1980; Moon Edley, Hall, Herkenham, & Pert, 1982; Zhang & Pasternak, 1980). Although both mu and delta receptors have been implicated in the mediation of reward (see section 1.3), morphine has a greater affinity for mu receptors than delta receptors (Akil, Bronstein, & Mansour, 1988; Atweh, 1983; Loh & Smith, 1990). Thus, morphine may lack rewarding efficacy in goldfish because it is relatively inactive at the delta opioid receptors which they possess. That an extremely large dose of morphine may be necessary to produce an effect at the delta opioid receptors in goldfish is suggested by the 30 mg/kg dose of morphine administered intracranially by Ehrensing, Michell, and Kastin (1982) to produce analgesia in goldfish.

The preceding experiments with morphine suggest that opioid mechanisms are not involved in mediating reward in goldfish. However, a more definitive statement awaits the results of studies with delta opioid agonist drugs which are more likely than morphine to activate the opioid receptors which are present in the goldfish brain.

CHAPTER 5

GENERAL DISCUSSION: PHYLOGENETIC IMPLICATIONS

In mammals, as discussed earlier (see Chapter 2), there is considerable evidence for a common reward system, called here the dopamine reward system. This system can be identified by three features: the dopamine mechanism, the opioid mechanism, and the functional dependence of the opioid mechanism on the dopamine mechanism. The purpose of the present research was to evaluate the possibility that the dopamine reward system is common to all vertebrates by testing for the presence of these three features in fish, the vertebrate class most distantly related to mammals. The present results have implications concerning dopamine and opioid reward mechanisms in fish as well as implications for the more general reward system of which they are a part. The implications for reward mechanisms are relatively straightforward and will be considered first. At the more general level of the integrated reward system, the implications of the results are more speculative and consideration of these will conclude this dissertation.

5.1 Dopamine Reward Mechanism in Goldfish

In five separate experiments (2a, 2b, 2c, 3, and 4) the indirect dopamine agonist, amphetamine, consistently produced rewarding effects in goldfish. These findings

suggest that goldfish possess a dopamine reward mechanism. The last two experiments in this series were designed to supply more specific evidence as to whether this rewarding effect was due to activation of dopamine mechanisms rather than due to some other effect of amphetamine. In Experiments 3 and 4, dopamine antagonists were administered before place-amphetamine pairings. Preadministration of these drugs would be expected to eliminate or reduce the reward effect in so far as it depended on dopamine mechanisms. However, neither haloperidol (Experiment 3) nor flupentixol (Experiment 4) had any such effect. This could mean that the amphetamine-induced place preference in goldfish is not mediated by dopamine. Alternatively, it is possible that some other factor could explain the ineffectiveness of the dopamine antagonists in fish; for example, the pharmacokinetics of the dopamine antagonists in fish may differ from those in rats.

In Experiments 5a, 5b, 5c, 5d, and 5e, apomorphine was used as the conditioning drug. Apomorphine is a direct dopamine agonist with effects more specific to dopamine synapses than amphetamine and cocaine. The results of this series of experiments provide evidence that activation of dopamine receptors is rewarding in fish, although this effect was evident only with doses between 0.4 and 0.5 mg/kg. A lower dose (0.25 mg/kg) and higher doses (1.0 and

2.0 mg/kg) were ineffective. It may be that the rewarding effect of higher doses of apomorphine is counteracted by aversive effects, because high doses of apomorphine do produce aversive effects in rats (Best, Best, & Mickley, 1973; Revusky & Gorry, 1973), other mammals (Borison & Wang, 1953), and in fish (Tiersch & Griffith, 1988).

Experiments 6a and 6b were designed to supply evidence as to whether cocaine (10 or 20 mg/kg) might have rewarding effects in goldfish. Cocaine was not effective in producing a rewarding effect in either experiment. However, a procedure parallel to the procedure used here also yielded negative results with rats (Nomikos & Spyraki, 1998a), so that the present negative results with goldfish are, arguably, not definitive (but see McMillen & Shore, 1979, for evidence of species differences in the behavioral effects of cocaine).

To summarize, the evidence for a dopamine reward mechanism in fish is not conclusive. Inconsistent with a dopamine mechanism are the failures to obtain conditioned place preference with cocaine and the failure of the dopamine antagonists haloperidol and flupentixol to block amphetamine-induced place preference. Although explanations for each of these negative findings have been offered, such explanations need independent confirmation. On the other hand, there are striking parallels between the rewarding

effects of the dopamine agonists, amphetamine and apomorphine, in goldfish and in rats and other mammals (see Experiments 2, 3, 4, and 5 for goldfish; sections 1.2.1 and 1.2.2 for rats; section 2.1 for other mammals). Amphetamine produces consistent rewarding effects in fish and mammals. Low doses of apomorphine are also rewarding to both fish and mammals, but high doses of apomorphine generally produce less consistent effects in both classes of vertebrates. At the present state of knowledge, the hypothesis that a dopamine mechanism mediates reward cannot be discarded. This is partly because of these parallel findings with amphetamine and apomorphine, but also because there appears to be no viable alternative to explain the present results with fish. The rewarding effects of amphetamine in particular seem reliable. Because the dose of flupentixol used in Experiment 4 was large enough to antagonize all known effects of amphetamine on transmitter systems, based on data from rats (Niemegeers & Janssen, 1979), the most reasonable explanation for the failure of the antagonists to interfere with the rewarding effects of amphetamine is that the antagonists were not reaching or were not effective at the relevant receptors. This interpretation is supported by casual observations that the behavior of the fish was not obviously affected by either haloperidol or flupentixol, whereas in rats the present dose of flupentixol would have

produced potent cataleptic effects (see section 4.5.2 for a more detailed discussion of this point). Thus, until the role of dopamine in mediating the rewarding effects of amphetamine and apomorphine can be ruled out more conclusively, the most reasonable working hypothesis is that fish possess a dopamine reward mechanism.

5.2 Opioid Reward Mechanism in Goldfish

Morphine, the only opioid agonist used in the present experiments, failed to produce a conditioned place preference in goldfish with doses ranging from 5.0 to 30.0 mg/kg, administered intraperitoneally, or with a 0.3 μg dose, administered intracranially. These results could mean that goldfish lack an opioid reward mechanism. A more guarded conclusion, however, may be appropriate in light of evidence that goldfish have delta opioid receptors, but lack mu receptors (Buatti & Pasternak, 1981), at which morphine is particularly effective. The present results may simply confirm morphine's ineffectiveness as a delta-receptor agonist (Akil, Bronstein, & Mansour, 1988; Atweh, 1983) and hence may not be definitive. Findings that other opioid agonists, less selective for the mu receptor, have effects on goldfish (Kavaliers, 1981; Olson, Kastin, Montalbano-Smith, Olson, Coy, & Michell, 1978; Rosenblum & Peter, 1989; Satake, 1979) support the contention that opioid effects in

goldfish may be mediated by receptors other than the mu receptor.

5.3 Speculations about the Dopamine Reward System

A dopamine reward system with a common evolutionary origin for all vertebrates would be inferred from the existence of many nontrivial similarities across vertebrate classes in the neurochemistry, neuroanatomical connections, and embryonic origins of the neurons involved in reward. Such an inference could be made even if not all characteristics of the system are identical for all classes (Bullock, 1984a; Echteler & Saidel, 1981; Northcutt, 1984; Northcutt & Braford, 1980; Striedter & Northcutt, 1991; Webster, 1979). Three neurochemical features of the dopamine reward system in mammals have been emphasized here. The present research has provided reasonable evidence for the presence of one of these in goldfish, a dopamine mechanism (see section 5.1); but it has also yielded evidence against the presence of the second feature, an opioid mechanism (see section 5.2), thus excluding the third feature, the functional dependence of the opioid mechanism on the dopamine mechanism. This makes any interpretation regarding the commonality of the dopamine reward system equivocal. The ambiguity would be reduced if either the evidence against a common dopamine reward system were

discounted, or the evidence for it were discounted. Each of these interpretations will be considered below.

5.3.1 The Interpretation that there is no Common Dopamine Reward System

That there is not a common dopamine reward system in fish and mammals was suggested primarily by the absence in goldfish of the opioid feature of the dopamine reward system in mammals. To confirm this interpretation, the presence of a dopamine reward mechanism in fish must be shown to be unrelated to the similar mechanism identified in the dopamine reward system in mammals. Such confirmation can be inferred from the major anatomical differences between the brains of teleost fish, like the goldfish, and those of other vertebrates.

One source of anatomical difference is found in the structure of the telencephalon. Teleosts belong to a subclass of actinopterygian fish. There are major structural differences between the brains of actinopterygian fish and the brains of other vertebrates. The differences appear to be due to a peculiarity of embryonic development in actinopterygian fish; the telencephalon undergoes a process of eversion, rather than the process of evagination-inversion observed in other vertebrates. The result is a different arrangement of brain structures in such fish

(e.g., Flood, Overmier, & Savage, 1976; Parent, 1986; Schroeder, 1980). It is not known to what extent this difference in arrangement is paralleled by differences in function but they complicate anatomical comparisons with the brains of other vertebrates. Uncertainty about how, or even if, the eversion process rearranges the parts of the brain involved (see, for example, Northcutt & Braford, 1980) complicates matters further.

A second source of difference lies in the distribution of dopamine and opioid neurotransmitters and receptors in the brains of teleost fish compared to those of mammals. Although both dopamine (Juorio, 1973; Kah, Chambolle, Thibault, & Geffard, 1984) and opioid peptides (Moon Edley, Hall, Herkenham, & Pert, 1982; Simantov, Goodman, Aposhian, & Snyder, 1976) are neurotransmitters in the teleost brain, they have not been localized in areas which might be expected to mediate reward on the basis of the mesolimbic reward system in the rat brain. For example, there is no evidence for dopamine neurons in the midbrain of goldfish (Hornby, Piekut, & Demski, 1987; Parent, Poitras, & Dubé, 1984), which is where the ventral tegmental dopamine cell bodies of the mesolimbic dopamine system are found in rats. Indeed, Parent, Poitras, and Dubé (1984) suggest that

mesotelencephalic dopamine projections, such as the mesolimbic dopamine system in the rat, are only to be found in terrestrial vertebrates (but see Meredith & Smeets, 1987).

The differences, outlined above, suggest that even if a dopamine reward mechanism is firmly established in fish, it may be part of a different system from the one identified in rats and other mammals. Of course, this leaves the question of why these different reward systems have in each case evolved to include a dopamine mechanism. This question cannot be answered definitively, because nothing is known about the normal functioning or location of the dopamine reward mechanism in goldfish. However, dopamine mechanisms are involved in many functions in goldfish, including reproductive and visual functions (Chang, Peter, & Crim, 1984; Kah, Chambolle, Thibault, & Geffard, 1984; Mangel & Dowling, 1985; McIntyre, Healy, & Saari, 1979; Morita & Finger, 1987; Yu & Peter, 1990). Thus, it is possible that activation of dopamine mechanisms associated with one or more of these other functions is responsible for the observed rewarding effects in the present experiments. In turn, this may mean that these effects are a tangential result of such activation or that a reward system in fish evolved in connection with one of these systems and thus has a different origin from the dopamine reward system in

mammals. In either case, according to this interpretation, the similarity in the rewarding effects of amphetamine and apomorphine in fish and mammals is merely a coincidence and does not reflect a common dopamine mechanism or reward system.

5.3.2 The Interpretation that there is a Common Dopamine Reward System

Although the case just made against a common dopamine reward system was formulated as strongly as was feasible, it cannot be definitive until more is known about the dopamine mechanism in fish and the significance of the anatomical differences between fish and other vertebrates. Thus, it seems, on balance, that it is premature to discard the similarities in the rewarding effects of amphetamine and apomorphine in fish and in mammals as merely coincidental. The original rationale for a dopamine reward system in fish similar to that in mammals, which was based on the evidence for the commonality of the dopamine reward system across mammals, and possibly birds, still seems persuasive (see section 2.3). Nevertheless, to postulate a common vertebrate dopamine reward system, it is necessary to discount the apparent lack of the opioid mechanism in fish.

It is possible that the opioid mechanism with its mu receptors is not an essential part of the phylogenetically old, common reward system. In support of this notion is evidence suggesting that mu opioid mechanisms may be phylogenetically recent modifications of the nervous system. Moon Edley, Hall, Herkenham, and Pert (1982) report that the relative proportion of mu to delta striatal opioid receptors increases with increasing relatedness to humans. Others have found a general increase in mu opioid receptors: from goldfish, with none, to mammals, with the most (Buatti & Pasternak, 1981). Such findings suggest that the role of mu opioid mechanisms in reward is a relatively recent evolutionary phenomenon, and thus that mu opioid receptors may constitute a modification of the reward system, but not a defining feature.

Other findings, however, suggest that mu opioid receptors are not phylogenetically recent but are simply more variable in their distribution (Simon & Hiller, 1984; Snyder, Pasternak, & Pert, 1975). For example, although goldfish lack them (Buatti & Pasternak, 1981; Moon Edley, Hall, Herkenham, & Pert, 1982), other fish (Bird, Jackson, Baker, & Buckingham, 1988), as well as invertebrates (Kavaliers, 1988; Kavaliers & Hirst, 1986; Kavaliers, Hirst, & Teskey, 1985; Kavaliers, Rangeley, Hirst, & Teskey, 1986; Stefano, Hall, Makman, & Dvorkin, 1981), appear to possess

them. It seems likely, therefore, that mu opioid receptors are phylogenetically old but are susceptible to evolutionary change and thus have been lost, or have had their distribution in the nervous system modified, in different taxonomic groups. This latter possibility is supported by findings of significant variability in the prevalence and distribution of mu opioid receptors among different mammalian orders and even among different species within the same order (Araki, Kato, Kogure, Shuto, & Ishida, 1992; Maurer, 1982; Pert, Aposhian, & Snyder, 1974). Variability in the behavioral effects of mu opioid agonists has also been found among mammals. For example, there are differences in the degree to which morphine produces rewarding effects (see section 2.2), as well as other effects, such as those on motor activity (see section 3.2.2). This variability across mammalian orders, in both the prevalence and distribution of mu opioid receptors and in the behavioral effects of the mu opioid agonist, morphine, supports the view that mu opioid mechanisms are susceptible to change or loss within evolutionary lines of descent.

Whether mu opioid mechanisms are phylogenetically recent or phylogenetically old but variable, the mu opioid mechanism that is characteristic of the rat dopamine reward system cannot be a defining feature of a common reward

system in all vertebrates. Such a common reward system would have undoubtedly undergone modification during the independent evolution of the various extant taxonomic groups, and the mu opioid reward mechanism could be one of those modifications.

When other neurochemical properties of the fish reward system and the input and output connections to that system are established, then comparisons of more features of the fish reward system will be possible. A delta opioid mechanism, for example, would be one feature to compare for commonality among vertebrates, because delta opioid mechanisms appear to be involved in mammalian reward (see section 1.3) and delta opioid mechanisms are also present in fish (Buatti & Pasternak, 1981; Moon Edley, Hall, Herkenham, & Pert, 1982).

In short, the present research provides evidence for the presence of a dopamine but not a mu opioid reward mechanism in fish. Although it remains possible that the dopamine reward mechanism in fish and mammals have a common evolutionary origin, the opposite conclusion has not been ruled out.

REFERENCES

Acquas, E., Carboni, E., Leone, P., & Di Chiara, G. (1989). SCH 23390 blocks drug-conditioned place-preference and place-aversion: Anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology, 99, 151-155.

Adams, W. J., Lorens, S. A., & Mitchell, C. L. (1972). Morphine enhances lateral hypothalamic self-stimulation in the rat. Proceedings of the Society for Experimental Biology, 140, 770-771.

Advokat, C. (1985). Evidence of place conditioning after chronic intrathecal morphine in rats. Pharmacology Biochemistry & Behavior, 22, 271-277.

Ågmo, A., & Berenfeld, R. (1990). Reinforcing properties of ejaculation in the male rat: Role of opioids and dopamine. Behavioral Neuroscience, 104, 177-182.

Agranoff, B. W., & Klinger, P. D. (1964). Puromycin effect on memory fixation in the goldfish. Science, 146, 952-953.

Akil, H., Bronstein, D., & Mansour, A. (1988). Overview of the endogenous opioid systems: Anatomical, biochemical and functional issues. In R. J. Rodgers & S. J. Cooper (Eds.), Endorphins, opiates and behavioural processes (pp. 1-23). New York: John Wiley & Sons.

Amalric, M., Cline, E. J., Martinez, J. L., Bloom, F. E., & Koob, G. F. (1987). Rewarding properties of b-endorphin as measured by conditioned place preference. Psychopharmacology, 91, 14-19.

Andén, N.-E. (1970). Effects of amphetamine and some other drugs on central catecholamine mechanisms. In E. Costa & S. Garattini (Eds.), Amphetamine and related compounds (pp. 447-462). New York: Raven Press.

Andén, N.-E., Butcher, S. G., Corrodi, H., Fuxe, K., & Ungerstedt, U. (1970). Receptor activity and turnover of dopamine and noradrenaline after neuroleptics. European Journal of Pharmacology, 11, 303-314.

Andén, N.-E., Rubenson, A., Fuxe, K., & Hökfelt, T. (1967). Evidence for dopamine receptor stimulation by apomorphine. Journal of Pharmacy and Pharmacology, 19, 627-629.

Andrew, R. J. (1967). Intracranial self-stimulation in the chick. Nature, 213, 847-848.

Andrew, R. J. (1969). Intracranial self-stimulation in the chick and the causation of emotional behavior. Annals of the New York Academy of Sciences, 150, 625-639.

Andrews, J. S., & Holtzman, S. G. (1987). Effects of naloxone and diprenorphine on amphetamine-stimulated behavior in guinea pigs and rats. Neuropharmacology, 26, 1115-1120.

Angrist, B., & Gershon, S. (1978). Four dopamine agonists: A clinical continuum. Neuropsychopharmacology: Vol. 1. Proceedings of the Tenth Congress of the Collegium Internationale Neuro-Psychopharmacologium (pp. 731-738). Oxford, Pergamon Press.

Antoniou, K., & Kafetzopoulos, E. (1991). A comparative study of the behavioral effects of d-amphetamine and apomorphine in the rat. Pharmacology Biochemistry & Behavior, 39, 61-70.

Araki, T., Kato, H., Kogure, K., Shuto, K., & Ishida, Y. (1992). Autoradiographic mapping of neurotransmitter system receptors in mammalian brain. Pharmacology Biochemistry & Behavior, 41, 539-542.

Arnt, J. (1982). Pharmacological specificity of conditioned avoidance response inhibition in rats: Inhibition by neuroleptics and correlation to dopamine receptor blockade. Acta Pharmacologia et Toxicologia, 51, 321-329.

Atweh, S. F. (1983). Characterization and distribution of brain opiate receptors and endogenous opioid peptides. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 59-87). Amsterdam: Elsevier Biomedical Press.

Aulisi, E. F., & Hoebel, B. G. (1983). Rewarding effects of amphetamine and cocaine in the nucleus accumbens and block by flupenthixol. Society for Neuroscience Abstracts, 9, 121.

Avis, H. H., & Peeke, H. V. S. (1975). Differentiation by morphine of two types of aggressive behavior in the convict cichlid (*Cichlasoma nigrofasciatum*). Psychopharmacologia (Berlin), 43, 287-288.

Axelrod, J. (1970). Amphetamine: Metabolism, physiological disposition and its effects on catecholamine storage. In E. Costa & S. Garattini (Eds.), Amphetamine and related compounds (pp. 207-216). New York: Raven Press.

Baldessarini, R. J., Arana, G. W., Kula, N. S., Campbell, A., & Harding, M. (1981). Preclinical studies of the pharmacology of aporphines. In G. U. Corsini & G. L. Gessa (Eds.), Apomorphine and other dopaminomimetics: Vol. 1. Basic pharmacology (pp. 219-228). New York: Raven Press.

Balster, R. L., Kilbey, M. M., & Ellinwood, E. H., Jr. (1976). Methamphetamine self-administration in the cat. Psychopharmacologia (Berlin), 46, 229-233.

Balster, R. L., & Schuster, C. R. (1973a). A comparison of d-amphetamine, l-amphetamine, and methamphetamine self-administration in rhesus monkeys. Pharmacology Biochemistry & Behavior, 1, 67-71.

Balster, R. L., & Schuster, C. R. (1973b). Fixed-interval schedule of cocaine reinforcement: Effect of dose and infusion duration. Journal of the Experimental Analysis of Behavior, 20, 119-129.

Bannon, M. J., Freeman, A. S., Chiodo, L. A., Bunney, B. S., & Roth, R. H. (1987). The electrophysiological and biochemical pharmacology of the mesolimbic and mesocortical dopamine neurons. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 19, pp. 329-374). New York: Plenum Press.

Bardo, M. T., Miller, J. S., & Neisewander, J. L. (1984). Conditioned place preference with morphine: The effect of extinction training on the reinforcing CR. Pharmacology Biochemistry & Behavior, 21, 545-549.

Bardo, M. T., & Neisewander, J. L. (1986). Single-trial conditioned place preference using intravenous morphine. Pharmacology Biochemistry & Behavior, 25, 1101-1105.

Barnes, N. J., Costall, B., Domeney, A. M., & Naylor, R. J. (1987). Behavioural consequences of the infusion of dopamine into the nucleus accumbens of the common marmoset (*Callithrix jacchus*). Neuropharmacology, 26, 1327-1335.

Barr, G. A., Paredes, W., & Bridger, W. H. (1985). Place conditioning with morphine and phencyclidine: Dose dependent effects. Life Sciences, 36, 363-368.

Baxter, B. L., Gluckman, M. I., & Scerni, R. A. (1976). Apomorphine self-injection is not affected by alpha-methylparatyrosine treatment: Support for dopaminergic reward. Pharmacology Biochemistry & Behavior, 4, 611-612.

Baxter, B. L., Gluckman, M. I., Stein, L., & Scerni, R. A. (1974). Self-injection of apomorphine in the rat: Positive reinforcement by a dopamine receptor stimulant. Pharmacology Biochemistry & Behavior, 2, 387-391.

Bechara, A., Harrington, F., Nader, K., & van der Kooy, D. (in press). Neurobiology of motivation: Double dissociation of two motivational mechanisms mediating opiate reward in drug-naive versus drug-dependent animals. Behavioral Neuroscience.

Bechara, A., & van der Kooy, D. (1985). Opposite motivational effects of endogenous opioids in brain and periphery. Nature, 314, 533-534.

Bechara, A., & van der Kooy, D. (1992a). A single brain stem substrate mediates the motivational effects of both opiates and food in nondeprived rats but not in deprived rats. Behavioral Neuroscience, 106, 351-363.

Bechara, A., & van der Kooy, D. (1992b). Chronic exposure to morphine does not alter the neural tissues subserving its acute rewarding properties: Apparent tolerance is overshadowing. Behavioral Neuroscience, 106, 364-373.

Bechara, A, & van der Kooy, D. (1992c). Lesions of the tegmental pedunculopontine nucleus: Effects on the locomotor activity induced by morphine and amphetamine. Pharmacology Biochemistry & Behavior, 42, 9-18.

Bechara, A., Zito, K. A., & van der Kooy, D. (1987). Peripheral receptors mediate the aversive conditioning effects of morphine in the rat. Pharmacology Biochemistry & Behavior, 28, 219-225.

Bedford, J. A. (1973). Drug self-administration in the pig. Dissertation Abstracts International, 34, 1767 B.

Belluzzi, J. D., & Stein, L. (1977). Enkephalin may mediate euphoria and drive-reduction reward. Nature, 206, 556-558.

Bendani, T., & Cazala, P. (1988). Differential effects of intracerebral microinjection of morphine on approach and escape responses induced by lateral hypothalamic stimulation in the mouse. Pharmacology Biochemistry & Behavior, 30, 397-401.

Beninger, R. J. (1983). The role of dopamine in locomotor activity and learning. Brain Research Reviews, 6, 173-196.

Beninger, R. J. (1989). Dissociating the effects of altered dopaminergic function on performance and learning. Brain Research Bulletin, 23, 365-371.

Beninger, R. J., Hoffman, D. C., & Mazurski, E. J. (1989). Receptor subtype-specific dopaminergic agents and conditioned behavior. Neuroscience & Biobehavioral Reviews, 13, 113-122.

Best, P. J., Best, M. R., & Mickley, G. A. (1973). Conditioned aversion to distinct environmental stimuli resulting from gastrointestinal distress. Journal of Comparative and Physiological Psychology, 85, 250-257.

Bird, D. J., Jackson, M., Baker, B. I., & Buckingham, J. C. (1988). Opioid binding sites in the fish brain: An autoradiographic study. General and Comparative Endocrinology, 70, 49-62.

Bishop, M. P., Elder, S. T., & Heath, R. G. (1963). Intracranial self-stimulation in man. Science, 140, 394-396.

Bitterman, M. E. (1975). The comparative analysis of learning: Are the laws of learning the same in all animals? Science, 188, 699-709.

Bitterman, M. E. (1984). Learning in man and other animals. In V. Sarris & A. Parducci (Eds.), Perspectives in psychological experimentation: Toward the year 2000 (pp. 59-70). Hillsdale, NJ: Lawrence Erlbaum Associates.

Blander, A., Hunt, T., Blair, R., & Amit, Z. (1984). Conditioned place preference: An evaluation of morphine's positive reinforcing properties. Psychopharmacology, 84, 124-127.

Borison, H. L., & Wang, S. C. (1953). Physiology and pharmacology of vomiting. Pharmacological Reviews, 5, 193-230.

Boyd, E. S., & Gardner, L. C. (1962). Positive and negative reinforcement from intracranial stimulation of a teleost. Science, 136, 648-649.

Bozarth, M. A. (1983). Opiate reward mechanisms mapped by intracranial self-administration. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 331-359). Amsterdam: Elsevier Biomedical Press.

Bozarth, M. A. (1985). Biological basis of cocaine addiction. In C. J. Brink (Ed.), Cocaine: A symposium (pp. 32-36). Madison WI: Wisconsin Institute on Drug Abuse.

Bozarth, M. A. (1986). Neural basis of psychomotor stimulant and opiate reward: Evidence suggesting the involvement of a common dopaminergic system. Behavioural Brain Research, 22, 107-116.

Bozarth, M. A. (1987a). Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. Brain Research, 414, 77-84.

Bozarth, M. A. (1987b). Ventral tegmental reward system. In J. Engel & L. Oreland (Eds.), Brain reward systems and abuse (pp. 1-17). New York: Raven Press.

Bozarth, M. A. (1988). Opioid reinforcement processes. In R. J. Rodgers & S. J. Cooper (Eds.), Endorphins, opiates and behavioural processes (pp. 54-75). New York: John Wiley & Sons.

Bozarth, M. A., & Wise, R. A. (1981a). Heroin reward is dependent on a dopaminergic substrate. Life Sciences, 29, 1881-1886.

Bozarth, M. A., & Wise, R. A. (1981b). Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sciences, 28, 551-555.

Bozarth, M. A., & Wise, R. A. (1982). Localization of the reward-relevant opiate receptors. In L. S. Harris (Ed.), Problems of drug dependence, 1981 (National Institute on Drug Abuse Research Monograph 41) (pp. 158-164). Rockville, MD: U.S. Government Printing Office.

Bozarth, M. A., & Wise, R. A. (1984). Anatomically distinct opiate receptor fields mediate reward and physical dependence. Science, 224, 516-517.

Bozarth, M. A., & Wise, R. A. (1986). Involvement of the ventral tegmental dopamine system in opioid and psychomotor stimulant reinforcement. In L. S. Harris (Ed.), Problems of drug dependence, 1985, (National Institute on Drug Abuse Research Monograph 67) (pp. 190-196). Washington, DC: US Government Printing Office.

Brady, J. V., Griffiths, R. R., Hienz, R. D., Ator, N. A., Lukas, S. E., & Lamb, R. J. (1987). Assessing drugs for abuse liability and dependence potential in laboratory primates. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 45-85). New York: Springer Verlag.

Britt, M. D., & Wise, R. A. (1983). Ventral tegmental site of opiate reward: Antagonism by a hydrophillic opiate receptor blocker. Brain Research, 258, 105-108.

Broekkamp, C. L. E. (1976). The effects of manipulations with the dopaminergic system on self-stimulation behavior. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 194-195). New York: Elsevier/North Holland.

Broekkamp, C. L., Phillips, A. G., & Cools, A. R. (1979). Facilitation of self-stimulation behavior following intracerebral microinjections of opioids into the ventral tegmental area. Pharmacology Biochemistry & Behavior, 11, 289-295.

Broekkamp, C. L. E., Pijnenburg, A. J. J., Cools, A. R., & van Rossum, J. M. (1975). The effect of microinjections of amphetamine into the neostriatum and the nucleus accumbens on self-stimulation behaviour. Psychopharmacologia (Berlin), 42, 179-183.

Broekkamp, C. L., Van Den Bogaard, J. H., Heijnen, H. J., Rops, R. H., Cools, A. R., & Van Rossum, J. M. (1976). Separation of inhibiting and stimulating effects of morphine on self-stimulation behaviour by intracerebral microinjections. European Journal of Pharmacology, 36, 443-446.

Broekkamp, C. L. E., & Van Rossum, J. M. (1974). Effects of apomorphine on self-stimulation behavior. Psychopharmacologia (Berlin), 34, 71-80.

Brown, P. L., & Jenkins, H. M. (1968). Auto-shaping of the pigeon's key-peck. Journal of the Experimental Analysis of Behavior, 11, 1-8.

Buatti, M. C., & Pasternak, G. W. (1981). Multiple opiate receptors: Phylogenetic differences. Brain Research, 218, 400-405.

Bullock, T. H. (1984a). The future of comparative neurology. American Zoologist, 24, 765-774.

Bullock, T. H. (1984b). Understanding brains by comparing taxa. Perspectives in Biology and Medicine, 27, 510-524.

Bunney, B. S. (1983). Neuroleptics: Electrophysiological pharmacology. In J. T. Coyle & S. J. Enna (Eds.), Neuroleptics: Neurochemical, behavioral, and clinical perspectives (pp. 157-181). New York: Raven Press.

Bunney, B. S. (1988). Effects of acute and chronic neuroleptic treatment on the activity of midbrain dopamine neurons. Annals of the New York Academy of Sciences, 537, 77-85.

Burg, B., Haase, C., Lindenblatt, U., & Delius, J. D. (1989). Sensitization to and conditioning with apomorphine in pigeons. Pharmacology Biochemistry & Behavior, 34, 59-64.

Bursten, B., & Delgado, J. M. R. (1958). Positive reinforcement induced by intracerebral stimulation in the monkey. Journal of Comparative and Physiological Psychology, 51, 6-10.

Burton, M. J., Mora, F., & Rolls, E. T. (1976). Neurophysiological convergence of natural and brain-stimulation reward on visual units in the lateral hypothalamus of squirrel and rhesus monkeys. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 101-104). New York: Elsevier/North Holland.

Carey, R. J. (1982). Rate dependent inhibition of self-stimulation by apomorphine. Pharmacology Biochemistry & Behavior, 16, 859-861.

Carey, R. J., Goodall, E., & Lorens, S. A. (1975). Differential effects of amphetamine and food deprivation on self-stimulation of the lateral hypothalamus and medial frontal cortex. Journal of Comparative and Physiological Psychology, 88, 224-230.

Carlsson, A. (1970). Amphetamine and brain catecholamines. In E. Costa & S. Garattini (Eds.), Amphetamine and related compounds (pp. 289-300). New York: Raven Press.

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

Carr, G. D., Phillips, A. G., & Fibiger, H. C. (1988). Independence of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. Psychopharmacology, 94, 221-226.

Carr, G. D., & White, N. M. (1983). Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. Life Sciences, 33, 2551-2557.

Carr, G. D., & White, N. M. (1986). Anatomical disassociation of amphetamine's rewarding and aversive effects: An intracranial microinjection study. Psychopharmacology, 89, 340-346.

Carr, G. D., & White, N. M., (1987). Effects of systemic and intracranial amphetamine injections on behavior in the open field: A detailed analysis. Pharmacology Biochemistry & Behavior, 27, 113-122.

Carroll, R. L. (1988). Vertebrate paleontology and evolution. New York: W. H. Freeman.

Cazala, P., Darracq, C., & Saint-Marc, M. (1987). Self-administration of morphine into the lateral hypothalamus in the mouse. Brain Research, 416, 283-288.

Chang, J. P., Peter, R. E., & Crim, L. W. (1984). Effects of dopamine and apomorphine on gonadotropin release from the transplanted pars distalis in goldfish. General and Comparative Endocrinology, 55, 347-350.

Christopher, S. M., & Butter, C. M. (1968). Consummatory behaviors and locomotor exploration evoked from self-stimulation sites in rats. Journal of Comparative and Physiological Psychology, 66, 335-339.

Colle, L. M., & Wise, R. A. (1988). Effects of nucleus accumbens amphetamine on lateral hypothalamic brain stimulation reward. Brain Research, 459, 361-368.

Colpaert, F. C., Van Bever, W. F. M., & Leysen, J. E. M. F. (1976). Apomorphine: Chemistry, pharmacology, biochemistry. International Review of Neurobiology, 19, 225-268.

Corbett, D., Stellar, J. R., Stinus, L., Kelley, A., & Fouriezos, G. (1983). Time course of alpha-flupenthixol action explains "response artifacts" of neuroleptic action on brain stimulation reward. Science, 222, 1251-1252.

Corrigall, W. A., & Vaccarino, F. J. (1988). Antagonist treatment in nucleus accumbens or periaqueductal grey affects heroin self-administration. Pharmacology Biochemistry & Behavior, 30, 443-450.

Costall, B., Domeney, A. M., & Naylor, R. J. (1984). Locomotor hyperactivity caused by dopamine infusion into the nucleus accumbens of rat brain: Specificity of action. Psychopharmacology, 82, 174-180.

Costall, B, & Naylor, R. J. (1973). On the mode of action of apomorphine. European Journal of Pharmacology, 21, 350-361.

Creese, I., Hamblin, M. W., Leff, S. E., & Sibley, D. R. (1983). CNS dopamine receptors. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 17, pp. 81-138). New York: Plenum Press.

Creese, I., & Iversen, S. D. (1975). The pharmacological and anatomical substrates of the amphetamine response in the rat. Brain Research, 83, 419-436.

Criswell, H. E., & Ridings, A. (1983). Intravenous self-administration of morphine by naive mice. Pharmacology Biochemistry & Behavior, 18, 467-170.

Crow, T. J. (1972). A map of the rat mesencephalon for electrical self-stimulation. Brain Research, 36, 265-273.

Crowder, W. F., & Hutto, C. W., Jr. (1992). Operant place conditioning measures examined using two nondrug reinforcers. Pharmacology Biochemistry & Behavior, 41, 817-824.

Csányi, V., Doka, A., Castellano, C., Puglisi-Allegra, S., & Oliverio, A. (1984). An ethopharmacological analysis of morphine effects in fish (*Macropodus opercularis*). Behavioural Processes, 9, 315-321.

Davis, W. M., & Smith, S. G. (1975). Effect of haloperidol on (+)-amphetamine self-administration. Journal of Pharmacy and Pharmacology, 27, 540-541.

Davis, W. M., & Smith, S. G. (1977). Catecholaminergic mechanisms of reinforcement: Direct assessment by drug self-administration. Life Sciences, 20, 483-492.

Davis, W. M., Smith, S. G., & Khalsa, J. H. (1975). Noradrenergic role in the self-administration of morphine or amphetamine. Pharmacology Biochemistry & Behavior, 3, 477-484.

De La Garza, R., & Johanson, C. E. (1982). Effects of haloperidol and physostigmine on self-administration of local anesthetics. Pharmacology Biochemistry & Behavior, 17, 1295-1299.

Delfs, J. M., Schreiber, L., & Kelley, A. (1990). Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. The Journal of Neuroscience, 10, 303-310.

Delgado, J. M. R. (1976). New orientations in brain stimulation in man. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 481-503). New York: Elsevier/North Holland.

Deneau, G., Yanagita, T., & Seevers, M. H. (1969). Self-administration of psychoactive substances by the monkey. Psychopharmacologia (Berlin), 16, 30-48.

de Wit, H., & Wise, R. A. (1977). Blockade of cocaine reinforcement in rats with the dopamine receptor blocker pimozide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. Canadian Journal of Psychology, 31, 195-203.

Dhasmana, K. M., Dixit, K. S., Jaju, B. P., & Gupta, M. L. (1972). Role of central dopaminergic receptors in manic response of cats to morphine. Psychopharmacologia (Berlin), 24, 380-383.

Di Chiara, G., Acquas, E., & Carboni, E. (1992). Drug motivation and abuse: A neurobiological perspective. Annals of the New York Academy of Sciences, 654, 207-219.

Di Chiara, G., & Gessa, G. L. (1978). Pharmacology and neurochemistry of apomorphine. Advances in Pharmacology and Chemotherapy, 15, 87-160.

Di Chiara, G., & Imperato, A. (1988a). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proceedings of the National Academy of Sciences of the United States of America, 85, 5274-5278.

Di Chiara, G., & Imperato, A. (1988b). Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. The Journal of Pharmacology and Experimental Therapeutics, 244, 1067-1080.

Di Chiara, G., Imperato, A., & Mulas, A. (1987). Preferential stimulation of dopamine release in the mesolimbic system: A common feature of drugs of abuse. In M. Sandler, C. Feuerstein, & B. Scatton (Eds.), Neurotransmitter interactions in the basal ganglia (pp. 171-182). New York: Raven Press.

Dilts, R. P., & Kalivas, P. W. (1988). Localization of mu opioid and neurotensin receptors within the A10 region of the rat. Annals of the New York Academy of Sciences, 537, 472-474.

Doka, A., Csányi, V., Castellano, C., & Oliverio, A. (1985). Effects of morphine in different strains of paradise fish (Macropodus Opercularis L.): An ethopharmacological analysis. Behavioural Processes, 11, 171-180.

Domesick, V. B. (1988). Neuroanatomical organization of dopamine neurons in the ventral tegmental area. Annals of the New York Academy of Sciences, 537, 10-26.

Domino, E. F., Vasko, M. R., & Wilson, A. E. (1976). Mixed depressant and stimulant actions of morphine and their relationship to brain acetylcholine. Life Sciences, 18, 361-376.

Dougherty, J., & Pickens, R. (1973). Fixed-interval schedules of intravenous cocaine presentation in rats. Journal of the Experimental Analysis of Behavior, 20, 111-118.

Druhan, J. P., & Stewart, J. (1990). A comparison of the stimulant-like effects produced by ventral tegmental and nucleus accumbens morphine injections. Psychopharmacology, suppl. to Vol. 101, p. S67, #258.

Dworkin, S. I., Goeders, N. E., & Smith, J. E. (1986). The reinforcing and rate effects of intracranial dopamine administration. In L. S. Harris, (Ed.), Problems of drug dependence, 1985, (National Institute on Drug Abuse Research Monograph 67) (pp. 242-248). Washington, DC: US Government Printing Office.

Dworkin, S. I., Guerin, G. F., Co, C., Goeders, N. E., & Smith, J. E. (1988). Lack of an effect of 6-hydroxydopamine lesions of the nucleus accumbens on intravenous morphine self-administration. Pharmacology Biochemistry & Behavior, 30, 1051-1057.

Dworkin, S. I., Guerin, G. F., Goeders, N. E., & Smith, J. E. (1988). Kainic acid lesions of the nucleus accumbens selectively attenuate morphine self-administration. Pharmacology Biochemistry & Behavior, 29, 175-181.

Dworkin, S. I., & Smith, J. E. (1988). Neurobehavioral pharmacology of cocaine. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 185-198). Washington, DC: U.S. Government Printing Office.

Echteler, S. M., & Saidel, W. M. (1981). Forebrain connections in the goldfish support telencephalic homologies with land vertebrates. Science, 212, 683-685.

Ehrensing, R. H., Michell, G. F., & Kastin, A. J. (1982). Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus*. Pharmacology Biochemistry & Behavior, 17, 757-761.

Ellenbroek, B. A., van Aanholt, H. T., & Cools, A. R. (1990). The role of dopamine in the occurrence of spontaneous stereotypies in a group of java monkeys (*M. fascicularis*). Psychopharmacology, suppl. to Vol. 101, p. S17, #58.

Enna, S. J., & Coyle, J. T. (1983). Neuroleptics. In J. T. Coyle & S. J. Enna (Eds.), Neuroleptics: Neurochemical, behavioral, and clinical perspectives (pp. 1-14). New York: Raven Press.

Esposito, R. U., Faulkner, W., & Kornetsky, C. (1979). Specific modulation of brain stimulation reward by haloperidol. Pharmacology Biochemistry & Behavior, 10, 937-940.

Esposito, R. U., & Kornetsky, C. (1978). Opioids and rewarding brain stimulation. Neuroscience & Biobehavioral Reviews, 2, 115-122.

Esposito, R. U., Motola, A. H. D., & Kornetsky, C. (1978). Cocaine: Acute effects on reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle. Pharmacology Biochemistry & Behavior, 8, 437-439.

Esposito, R. U., Perry, W., & Kornetsky, C. (1980). Effects of d-amphetamine and naloxone on brain stimulation reward. Psychopharmacology, 69, 187-191.

Ettenberg, A. (1989). Dopamine, neuroleptics and reinforced behavior. Neuroscience & Biobehavioral Reviews, 13, 105-111.

Ettenberg, A., & Duvauchelle, C. L. (1988). Haloperidol blocks the conditioned place preferences induced by rewarding brain stimulation. Behavioral Neuroscience, 102, 687-691.

Ettenberg, A., Koob, G. F., & Bloom, F. E. (1981). Response artifact in the measurement of neuroleptic-induced anhedonia. Science, 213, 357-359.

Ettenberg, A., Pettit, H. O., Bloom, F. E., & Koob, G. F. (1982). Heroin and cocaine intravenous self-administration in rats: Mediation by separate neural systems. Psychopharmacology, 78, 204-209.

Evans, K. R., & Vaccarino, F. J. (1990). Amphetamine- and morphine-induced feeding: Evidence for involvement of reward mechanisms. Neuroscience & Biobehavioral Reviews, 14, 9-22.

Fibiger, H. C., & Phillips, A. G. (1988). Mesocorticolimbic dopamine systems and reward. Annals of the New York Academy of Sciences, 537, 206-215.

Fielding, S., & Lal, H. (1978). Behavioral actions of neuroleptics. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 10, pp. 91-128). New York: Plenum Press.

Fink, J. S., & Smith, G. P. (1980). Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioral responses to amphetamine and apomorphine. Brain Research, 201, 107-127.

Finlay, J. M., Jakubovic, A., Phillips, A. G., & Fibiger, H. C. (1988). Fentanyl-induced conditional place preference: Lack of associated conditional neurochemical events. Psychopharmacology, 96, 534-540.

Fischman, M. W. (1984). The behavioral pharmacology of cocaine in humans. In J. Grabowski (Ed.), Cocaine: Pharmacology, effects, and treatment of abuse (National Institute on Drug Abuse Research Monograph 50) (pp. 72-91). Washington, DC: U. S. Government Printing Office.

Flood, N. C., Overmier, J. B., & Savage, G. E. (1976). Teleost telencephalon and learning: An interpretive review of data and hypotheses. Physiology & Behavior, 16, 783-798.

Fouriezos, G., Hansson, P., & Wise, R. A. (1978). Neuroleptic-induced attenuation of brain stimulation reward in rats. Journal of Comparative and Physiological Psychology, 92, 661-671.

Frank, R. A., Manderscheid, P. Z., Panicker, S., Williams, H. P., & Kokoris, D. (1992). Cocaine euphoria, dysphoria, and tolerance assessed using drug-induced changes in brain-stimulation reward. Pharmacology Biochemistry & Behavior, 42, 771-779.

Franklin, K. B. J. (1978). Catecholamines and self-stimulation: Reward and performance effects dissociated. Pharmacology Biochemistry & Behavior, 9, 813-820.

Fray, P. J., Sahakian, B. J., Robbins, T. W., Koob, G. F., & Iversen, S. D. (1980). An observational method for quantifying the behavioural effects of dopamine agonists: Contrasting effects of d-amphetamine and apomorphine. Psychopharmacology, 69, 253-259.

Fujiwara, H. (1992). Comparative studies of sulpiride and classical neuroleptics on induction of catalepsy, locomotor activity, and brain dopamine metabolism in mice. Pharmacology Biochemistry & Behavior, 41, 301-308.

Gallistel, C. R. (1983). Self-stimulation. In J. A. Deutsch (Ed.), The physiological basis of memory (pp. 269-349). New York: Academic Press.

Gallistel, C. R., Boytim, M., Gomita, Y., & Klebanoff, L. (1982). Does pimozide block the reinforcing effect of brain stimulation? Pharmacology Biochemistry & Behavior, 17, 769-781.

Gallistel, C. R., & Freyd, G. (1987). Quantitative determination of the effects of catecholaminergic agonists and antagonists on the rewarding efficacy of brain stimulation. Pharmacology Biochemistry & Behavior, 26, 731-741.

Gallistel, C. R., & Karras, D. (1984). Pimozide and amphetamine have opposing effects on the reward summation function. Pharmacology Biochemistry & Behavior, 20, 73-77.

Garattini, S. (1978). Some examples of resistance to psychotropic drugs in animals and man. Neuropsychopharmacology: Vol. 1. Proceedings of the Tenth Congress of the Collegium Internationale Neuro-Psychopharmacologicum (pp. 311-319). Oxford, Pergamon Press.

Garcia, J., Ervin, F. R., & Koelling, R. A. (1966). Learning with prolonged delay of reinforcement. Psychonomic Science, 5, 121-122.

Garcia, J., Hankins, W. G., & Coil, J. D. (1977). Koalas, men, and other conditioned gastronomes. In N. W. Milgram, L. Krames, & T. M. Alloway (Eds.), Food aversion learning (pp. 195-218). New York: Plenum Press.

Gerber, G. J., & Wise, R. A. (1989). Pharmacological regulation of intravenous cocaine and heroin self-administration in rats: A variable dose paradigm. Pharmacology Biochemistry & Behavior, 32, 527-531.

Gianutsos, G., & Moore, K. E. (1980). Differential behavioral and biochemical effects of four dopaminergic agonists. Psychopharmacology, 68, 139-146.

Gilbert, D., & Cooper, S. J. (1983). B-phenylethylamine, d-amphetamine- and l-amphetamine-induced place preference conditioning in rats. European Journal of Pharmacology, 95, 311-314.

Gill, C. A., Holz, W. C., Zirkle, C. L., & Hill, H. (1978). Pharmacological modification of cocaine and apomorphine self-administration in the squirrel monkey. Neuropsychopharmacology: Vol. 1. Proceedings of the Tenth Congress of the Collegium Internationale Neuro-Psychopharmacologicum (pp. 1477-1484). Oxford, Pergamon Press.

Glimcher, P. W., Giovino, A. A., Margolin, D. H., & Hoebel, B. G. (1984). Endogenous opiate reward induced by an enkephalinase inhibitor, thiorphan, injected into the ventral midbrain. Behavioral Neuroscience, 98, 262-268.

Goeders, N. E. (1988). Intracranial cocaine self-administration. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 199-216). Washington, DC: U.S. Government Printing Office.

Goeders, N. E., Lane, J. D., & Smith, J. E. (1984). Self-administration of methionine enkephalin in the nucleus accumbens. Pharmacology Biochemistry & Behavior, 20, 451-455.

Goeders, N. E., & Smith, J. E. (1983). Cortical dopaminergic involvement in cocaine reinforcement. Science, 221, 773-775.

Goeders, N. E., & Smith, J. E. (1984). Effects of 6-hydroxydopamine lesions of the medial prefrontal cortex on intracranial cocaine self-administration. Society for Neuroscience Abstracts, 10, 1206.

Goldberg, S. R. (1973). Comparable behavior maintained under fixed-ratio and second-order schedules of food presentation, cocaine injection or d-amphetamine injection in the squirrel monkey. The Journal of Pharmacology and Experimental Therapeutics, 186, 18-30.

Goldberg, S. R., & Kelleher, R. T. (1976). Behavior controlled by scheduled injections of cocaine in squirrel and rhesus monkeys. Journal of the Experimental Analysis of Behavior, 25, 93-104.

Goldberg, S. R., Spealman, R. D., & Kelleher, R. T. (1979). Enhancement of drug-seeking behavior by environmental stimuli associated with cocaine or morphine injections. Neuropharmacology, 18, 1015-1017.

Goodman, R. R., Snyder, S. H., Kuhar, M. J., & Young, S. (1980). Differentiation of delta and mu opiate receptor localizations by light microscopic autoradiography. Proceedings of the National Academy of Sciences of the United States of America, 77, 6239-6243.

Gordon, D. (1979). Effects of forebrain ablation on taste aversion in goldfish (*Carassius auratus*). Experimental Neurology, 63, 356-366.

Grace, A. A. (1988). *In vivo* and *in vitro* intracellular recordings from rat midbrain dopamine neurons. Annals of the New York Academy of Sciences, 537, 51-76.

Gratton, A., & Wise, R. A. (1988). Comparisons of refractory periods for medial forebrain bundle fibers subserving stimulation-induced feeding and brain stimulation reward: A psychophysical study. Brain Research, 438, 256-263.

Griffiths, R. R. & Balster, R.L. (1979). Opioids: Similarity between evaluation of subjective effects and animal self-administration results. Clinical Pharmacology and Therapeutics, 25, 611-617.

Griffiths, R. R., Bigelow, G. E., & Henningfield, J. E. (1980). Similarities in animal and human drug-taking behavior. In N. K. Mello (Ed.), Substance abuse: Vol. 1 (pp. 1-90). Greenwich, CT: JAI Press.

Griffiths, R. R., Bradford, L. D., & Brady, J. V. (1979). Progressive ratio and fixed ratio schedules of cocaine-maintained responding in baboons. Psychopharmacology, 65, 125-136.

Griffiths, R. R., Winger, G., Brady, J. V., & Snell, J. D. (1976). Comparison of behavior maintained by infusions of eight phenylethylamines in baboons. Psychopharmacology, 50, 251-258.

Groves, P. M., & Tepper, J. M. (1983). Neuronal mechanisms of action of amphetamine. In I. Creese (Ed.), Stimulants: Neurochemical, behavioral, and clinical perspectives (pp. 81-129). New York: Raven Press.

Guerin, G. F., Goeders, N. E., Dworkin, S. I., & Smith, J. E. (1984). Intracranial self-administration of dopamine into the nucleus accumbens. Society for Neuroscience Abstracts, 10, 1072.

Gunne, L.-M., Änggård, W., & Jönsson, L. E. (1972). Clinical trials with amphetamine-blocking drugs. Psychiatria, Neurologia, Neurochirurgia, 75, 225-226.

Hall, F. S., Stellar, J. R., Rice, M., Meyers, C., & Coffey, S. (1988). Amphetamine, apomorphine, and cholecystokinin modulation of dopamine systems and lateral hypothalamic reward. Society for Neuroscience Abstracts, 14, 1103.

Hand, T. H., & Franklin, K. B. J. (1983). The influence of amphetamine on preference for lateral hypothalamic versus prefrontal cortex or ventral tegmental area self-stimulation. Pharmacology Biochemistry & Behavior, 18, 695-699.

Hand, T. H., & Franklin, K. B. J. (1985). 6-OHDA lesions of the ventral tegmental area block morphine-induced but not amphetamine-induced facilitation of self-stimulation. Brain Research, 328, 233-241.

Hand, T. H., Stinus, L., & Le Moal, M. (1989). Differential mechanisms in the acquisition and expression of heroin-induced place preference. Psychopharmacology, 98, 61-67.

Hanson, H. M., & Cimini-Venema, C. A. (1972). Effects of haloperidol on self-administration of morphine in rats. Federation Proceedings, 31, 503.

Harrigan, S. E., & Downs, D. A. (1978). Self-administration of heroin, acetylmethadol, morphine, and methadone in rhesus monkeys. Life Sciences, 22, 619-624.

Heikkila, R. E., Orlansky, H., & Cohen, G. (1975). Studies on the distinction between uptake inhibition and release of [^{3}H]dopamine in rat brain tissue slices. Biochemical Pharmacology, 24, 847-852.

Henningfield, J. E., Nemeth-Coslett, R., Katz, J. L., & Goldberg, S. R. (1987). Intravenous cocaine self-administration by human volunteers: Second-order schedules of reinforcement. In L. S. Harris (Ed.), Problems of drug dependence, 1986 (National Institute on Drug Abuse Research Monograph 76) (pp. 266-273). Washington, DC: US Government Printing Office.

Hernandez, L., Guzman, N. A., & Hoebel, B. G. (1991). Bidirectional microdialysis in vivo shows differential dopaminergic potency of cocaine, procaine and lidocaine in the nucleus accumbens using capillary electrophoresis for calibration of drug outward diffusion. Psychopharmacology, 105, 264-268.

Hernandez, L., & Hoebel, B. G. (1988a). Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiology & Behavior, 44, 599-606.

Hernandez, L., & Hoebel, B. G. (1988b). Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. Life Sciences, 42, 1705-1712.

Hernandez, L., Lee, F., & Hoebel, B. G. (1988). Microdialysis in the nucleus accumbens during feeding or drugs of abuse: Amphetamine, cocaine, and phencyclidine. Annals of the New York Academy of Sciences, 537, 508-511.

Hienz, R. D., Turkkan, J. S., Spear, D. J., Sannerud, C. A., Kaminski, B. J., & Allen, R. P. (1992). General activity in baboons measured with a computerized, lightweight piezoelectric motion sensor: Effects of drugs. Pharmacology Biochemistry & Behavior, 42, 497-507.

Hoebel, B. G., Hernandez, L., McLean, S., Stanley, B. G., Aulissi, E. F., Glimcher, P., & Margolin, D. (1982). Catecholamines, enkephalin and neurotensin in feeding and reward. In B. G. Hoebel & D. Novin (Eds.), The neural basis of feeding and reward (pp. 465-478). Brunswick ME: Haer Institute.

Hoffman, D. C. (1989). The use of place conditioning in studying the neuropharmacology of drug reinforcement. Brain Research Bulletin, 23, 373-387.

Hoffman, D. C., & Beninger, R. J. (1988). Selective D1 and D2 dopamine agonists produce opposing effects in place conditioning but not in conditioned taste aversion learning. Pharmacology Biochemistry & Behavior, 31, 1-8.

Hoffman, D. C., & Beninger, R. J. (1989). The effects of selective dopamine D1 or D2 receptor antagonists on the establishment of agonist-induced place conditioning in rats. Pharmacology Biochemistry & Behavior, 33, 273-279.

Hoffmeister, F., & Goldberg, S. R. (1973). A comparison of chlorpromazine, imipramine, morphine, and d-amphetamine self-administration in cocaine-dependent rhesus monkeys. The Journal of Pharmacology and Experimental Therapeutics, 187, 8-14.

Holmes, J. C., & Rutledge, C. O. (1976). Effects of the d- and l-isomers of amphetamine on uptake, release and catabolism of norepinephrine, dopamine and 5-hydroxytryptamine in several regions of rat brain. Biochemical Pharmacology, 25, 447-451.

Hornby, P. J., Piekut, D. T., & Demski, L. S. (1987). Localization of immunoreactive tyrosine hydroxylase in the goldfish brain. Journal of Comparative Neurology, 261, 1-14.

Horvitz, J. C., & Ettenberg, A. (1989). Haloperidol blocks the response-reinstating effects of food reward: A methodology for separating neuroleptic effects on reinforcement and motor processes. Pharmacology Biochemistry & Behavior, 31, 861-865.

Houdi, A. A., Bardo, M. T., & Van Loon, G. R. (1989). Opioid mediation of cocaine-induced hyperactivity and reinforcement. Brain Research, 497, 195-198.

Hu, X. T., & Wang, R. Y. (1984). Comparison of morphine-induced effects on dopamine and non-dopamine neurons in the rat ventral-tegmental area. Society for Neuroscience Abstracts, 10, 66.

Hubner, C. B., & Moreton, J. E. (1991). Effects of selective D1 and D2 dopamine antagonists on cocaine self-administration in the rat. Psychopharmacology, 105, 151-156.

Hunt, G. E., & Atrens, D. M. (1992). Reward summation and the effects of pimozide, clonidine, and amphetamine on fixed-interval responding for brain stimulation. Pharmacology Biochemistry & Behavior, 42, 563-577.

Idemudia, S. O., & McMillan, D. E. (1984). Effects of d-amphetamine on spontaneous motor activity in pigeons. Psychopharmacology, 84, 315-317.

Isaac, W., Neisewander, J. L., Landers, T., Alcala, R., Bardo, M., & Nonneman, A. (1984). Mesocortical dopamine system lesions disrupt cocaine reinforced conditioned place preference. Society for Neuroscience Abstracts. 10, 1206.

Iversen, S. D. (1983). Brain endorphins and reward function: Some thoughts and speculations. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 439-468). Amsterdam: Elsevier Biomedical Press.

Jasinski, D. R., Johnson, R. E., & Henningfield, J. E. (1984). Abuse liability assessment in human subjects. Trends in Pharmacological Sciences, 5, 1-5.

Jenck, F., Gratton, A., & Wise, R. A. (1987). Opioid receptor subtypes associated with ventral tegmental facilitation of lateral hypothalamic brain stimulation reward. Brain Research, 423, 34-38.

Johanson, C. E. (1976). Pharmacological and environmental variables affecting drug preference in rhesus monkeys. Pharmacological Reviews, 27, 343-355.

Johnson, R. P., Sar, M., & Stumpf, W. E. (1980). A topographic localization of enkephalin on the dopamine neurons of the rat substantia nigra and ventral tegmental area demonstrated by combined histofluorescence-immunocytochemistry. Brain Research, 194, 566-571.

Jones, B. E., & Prada, J. A. (1973). Relapse to morphine use in dog. Psychopharmacologia (Berlin), 30, 1-12.

Jones, B. E., & Prada, J. A. (1981). Characteristics of chronic self-administration of morphine by dogs. Psychopharmacology, 74, 204-207.

Jones, R. T. (1984). The pharmacology of cocaine. In J. Grabowski (Ed.), Cocaine: Pharmacology, effects, and treatment of abuse (National Institute on Drug Abuse Research Monograph 50) (pp. 34-53). Washington, DC: US Government Printing Office.

Jönsson, L-E. (1972). Pharmacological blockade of amphetamine effects in amphetamine dependent subjects. European Journal of Clinical Pharmacology, 4, 206-211.

Jönsson, L-E., Änggård, E., & Gunne, L-M. (1971). Blockade of intravenous amphetamine euphoria in man. Clinical Pharmacology and Therapeutics, 12, 889-896.

Juorio, A. V. (1973). The distribution of catecholamines in the hypothalamus and other brain areas of some lower vertebrates. Journal of Neurochemistry, 20, 641-645.

Kah, O., Chambolle, P., Thibault, J., & Geffard, M. (1984). Existence of dopaminergic neurons in the preoptic region of the goldfish. Neuroscience Letters, 48, 293-298.

Kalivas, P. W. (1985). Interactions between neuropeptides and dopamine neurons in the ventromedial mesencephalon. Neuroscience & Biobehavioral Reviews, 9, 573-587.

Kalivas, P. W., & Bronson, M. (1985). Mesolimbic dopamine lesions produce an augmented behavioral response to enkephalin. Neuropharmacology, 24, 931-936.

Kalivas, P. W., Widerlöv, E., Stanley, D., Breese, G., & Prange, A. J., Jr. (1983). Enkephalin action on the mesolimbic system: A dopamine-dependent and a dopamine-independent increase in locomotor activity. The Journal of Pharmacology and Experimental Therapeutics, 227, 229-237.

Katz, J. L. (1979). A comparison of responding maintained under second-order schedules of intramuscular cocaine injection or food presentation in squirrel monkeys. Journal of The Experimental Analysis of Behavior, 32, 419-431.

Katz, J. L. (1989). Drugs as reinforcers: Pharmacological and behavioural factors. In J. M. Leibman & S. J. Cooper (Eds.), The neuropharmacological basis of reward (pp. 164-213). Oxford: Clarendon Press.

Katz, R. J., & Gormezano, G. (1979). A rapid and inexpensive technique for assessing the reinforcing effects of opiate drugs. Pharmacology Biochemistry & Behavior, 11, 231-233.

Kavaliers, M. (1981). Schooling behavior of fish: An opiate dependent activity? Behavioral and Neural Biology, 33, 397-401.

Kavaliers, M. (1988). Evolutionary and comparative aspects of nociception. Brain Research Bulletin, 21, 923-931.

Kavaliers, M., & Hirst, M. (1986). Environmental specificity of tolerance to morphine-induced analgesia in a terrestrial snail: Generalization of the behavioral model of tolerance. Pharmacology Biochemistry & Behavior, 25, 1201-1206.

Kavaliers, M., Hirst, M., & Teskey, G. C. (1985). Opioid systems and feeding in the slug, *Limax maximus*: Similarities to and implications for mammalian feeding. Brain Research Bulletin, 14, 681-685.

Kavaliers, M., Rangeley, R. W., Hirst, M., & Teskey, G. C. (1986). Mu- and kappa-opiate agonists modulate ingestive behaviors in the slug, *Limax maximus*. Pharmacology Biochemistry & Behavior, 24, 561-566.

Kilbey, M. M., & Ellinwood, E. H., Jr. (1980). Self-administration of morphine in the cat. The International Journal of the Addictions, 15, 447-460.

Koob, G. F., & Hubner, C. B. (1988). Reinforcement pathways for cocaine. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 137-159). Washington, DC: US Government Printing Office.

Koob, G. F., Pettit, H. O., Ettenberg, A., & Bloom, F. E. (1984). Effects of opiate antagonists and their quaternary derivatives on heroin self-administration in the rat. The Journal of Pharmacology and Experimental Therapeutics, 229, 481-486.

Koob, G. F., Vaccarino, F., Amalric, M., & Bloom, F. E. (1987). Positive reinforcement properties of drugs: Search for neural substrates. In J. Engel & L. Oreland (Eds.), Brain reward systems and abuse (pp. 35-50). New York: Raven Press.

Kornetsky, C., & Bain, G. (1983). Effects of opiates on rewarding brain stimulation. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 237-256). Amsterdam: Elsevier Biomedical Press.

Kruszewska, A., Romandini, S., & Samanin, R. (1986). Different effects of zimelidine on the reinforcing properties of d-amphetamine and morphine on conditioned place preference in rats. European Journal of Pharmacology, 125, 283-286.

Kuczenski, R. (1983). Biochemical actions of amphetamine and other stimulants. In I. Creese (Ed.), Stimulants: Neurochemical, behavioral, and clinical perspectives (pp. 31-61). New York: Raven Press.

Kuhar, M. J., Ritz, M.C., & Sharkey, J. (1988). Cocaine receptors on dopamine transporters mediate cocaine-reinforced behavior. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 14-22). Washington, DC: U.S. Government Printing Office.

Kumar, R. (1972). Morphine dependence in rats: Secondary reinforcement from environmental stimuli. Psychopharmacologia (Berlin), 25, 332-338.

Kurumiya, S., & Nakajima, S. (1988). Dopamine D_1 receptors in the nucleus accumbens: Involvement in the reinforcing effect of tegmental stimulation. Brain Research, 448, 1-6.

Kuzmin, A., Zvartau, E., Gessa, G. L., Martellotta, M. C., & Fratta, W. (1992). Calcium antagonists isradipine and nimodipine suppress cocaine and morphine intravenous self-administration in drug-naive mice. Pharmacology Biochemistry & Behavior, 41, 497-500.

Laming, P. R. (1981). An introduction to the functional anatomy of the brains of fish and amphibians (with comments on the reptiles). In P. R. Laming (Ed.), Brain mechanisms of behaviour in lower vertebrates (pp. 7-32). Cambridge: Cambridge University Press.

Leith, N. J. (1983). Effects of apomorphine on self-stimulation responding: Does the drug mimic the current? Brain Research, 277, 129-136.

Le Moal, M., Stinus, L., & Hand, T. H. (1988). Different mechanisms underlie the acquisition and expression of heroin-induced place preference. Society for Neuroscience Abstracts, 14, 661.

Leone, P., & Di Chiara, G. (1987). Blockade of D-1 receptors by SCH 23390 antagonizes morphine- and amphetamine-induced place preference conditioning. European Journal of Pharmacology, 135, 251-254.

Leone, P., Pocock, D., & Wise, R. A. (1991). Morphine-dopamine interaction: Ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacology Biochemistry & Behavior, 39, 469-472.

Lepore, M., & Franklin, K. B. J. (1992). Modelling drug kinetics with brain stimulation: Dopamine antagonists increase self-stimulation. Pharmacology Biochemistry & Behavior, 41, 489-496.

Lett, B. T. (1988). Enhancement of conditioned preference for a place paired with amphetamine produced by blocking the association between place and amphetamine-induced sickness. Psychopharmacology, 95, 390-394.

Lett, B. T., & Grant, V. L. (1988). The hedonic effects of amphetamine and pentobarbital in goldfish. Pharmacology Biochemistry & Behavior, 32, 355-356.

Leysen, J. E., Niemegeers, C. J. E., Tollenaere, J. P., & Laduron, P. M. (1978). Serotonergic component of neuroleptic receptors. Nature, 272, 168-171.

Lilly, J. C., & Miller, A. M. (1962). Operant conditioning of the bottlenose dolphin with electrical stimulation of the brain. Journal of Comparative and Physiological Psychology, 55, 73-79.

Lindenblatt, U., & Delius, J. D. (1988). Nucleus vasalis prosencephali, a substrate of apomorphine-induced pecking in pigeons. Brain Research, 453, 1-8.

Loh, H. H., & Smith, A. P. (1990). Molecular characterization of opioid receptors. Annual Review of Pharmacology and Toxicology, 30, 123-147.

Lukas, S. E., Brady, J. V., & Griffiths, R. R. (1986). Comparison of opioid self-injection and disruption of schedule-controlled performance in the baboon. The Journal of Pharmacology and Experimental Therapeutics, 238, 924-931.

Lynch, M. R., & Wise, R. A. (1985). Relative effectiveness of pimozide, haloperidol and trifluoperazine on self-stimulation rate-intensity functions. Pharmacology Biochemistry & Behavior, 23, 777-780.

Lyness, W. H., Friedle, N. M., & Moore, K. E. (1979). Destruction of dopaminergic nerve terminals in nucleus accumbens: Effect on d-amphetamine self-administration. Pharmacology Biochemistry & Behavior, 11, 553-556.

Mackey, W. B., & van der Kooy, D. (1985). Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. Pharmacology Biochemistry & Behavior, 22, 101-105.

MacPhail, E. M. (1967). Positive and negative reinforcement from intracranial stimulation in pigeons. Nature, 213, 947-948.

MacPhail, E. M. (1982). Brain and intelligence in vertebrates. Oxford: Oxford University Press.

Mangel, S. C., & Dowling, J. E. (1985). Responsiveness and receptive field size of carp horizontal cells are reduced by prolonged darkness and dopamine. Science, 229, 1107-1109.

Marcais, H., Bonnet, J. J., & Constentin, J. (1981). Evidence for sedative effects of low doses of morphine in mice involving receptors sensitive to naloxone. Life Sciences, 28, 2737-2742.

Maroli, A. N., Tsang, W.-K., & Stutz, R. M. (1978). Morphine and self-stimulation: Evidence for action on a common neural substrate. Pharmacology Biochemistry & Behavior, 8, 119-123.

Matthews, R. T., & German, D. C. (1984). Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience, 11, 617-625.

Maurer, R. (1982). Multiplicity of opiate receptors in different species. Neuroscience Letters, 30, 303-307.

Mazurski, E. J., & Beninger, R. J. (1988). Stimulant effects of apomorphine and (+)-amphetamine in rats with varied habituation to test environment. Pharmacology Biochemistry & Behavior, 29, 249-255.

McIntyre, D. C., Healy, L. M., & Saari, M. (1979). Intraspecies aggression and monoamine levels in rainbow trout (*Salmo gairdneri*) fingerlings. Behavioral and Neural Biology, 25, 90-98.

McMillen, B. A. (1983). CNS stimulants: Two distinct mechanisms of action for amphetamine-like drugs. Trends in Pharmacological Sciences, 4, 429-432.

McMillen, B. A., & Shore, P. A. (1979). Species-dependent responsiveness to nonamphetamine central stimulants: Relationship between uptake and release of brain dopamine. Pharmacology, 19, 261-266.

Mello, N. K., & Mendelson, J. H. (1978). Self-administration of an enkephalin analog by rhesus monkey. Pharmacology Biochemistry & Behavior, 9, 579-586.

Mello N. K., & Mendelson, J. H. (1987). Operant analysis of human drug self-administration: Marijuana, alcohol, heroin, and polydrug use. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 525-558). New York: Springer-Verlag.

Meredith, G. E., & Smeets, W. J. A. J. (1987). Immunocytochemical analysis of the dopamine system in the forebrain and midbrain of *Raja radiata*: Evidence for a substantia nigra and ventral tegmental area in cartilaginous fish. Journal of Comparative Neurology, 265, 530-548.

Miller, R. L., & Baum, M. J. (1987). Naloxone inhibits mating and conditioned place preference for an estrous female in male rats soon after castration. Pharmacology Biochemistry & Behavior, 26, 781-789.

Mithani, S., Martin-Iverson, M. T., Phillips, A. G., & Fibiger, H. C. (1986). The effects of haloperidol on amphetamine-induced place preferences and locomotor activity. Psychopharmacology, 90, 247-252.

Møller-Nielson, I., Pedersen, V., Nymark, M., Franck, K. F., Boeck, V., Fjalland, B., & Christensen, A. V. (1973). The comparative pharmacology of flupenthixol and some reference neuroleptics. Acta Pharmacologia et Toxicologia, 33, 353-362.

Monaco, A. P., Hernandez, L., & Hoebel, B. G. (1981). Nucleus accumbens: Site of amphetamine self-injection: Comparison with the lateral ventricle. In R. B. Chronister & J. F. DeFrance (Eds.), The neurobiology of the nucleus accumbens (pp. 338-342). Brunswick, ME: Haer Institute.

Moon Edley, S., Hall, L., Herkenham, M., & Pert, C. B. (1982). Evolution of striatal opiate receptors. Brain Research, 249, 184-188.

Moore, K. E. (1978). Amphetamines: Biochemical and behavioral actions in animals. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 11, pp. 41-98). New York: Plenum Press.

Moore, K. E., Chiueh, C. C., & Zeldes, G. (1977). Release of neurotransmitters from the brain in vivo by amphetamine, methylphenidate and cocaine. In E. H. Ellinwood, Jr. & M. M. Kilbey (Eds.), Cocaine and other stimulants (pp. 143-160). New York: Plenum Press.

Morency, M. A., & Beninger, R. J. (1986). Dopaminergic substrates of cocaine-induced place conditioning. Brain Research, 399, 33-41.

Morita, Y., & Finger, T. E. (1987). Area postrema of the goldfish, *Carassius auratus*: Ultrastructure, fiber connections, and immunocytochemistry. Journal of Comparative Neurology, 256, 104-116.

Motles, E., Martinez, I., Concha, E., Mejias, B., & Torres, P. (1989). Comparative study of the behavioral changes evoked by d-amphetamine and apomorphine in adult cats. Dose-response relationship. Pharmacology Biochemistry & Behavior, 33, 115-121.

Mucha, R. F., & Herz, A. (1985). Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology, 86, 274-280.

Mucha, R. F., & Herz, A. (1986). Preference conditioning produced by opioid active and inactive isomers of levorphanol and morphine in rat. Life Sciences, 38, 241-249.

Mucha, R. F., & Iversen, S. D. (1984). Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: A procedural examination. Psychopharmacology, 82, 241-247.

Mucha, R. F., van der Kooy, D., O'Shaughnessy, M., & Bucenieks, P. (1982). Drug reinforcement studied by the use of place conditioning in rat. Brain Research, 243, 91-105.

Munro, A. D. (1986). The effects of apomorphine, d-amphetamine and chlorpromazine on the aggressiveness of isolated *Aequidens pulcher* (teleostei, cichlidae). Psychopharmacology, 88, 124-128.

Nakajima, S. (1989). Subtypes of dopamine receptors involved in the mechanism of reinforcement. Neuroscience & Biobehavioral Reviews, 13, 123-128.

Nakajima, S., & Wise, R. A. (1987). Heroin self-administration in the rat suppressed by SCH23390. Society for Neuroscience Abstracts, 13, 1545.

Nazzaro, J. M., Seeger, T. F., & Gardner, E. L. (1981). Morphine differentially affects ventral tegmental and substantia nigra brain reward thresholds. Pharmacology Biochemistry & Behavior, 14, 325-331.

Neumeyer, J. L., Law, S. J., & Lamont, J. S. (1981). Apomorphine and related aporphines as probes of the dopamine receptor. In G. U. Corsini & G. L. Gessa (Eds.), Apomorphine and other dopaminomimetics: Vol. 1. Basic pharmacology (pp. 209-2183). New York: Raven Press.

Niemegeers, C. J. E., & Janssen, P. A. J. (1979). A systematic study of the pharmacological activities of dopamine antagonists. Life Sciences, 24, 2201-2216.

Nomikos, G. G., & Spyraki, C. (1988a). Cocaine-induced place conditioning: Importance of route of administration and other procedural variables. Psychopharmacology, 94, 119-125.

Nomikos, G. G., & Spyraki, C. (1988b). Effects of ritanserin on the rewarding properties of d-amphetamine, morphine and diazepam revealed by conditioned place preference in rats. Pharmacology Biochemistry & Behavior, 30, 853-858.

North, R. A. (1992). Cellular actions of opiates and cocaine. Annals of the New York Academy of Sciences, 654, 1-6.

Northcutt, R. G. (1984). Evolution of the vertebrate central nervous system: Patterns and processes. American Zoologist, 24, 701-716.

Northcutt, R. G., & Braford, M. R., Jr. (1980). New observations on the organization and evolution of the telencephalon of actinopterygian fishes. In S. O. E. Ebbesson (Ed.), Comparative neurology of the telencephalon (pp. 41-98). New York: Plenum Press.

Offermeier, J., & van Rooyen, J. M. (1986). A comparative study of the locomotor activity effects of apomorphine and the "atypical dopamine agonists" (piribedil and S3608). Life Sciences, 38, 895-903.

Olds, J. (1956). Pleasure centers in the brain. Scientific American, 195, 105-116.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of Comparative and Physiological Psychology, 47, 419-427.

Olds, M. E. (1982). Reinforcing effects of morphine in the nucleus accumbens. Brain Research, 237, 428-440.

Olds, M. E., & Williams, K. N. (1980). Self-administration of D-Ala^2-met-enkephalinamide at hypothalamic self-stimulation sites. Brain Research, 194, 155-170.

Olson, R. D., Kastin, A. J., Montalbano-Smith, D., Olson, G. A., Coy, D. H., & Michell, G. F. (1978). Neuropeptides and blood-brain barrier in goldfish. Pharmacology Biochemistry & Behavior, 9, 521-524.

Ostrowski, N. L., Hatfield, C. B., & Caggiula, A. R. (1982). The effects of low doses of morphine on the activity of dopamine-containing cells and on behavior. Life Sciences, 31, 2347-2350.

Papp, M. (1988). Different effects of short- and long-term treatment with imipramine on the apomorphine- and food-induced place preference conditioning in rats. Pharmacology Biochemistry & Behavior, 30, 889-893.

Parada, M., Hernandez, L., Schwartz, D., & Hoebel, B. G. (1988). Hypothalamic infusion of amphetamine increases serotonin, dopamine and norepinephrine. Physiology & Behavior, 44, 607-610.

Parent, A. (1986). Comparative neurobiology of the basal ganglia. Toronto: John Wiley and Sons.

Parent, A., Poitras, D., & Dubé, L. (1984). Comparative anatomy of central monoaminergic systems. In A. Björklund & T. Hökfelt (Eds.), Handbook of chemical neuroanatomy: Vol. 2. Classical transmitters in the CNS, Part 1 (pp. 409-439). Amsterdam: Elsevier.

Parker, L. A. (1992). Place conditioning in a three- or four-choice apparatus: Role of stimulus novelty in drug-induced place conditioning. Behavioral Neuroscience, 106, 294-306.

Peroutka, S. J., & Snyder, S. H. (1980). Relationship of neuroleptic drug effects at brain dopamine, serotonin, α-adrenergic, and histamine receptors to clinical potency. American Journal of Psychiatry, 137, 1518-1522.

Pert, C. B., Aposhian, D., & Snyder, S. H. (1974). Phylogenetic distribution of opiate receptor binding. Brain Research, 75, 356-361.

Pettit, H. O., Ettenberg, A., Bloom, F. E., & Koob, G. F. (1984). Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology, 84, 167-173.

Phillips, A. G., Blaha, C. D., & Fibiger, H. C. (1989). Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. Neuroscience & Biobehavioral Reviews, 13, 99-104.

Phillips, A. G., Broekkamp, C. L., & Fibiger, H. C. (1983). Strategies for studying the neurochemical substrates of drug reinforcement in rodents. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 7, 585-590.

Phillips, A. G., & Fibiger, H. C. (1973). Dopaminergic and noradrenergic substrates of positive reinforcement: Differential effects of d- and l-amphetamine. Science, 179, 575-576.

Phillips, A. G., & Fibiger, H. C. (1987). Anatomical and neurochemical substrates of drug reward determined by the conditioned place preference technique. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 275-290). New York: Springer Verlag.

Phillips, A. G., & Fibiger, H. C. (1989). Neuroanatomical bases of intracranial self-stimulation: Untangling the Gordian knot. In J. M. Leibman & S. J. Cooper (Eds.), The neuropharmacological basis of reward (pp. 66-105). Oxford: Clarendon Press.

Phillips, A. G., Jakubovic, A., & Fibiger, H. C. (1987). Increased in vivo tyrosine hydroxylase activity in rat telencephalon produced by self-stimulation of the ventral tegmental area. Brain Research, 402, 109-116.

Phillips, A. G., & LePiane, F. G. (1980). Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacology Biochemistry & Behavior, 12, 965-968.

Phillips, A. G., & LePiane, F. G. (1982). Reward produced by microinjection of (D-Ala^2),Met^5-enkephalinamide into the ventral tegmental area. Behavioural Brain Research, 5, 225-229.

Phillips, A. G., LePiane, F. G., & Fibiger, H. C. (1983). Dopaminergic mediation of reward produced by direct injection of enkephalin into the ventral tegmental area of the rat. Life Sciences, 33, 2505-2511.

Phillips, A. G., Spyraki, C., & Fibiger, H. C. (1982). Conditioned place preference with amphetamine and opiates as reward stimuli: Attenuation by haloperidol. In B. G. Hoebel & D. Novin (Eds.), The neural basis of feeding and reward (pp. 455-464). Brunswick, ME: Haer Institute.

Pickens, R., Meisch, R. A., & Dougherty, J. A., Jr. (1968). Chemical interactions in methamphetamine reinforcement. Psychological Reports, 23, 1267-1270.

Pough, F. H., Heiser, J. B., & McFarland, W. N. (1989). Vertebrate life (3rd Ed.). New York: Macmillan.

Pozuelo, J., & Kerr, F. W. L. (1972). Suppression of craving and other signs of dependence in morphine-addicted monkeys by administration of alpha-methyl-para-tyrosine. Mayo Clinic Proceedings, 47, 621-628.

Reicher, M. A., & Holman, E. W. (1977). Location preference and flavor aversion reinforced by amphetamine in rats. Animal Learning and Behavior, 5, 343-346.

Reid, L. D. (1987). Tests involving pressing for intracranial stimulation as an early procedure for screening likelihood of addiction of opioids and other drugs. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 391-420). New York: Springer Verlag.

Reid, L. D., Marglin, S. H., Mattie, M. E., & Hubbell, C. L. (1989). Measuring morphine's capacity to establish a place preference. Pharmacology Biochemistry & Behavior, 33, 765-775.

Revusky, S., & Gorry, T. (1973). Flavor aversions produced by contingent drug injection: Relative effectiveness of apomorphine, emetine, and lithium. Behaviour Research and Therapy, 11, 403-409.

Riffee, W. H., & Wilcox, R. E. (1985). Effects of multiple pretreatment with apomorphine and amphetamine on amphetamine-induced locomotor activity and its inhibition by apomorphine. Psychopharmacology, 85, 97-101.

Risner, M. E., & Goldberg, S. R. (1983). A comparison of nicotine and cocaine self-administration in the dog: Fixed-ratio and progressive-ratio schedules of intravenous drug infusion. The Journal of Pharmacology and Experimental Therapeutics, 224, 319-326.

Risner, M. E., & Jones, B. E. (1976). Role of noradrenergic and dopaminergic processes in amphetamine self-administration. Pharmacology Biochemistry & Behavior, 5, 477-482.

Risner, M. E., & Jones, B. E. (1980). Intravenous self-administration of cocaine and norcocaine by dogs. Psychopharmacology, 71, 83-89.

Risner, M. E., & Silcox, D. L. (1981). Psychostimulant self-administration by beagle dogs in a progressive-ratio paradigm. Psychopharmacology, 75, 25-30.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science, 237, 1219-1223.

Roberts, D. C. S., Corcoran, M. E., & Fibiger, H. C. (1977). On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacology Biochemistry & Behavior, 6, 615-620.

Roberts, D. C. S., & Koob, G. F. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacology Biochemistry & Behavior, 17, 901-904.

Roberts, D. C. S., Koob, G. F., Klonoff, P., & Fibiger, H. C. (1980). Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacology Biochemistry & Behavior, 12, 781-787.

Roberts, D. C. S., & Vickers, G. (1988). Increased motivation to self-administer apomorphine following 6-hydroxydopamine lesions of the nucleus accumbens. Annals of the New York Academy of Sciences, 537, 523-524.

Roberts, W. W. (1958). Both rewarding and punishing effects from stimulation of posterior hypothalamus of cat with same electrode at same intensity. Journal of Comparative and Physiological Psychology, 51, 400-407.

Robledo, P., Maldonado-Lopez, R., & Koob, G. F. (1992). Role of dopamine receptors in the nucleus accumbens in the rewarding properties of cocaine. Annals of the New York Academy of Sciences, 654, 509-512.

Robson, L. E., Paterson, S. J., & Kosterlitz, H. W. (1983). Opiate receptors. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of Psychopharmacology (Vol. 17, pp. 13-80). New York: Plenum Press.

Rompré, P.-P., & Wise, R. A. (1989a). Behavioral evidence for midbrain dopamine depolarization inactivation. Brain Research, 477, 152-156.

Rompré, P.-P., & Wise, R. A. (1989b). Opioid-neuroleptic interaction in brainstem self-stimulation. Brain Research, 477, 144-151.

Rosenblum, P. M., & Peter, R. E. (1989). Evidence for the involvement of endogenous opioids in the regulation of gonadotropin secretion in male goldfish, *Carassius auratus*. General and Comparative Endocrinology, 73, 21-27.

Rosenthal, R. (1984). Applied social research methods series: Vol. 6. Meta-analytic procedures for social research. Beverly Hills, CA: Sage.

Rossi, N. A., & Reid, L. D. (1976). Affective states associated with morphine injections. Physiological Psychology, 4, 269-274.

Roth, R. H. (1983). Neuroleptics: Functional neurochemistry. In J. T. Coyle & S. J. Enna (Eds.), Neuroleptics: Neurochemical, behavioral and clinical perspectives (pp. 119-155). New York: Raven Press.

Salamone, J.D. (1987). The actions of neuroleptic drugs on appetitive instrumental behaviors. In L. L. Iversen, S. D. Iversen, S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 19, pp. 575-608). New York: Plenum Press.

Salamone, J. D. (1988). Dopaminergic involvement in activational aspects of motivation: Effects of haloperidol on schedule-induced activity, feeding, and foraging in rats. Psychobiology, 16, 196-206.

Sanberg, P. R. (1983). Dopaminergic and cholinergic influences on motor behavior in chickens. Journal of Comparative Psychology, 97, 59-68.

Sanberg, P. R., Henault, M. A., Hagenmeyer-Houser, S. H., & Russell, K. H. (1987). The topography of amphetamine and scopolamine-induced hyperactivity: Toward an activity print. Behavioral Neuroscience, 101, 131-133.

Satake, N. (1979). Effect of melatonin and methionine-enkephalin on surfacing responses in goldfish. Physiology & Behavior, 23, 995-999.

Savage, G. E. (1971). Behavioural effects of electrical stimulation of the telencephalon of the goldfish, *Carassius auratus*. Animal Behaviour, 19, 661-668.

Savage, G. E., & Roberts, M. G. (1975). Behavioural effects of electrical stimulation of the hypothalamus of the goldfish (*Carassius auratus*). Brain Behavior & Evolution, 12, 42-56.

Schaeffer, B. (1969). Adaptive radiation of the fishes and the fish-amphibian transition. Annals of the New York Academy of Sciences, 167, 5-17.

Scheel-Krüger, J. (1972). Behavioural and biochemical comparison of amphetamine derivatives, cocaine, benztropine and tricyclic anti-depressant drugs. European Journal of Pharmacology, 18, 63-73.

Scheel-Krüger, J., Braestrup, C., Nielson, M., Golembiowska, K., & Mogilnicka, E. (1977). Cocaine: Discussion on the role of dopamine in the biochemical mechanism of action. In E. M. Ellinwood & M. M. Kilbey (Eds.), Cocaine and other stimulants (pp. 373-408). New York: Plenum Press.

Schenk, S., Hunt, T., Colle, L., & Amit, Z. (1983). Isolation versus grouped housing in rats: Differential effects of low doses of heroin in the place preference paradigm. Life Sciences, 32, 1129-1134.

Schenk, S., Hunt, T., Malovechko, R., Robertson, A., Klukowski, G., & Amit, Z. (1986). Differential effects of isolation housing on the conditioned place preference produced by cocaine and amphetamine. Pharmacology Biochemistry & Behavior, 24, 1793-1796.

Schnur, P., Bravo, F., & Trujillo, M. (1983). Tolerance and sensitization to the biphasic effects of low doses of morphine in the hamster. Pharmacology Biochemistry & Behavior, 19, 435-439.

Schnur, P., & Martinez, R. A. (1989). Independent effects of morphine and apomorphine on stereotyped gnawing in the hamster. Pharmacology Biochemistry & Behavior, 32, 589-594.

Schnur, P., & Morrell, J. (1990). Morphine conditioned place preference in the hamster. Pharmacology Biochemistry & Behavior, 37, 383-385.

Schroeder, D. M. (1980). The telencephalon of teleosts. In S. O. E. Ebbesson (Ed.), Comparative neurology of the telencephalon (pp. 99-115). New York: Plenum Press.

Schuster, C. R., & Johanson, C. E. (1981). An analysis of drug seeking behavior in animals. Neuroscience and Biobehavioral Reviews, 5, 315-323.

Schwartz, A. S., & Marchok, P. L. (1974). Depression of morphine-seeking behaviour by dopamine inhibition. Nature, 248, 257-258.

Shannon, H. E., Bemis, K. G., & Peters, S. C. (1991). Potency and efficacy of dopamine agonists in mouse strains differing in dopamine cell and receptor number. Pharmacology Biochemistry & Behavior, 40, 103-107.

Shannon, H. E., & Risner, M. E. (1984). Comparison of behavior maintained by intravenous cocaine and d-amphetamine in dogs. The Journal of Pharmacology and Experimental Therapeutics, 229, 422-432.

Sherman, J. E., Pickman, C., Rice, A., Liebeskind, J. C., & Holman, E. W. (1980). Rewarding and aversive effects of morphine: Temporal and pharmacological properties. Pharmacology Biochemistry & Behavior, 13, 501-505.

Sherman, J. E., Roberts, T., Roskam, S. E., & Holman, E. W. (1980). Temporal properties of the rewarding and aversive effects of amphetamine in rats. Pharmacology Biochemistry & Behavior, 13, 597-599.

Shippenberg, T. S., Bals-Kubik, R., & Herz, A. (1987). Motivational properties of opioids: Evidence that an activation of delta-receptors mediates reinforcement processes. Brain Research, 436, 234-239.

Shippenberg, T. S., & Herz, A. (1987). Place preference conditioning reveals the involvement of D_1-dopamine receptors in the motivational properties of μ- and κ-opioid agonists. Brain Research, 436, 169-172.

Shippenberg, T. S., & Herz, A. (1988). Motivational effects of opioids: Influence of D-1 versus D-2 receptor antagonists. European Journal of Pharmacology, 151, 233-242.

Shippenberg, T. S., Herz, A., Spanagel, R., Bals-Kubik, R., & Stein, C. (1992). Conditioning of opioid reinforcement: Neuroanatomical and neurochemical substrates. Annals of the New York Academy of Sciences, 654, 347-356.

Simantov, R., Childers, S. R., & Snyder, S. H. (1978). The opiate receptor binding interactions of ^{3}H-methionine enkephalin, an opioid peptide. European Journal of Pharmacology, 47, 319-331.

Simantov, R., Goodman, R., Aposhian, D., & Snyder, S. H. (1976). Phylogenetic distribution of a morphine-like peptide 'enkephalin'. Brain Research, 111, 204-211.

Simon, E. J., & Hiller, J. M. (1984). Multiple opioid receptors. In J. Hughes, H. O. J. Collier, M. J. Rance, & M. B. Tyers (Eds.), Opioids past, present and future (pp. 33-52). London: Taylor & Francis.

Skirboll, L. R., Grace, A. A., & Bunney, B. S. (1979). Dopamine auto- and postsynaptic receptors: Electrophysiological evidence for differential sensitivity to dopamine agonists. Science, 206, 80-82.

Smith, G. P., & Schneider, L. H. (1988). Relationships between mesolimbic dopamine function and eating behavior. Annals of the New York Academy of Sciences, 537, 254-261.

Smith, J. E., Guerin, G. F., Co, C., Barr, T. S., & Lane, J. D. (1985). Effects of 6-OHDA lesions of the central medial nucleus accumbens on rat intravenous morphine self-administration. Pharmacology Biochemistry & Behavior, 23, 843-849.

Smith, J. E., & Lane, J. D. (1983). Brain neurotransmitter turnover correlated with morphine self-administration. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 361-402). Amsterdam: Elsevier Biomedical Press.

Smith, R. J., & Parker, L. A. (1985). Chin rub CRs are elicited by flavors associated with apomorphine, scopolamine, methscopolamine, physostigmine and neostigmine. Pharmacology Biochemistry & Behavior, 23, 583-589.

Smith, R. V., Wilcox, R. E., Soine, W. H., Riffee, W. H., Baldessarini, R. J., & Kula, N. S. (1979). Plasma levels of apomorphine following intravenous, intraperitoneal and oral administration to mice and rats. Research Communications in Chemical Pathology and Pharmacology, 24, 483-499.

Smith, S. G., & Davis, W. M. (1973). Haloperidol effects on morphine self-administration: Testing for pharmacological modification of the primary reinforcement mechanism. The Psychological Record, 23, 215-221.

Snyder, S. H., Pasternak, G. W., & Pert, C. B. (1975). Opiate receptor mechanisms. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 5, pp. 329-360). New York: Plenum Press.

Spanagel, R., Herz, A., Bals-Kubik, R., & Shippenberg, T. S. (1991). ß-endorphin-induced locomotor stimulation and reinforcement are associated with an increase in dopamine release in the nucleus accumbens. Psychopharmacology, 104, 51-56.

Spencer, J., & Revzin, A. (1976). Amphetamine, chlorpromazine and clonidine effects on self-stimulation in caudate or hypothalamus of the squirrel monkey. Pharmacology Biochemistry & Behavior, 5, 149-156.

Spyraki, C., Fibiger, H. C., & Phillips, A. G. (1982a). Attenuation by haloperidol of place preference conditioning using food reinforcement. Psychopharmacology, 77, 379-382.

Spyraki, C., Fibiger, H. C., & Phillips, A. G. (1982b). Cocaine-induced place preference conditioning: Lack of effects of neuroleptics and 6-hydroxydopamine lesions. Brain Research, 253, 195-203.

Spyraki, C., Fibiger, H. C., & Phillips, A. G. (1982c). Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research, 253, 185-193.

Spyraki, C., Fibiger, H. C., & Phillips, A. G. (1983). Attenuation of heroin reward in rats by disruption of the mesolimbic dopamine system. Psychopharmacology, 79, 278-283.

Spyraki, C., Nomikos, G. G., Galanopoulou, P., & Daïfotis, Z. (1988). Drug-induced place preference in rats with 5,7-dihydroxytryptamine lesions of the nucleus accumbens. Behavioural Brain Research, 29, 127-134.

Spyraki, C., Nomikos, G. G., & Varonos, D. D. (1987). Intravenous cocaine-induced place preference: Attenuation by haloperidol. Behavioural Brain Research, 26, 57-62.

St. Laurent, J., Le Clerc, R. R., Mitchell, M. L., & Milliaressis, T. (1973). Effects of apomorphine on self-stimulation. Pharmacology Biochemistry & Behavior, 1, 581-585.

Ståhle, L., & Ungerstedt, U. (1986). Effects of neuroleptic drugs on the inhibition of exploratory behavior induced by a low dose of apomorphine: Implications for the identity of dopamine receptors. Pharmacology Biochemistry & Behavior, 25, 473-480.

Stapleton, J. M., Lind, M. D., Merriman, V. J., Bozarth, M. A., & Reid, L. D. (1979). Affective consequences and subsequent effects on morphine self-administration of d-ala^2-methionine enkephalin. Physiological Psychology, 7, 146-152.

Stefano, G. B., Hall, B., Makman, M. H., & Dvorkin, B. (1981). Opioid inhibition of dopamine release from nervous tissue of *Mytilus edulis* and *Octopus bimaculatus*. Science, 213, 928-930.

Stellar, J. R., Kelley, A. E., & Corbett, D. (1983). Effects of peripheral and central dopamine blockade on lateral hypothalamic self-stimulation: Evidence for both reward and motor deficits. Pharmacology Biochemistry & Behavior, 18, 433-442.

Stellar, J. R., & Rice, M. B. (1989). Pharmacological basis of intracranial self-stimulation reward. In J. M. Leibman & S. J. Cooper (Eds.), The neuropharmacological basis of reward (pp. 14-65). Oxford: Clarendon Press.

Stewart, J. (1984). Reinstatement of heroin and cocaine self-administration behavior in the rat by intracerebral application of morphine in the ventral tegmental area. Pharmacology Biochemistry & Behavior, 20, 917-923.

Stewart, J. (1992). Neurobiology of conditioning to drugs of abuse. Annals of the New York Academy of Sciences, 654, 335-346.

Stewart, J., de Wit, H., & Eikelboom, R. (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychological Review, 91, 251-268.

Stewart, J., & Eikelboom, R. (1987). Conditioned drug effects. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), Handbook of psychopharmacology (Vol. 19, pp. 1-57). New York: Plenum Press.

Stinus, L., Cador, M., & Le Moal, M. (1992). Interaction between endogenous opioids and dopamine within the nucleus accumbens. Annals of the New York Academy of Sciences, 654, 254-273.

Stinus, L., Nadaud, D., Deminière, J., Jauregui, J., Hand, T. H., & Le Moal, M. (1989). Chronic flupenthixol treatment potentiates the reinforcing properties of systemic heroin administration. Biological Psychiatry, 26, 363-371.

Stinus, L., Nadaud, D., Jauregui, J., & Kelley, A. E. (1986). Chronic treatment with five different neuroleptics elicits behavioral supersensitivity to opiate infusion into the nucleus accumbens. Biological Psychiatry, 21, 34-48.

Stinus, L., Winnock, M., & Kelley, A. E. (1985). Chronic neuroleptic treatment and mesolimbic dopamine denervation induce behavioural supersensitivity to opiates. Psychopharmacology, 85, 323-328.

Stolerman, I. P. (1985). Motivational effects of opioids: Evidence on the role of endorphins in mediating reward or aversion. Pharmacology Biochemistry & Behavior, 23, 877-881.

Strecker, R. E., Roberts, D. C. S., & Koob, G. F. (1982). Apomorphine-induced facilitation of intracranial self-stimulation following dopamine denervation of the nucleus accumbens. Pharmacology Biochemistry & Behavior, 17, 1015-1018.

Stretch, R. (1977). Discrete-trial control of cocaine self-injection behaviour in squirrel monkeys: Effects of morphine, naloxone, and chlorpromazine. Canadian Journal of Physiology and Pharmacology, 55, 778-790.

Striedter, G. F., & Northcutt, R. G. (1991). Biological hierarchies and the concept of homology. Brain Behavior & Evolution, 38, 177-189.

Swerdlow, N. R., Gilbert, D., & Koob, G. F. (1989). Conditioned drug effects on spatial preferences: Critical evaluation. In A. A. Boulton, G. B. Baker, & A. J. Greenshaw (Eds.), Neuromethods - Psychopharmacology (Vol. 13, pp. 399-446). Clifton, NJ: Humana.

Swerdlow, N. R., Swanson, L. W., & Koob, G. F. (1984). Electrolytic lesions of the substantia innominata and lateral preoptic area attenuate the 'supersensitive' locomotor response to apomorphine resulting from denervation of the nucleus accumbens. Brain Research, 306, 141-148.

Swerdlow, N. R., Vaccarino, F. J., Amalric, M., & Koob, G. F. (1986). The neural substrates for the motor-activating properties of psychostimulants: A review of recent findings. Pharmacology Biochemistry & Behavior, 25, 233-248.

Tiersch, T. R., & Griffith, J. S. (1988). Apomorphine-induced vomiting in rainbow trout (*Salmo Gairdneri*). Comparative Biochemistry and Physiology, 91A, 721-725.

Titeler, M. (1983). Multiple dopamine receptors: Receptor binding studies in dopamine pharmacology. New York: Marcel Dekker, Inc.

Tombaugh, T. N., Grandmaison, L. J., & Zito, K. A. (1982). Establishment of secondary reinforcement in sign tracking and place preference tests following pimozide treatment. Pharmacology Biochemistry & Behavior, 17, 665-670.

Tortella, F. C., & Moreton, J. E. (1980). D-Ala^2-methionine enkephalinamide self-administration in the morphine-dependent rat. Psychopharmacology, 69, 143-147.

Trujillo, K. A., Belluzzi, J. D., & Stein, L. (1991). Naloxone blockade of amphetamine place preference conditioning. Psychopharmacology, 104, 265-274.

Ukai, M., Toyoshi, T., & Kameyama, T. (1991). Effects of [D-Ala^2,D-Leu^5]enkephalin and [D-Pen^2,L-Pen^5]enkephalin on apomorphine-induced motor activity in the mouse. Pharmacology Biochemistry & Behavior, 41, 171-176.

Ungerstedt, U. (1979). Central dopamine mechanisms and unconditioned behaviour. In A. S. Horn, J. Korf, & B. H. C. Westerink (Eds.), The neurobiology of dopamine (pp. 577-596). London: Academic Press.

Ungerstedt, U., Herrera-Marschitz, M., & Brugue, M. C. (1981). Are apomorphine, bromocriptine, and the methylzanthines agonists at the same dopamine receptor? In G. U. Corsini & G. L. Gessa (Eds.), Apomorphine and other dopaminomimetics: Vol. 1. Basic pharmacology (pp. 85-93). New York: Raven Press.

Vaccarino, F. J., Bloom, F. E., & Koob, G. F. (1985). Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. Psychopharmacology, 86, 37-42.

Vaccarino, F. J., & Corrigall, W. A. (1987). Effects of opiate antagonist treatment into either the periaqueductal grey or nucleus accumbens on heroin-induced locomotor activation. Brain Research Bulletin, 19, 545-549.

Vaccarino, F. J., Schiff, B. B., & Glickman, S. E. (1989). Biological view of reinforcement. In S. B. Klein & R. R. Mowrer (Eds.), Contemporary learning theories: Instrumental conditioning theory and the impact of biological constraints on learning (Vol. 2, pp. 111-142). Hillsdale, NJ: Lawrence Erlbaum Associates.

Vandenbussche, E. (1976). Intracranial self-stimulation behaviour in cats. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 425-427). New York: Elsevier/North Holland.

van der Kooy, D. (1987). Place conditioning: A simple and effective method for assessing the motivational properties of drugs. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 229-240). New York: Springer Verlag.

van der Kooy, D., Mucha, R. F., O'Shaughnessy, M., & Bucenieks, P. (1982). Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. Brain Research, 243, 107-117.

van der Kooy, D., Swerdlow, N. R., & Koob, G. F. (1983). Paradoxical reinforcing properties of apomorphine: Effects of nucleus accumbens and area postrema lesions. Brain Research, 259, 111-118.

Van Wolfswinkel, L., Seifert, W. F., & Van Ree, J. M. (1988). Catecholamines and endogenous opioids in ventral tegmental self-stimulation reward. Pharmacology Biochemistry & Behavior, 30, 589-595.

Van Wolfswinkel, L., & Van Ree, J. M. (1985). Site of rewarding action of morphine in the mesolimbic system determined by intracranial electrical self-stimulation. Brain Research, 358, 349-353.

Vasko, M. R., & Domino, E. F. (1978). Tolerance development to the biphasic effects of morphine on locomotor activity and brain acetylcholine in the rat. The Journal of Pharmacology and Experimental Therapeutics, 207, 848-858.

Vezina, P., & Stewart, J. (1984). Conditioning and place-specific sensitization of increases in activity induced by morphine in the VTA. Pharmacology Biochemistry & Behavior, 20, 925-934.

Vezina, P., & Stewart, J. (1987). Morphine conditioned place preference and locomotion: The effect of confinement during training. Psychopharmacology, 93, 257-260.

Wauquier, A. (1976). The influence of psychoactive drugs on brain self-stimulation in rats: A review. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 123-170). New York: Elsevier/North-Holland.

Wauquier, A., Melis, W., Desmedt, L. K. C., & Sadowski, B. (1976). Self-stimulation in dogs: Behavioural effects of anterior basal forebrain, amygdala and lateral hypothalamus implantations. In A. Wauquier & E. T. Rolls (Eds.), Brain-stimulation reward (pp. 427-430). New York: Elsevier/North Holland.

Wauquier, A., & Niemegeers, C. J. E. (1973a). Intracranial self-stimulation in rats as a function of various stimulus parameters. II. Influence of haloperidol, pimozide and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacologia (Berlin), 27, 191-202.

Wauquier, A., & Niemegeers, C. J. E. (1973b). Intracranial self-stimulation in rats as a function of various stimulus parameters. III. Influence of apomorphine on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacologia (Berlin), 30, 163-172.

Webster, K. E. (1979). Some aspects of the comparative study of the corpus striatum. In I. Divac & R. G. E. Öberg (Eds.), The neostriatum (pp. 107-126). Oxford: Pergamon Press.

Weeks, J. R., & Collins, R. J. (1987). Screening for drug reinforcement using intravenous self-administration in the rat. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 35-43). New York: Springer Verlag.

Weiss, F., Hurd, Y. L., Ungerstedt, U., Markou, A., Plotsky, P. M., & Koob, G. F. (1992). Neurochemical correlates of cocaine and ethanol self-administration. Annals of the New York Academy of Sciences, 654, 220-241.

Wilson, M. C., & Schuster, C. R. (1972). The effects of chlorpromazine on psychomotor stimulant self-administration in the rhesus monkey. Psychopharmacologia (Berlin), 26, 115-126.

Wilson, W. J., & Soltysik, S. S. (1985). Pharmacological manipulations of the nucleus accumbens: Effects on classically conditioned responses and locomotor activity in the cat. Acta Neurobiologiae Experimentalis, 45, 91-105.

Winger, G. (1988). Pharmacological modification of cocaine and opioid self-administration. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 125-136). Washington, DC: U.S. Government Printing Office.

Winn, P., Williams, S. F., & Herberg, L. J. (1982). Feeding stimulated by very low doses of d-amphetamine administered systemically or by microinjection into the striatum. Psychopharmacology, 78, 336-341.

Winsauer, P. J., & Thompson, D. M. (1991). Cocaine self-administration in the pigeon. Pharmacology Biochemistry & Behavior, 40, 41-52.

Wise, R. A. (1978). Catecholamine theories of reward: A critical review. Brain Research, 152, 215-247.

Wise, R. A. (1982a). Common neural basis for brain stimulation reward, drug reward, and food reward. In B. G. Hoebel & D. Novin (Eds.), The neural basis of feeding and reward (pp. 445-454). Brunswick, ME: Haer Institute.

Wise, R. A. (1982b). Neuroleptics and operant behavior: The anhedonia hypothesis. The Behavioral and Brain Sciences, 5, 39-87.

Wise, R. A. (1983). Brain neuronal systems mediating reward processes. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 405-437). Amsterdam: Elsevier Biomedical Press.

Wise, R. A. (1988). Psychomotor stimulant properties of addictive drugs. Annals of the New York Academy of Sciences, 537, 228-234.

Wise, R. A. (1939a). The brain and reward. In J. M. Leibman & S. J. Cooper (Eds.), The neuropharmacological basis of reward (pp. 377-424). Oxford: Clarendon Press

Wise, R. A. (1989b). Opiate reward: Sites and substrates. Neuroscience & Biobehavioral Reviews, 13, 129-133.

Wise, R. A., Bauco, P., Carlezon, W. A., Jr., & Trojniar, W. (1992). Self-stimulation and drug reward mechanisms. Annals of the New York Academy of Sciences, 654, 192-198.

Wise, R. A., & Bozarth, M. A. (1981). Brain substrates for reinforcement and drug self-administration. Progress in Neuro-psychopharmacology and Biological Psychiatry, 5, 467-474.

Wise, R. A., & Bozarth, M. A. (1982). Action of drugs of abuse on brain reward systems: An update with specific attention to opiates. Pharmacology Biochemistry & Behavior, 17, 239-243.

Wise, R. A., & Bozarth, M. A. (1984). Brain reward circuitry: Four circuit elements "wired" in apparent series. Brain Research Bulletin, 12, 203-208.

Wise, R. A., & Bozarth, M. A. (1987). A psychomotor stimulant theory of addiction. Psychological Review, 94, 469-492.

Wise, R. A., Jenck, F., & Raptis, L. (1986). Morphine potentiates feeding via the opiate reinforcement mechanism. In L. S. Harris, (Ed.), Problems of drug dependence, 1985, (National Institute on Drug Abuse Research Monograph 67) (pp. 228-234). Washington, DC: US Government Printing Office.

Wise, R. A., & Rompré, P.-P. (1989). Brain dopamine and reward. Annual Review of Psychology, 40, 191-225.

Wise, R. A., Yokel, R. A., & de Wit, H. (1976). Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. Science, 191, 1273-1275.

Witkin, J. M., Goldberg, S. R., & Katz, J. L. (1989). Lethal effects of cocaine are reduced by the dopamine-1 receptor antagonist SCH 23390 but not by haloperidol. Life Sciences, 44, 1285-1291.

Wood, P. L. (1983). Opioid regulation of CNS dopaminergic pathways: A review of methodology, receptor types, regional variations and species differences. Peptides, 4, 595-601.

Woodard, W. T., & Bitterman, M. E. (1974). Autoshaping in the goldfish. Behavior Research Methods & Instrumentation, 6, 409-410.

Woods, J. H., Herling, S., & Winger, G. (1978). Chlorpromazine- and haloperidol-induced changes in some behavioral effects of cocaine and amphetamine. Neuropsychopharmacology: Vol. 2. Proceedings of the Tenth Congress of the Collegium Internationale Neuro-Psychopharmacologicum (pp. 1485-1502). Oxford, Pergamon Press.

Woolverton, W. L. (1986). Effects of a D_1 and a D_2 dopamine antagonist on the self-administration of cocaine and piribedil by rhesus monkeys. Pharmacology Biochemistry & Behavior, 24, 531-535.

Woolverton, W. L., Goldberg, L. I., & Ginos, J. Z. (1984). Intravenous self-administration of dopamine receptor agonists by rhesus monkeys. The Journal of Pharmacology and Experimental Therapeutics, 230, 678-683.

Woolverton, W. L., & Kleven, M. S. (1988). Multiple dopamine receptors and the behavioral effects of cocaine. In D. Cluet, K. Asghar, & R. Brown (Eds.), Mechanisms of cocaine abuse and toxicity (National Institute on Drug Abuse Research Monograph 88) (pp. 160-184). Washington, DC: U.S. Government Printing Office.

Woolverton, W. L., & Virus, R. M. (1989). The effects of a D_1 and a D_2 dopamine antagonist on behavior maintained by cocaine or food. Pharmacology Biochemistry & Behavior, 32, 691-697.

Worms, P., Broekkamp, C. L. E., & Lloyd, K. G. (1983). Behavioral effects of neuroleptics. In J. T. Coyle & S. J. Enna (Eds.), Neuroleptics: Neurochemical, behavioral, and clinical perspectives (pp. 93-117). New York: Raven Press.

Yokel, R. A. (1987). Intravenous self-administration: Response rates, the effects of pharmacological challenges, and drug preference. In M. A. Bozarth (Ed.), Methods of assessing the reinforcing properties of abused drugs (pp. 1-33). New York: Springer Verlag.

Yokel, R. A., & Wise, R. A. (1975). Increased lever pressing for amphetamine after pimozide in rats: Implications for a dopamine theory of reward. Science, 187, 547-549.

Yokel, R. A., & Wise, R. A. (1976). Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. Psychopharmacology, 48, 311-318.

Yokel, R. A., & Wise, R. A. (1978). Amphetamine-type reinforcement by dopaminergic agonists in the rat. Psychopharmacology, 58, 289-296.

Young, A. M., Woods, J. H., Herling, S., & Hein, D. W. (1983). Comparison of the reinforcing and discriminative stimulus properties of opioids and opioid peptides. In J. E. Smith & J. D. Lane (Eds.), The neurobiology of opiate reward processes (pp. 147-174). Amsterdam: Elsevier Biomedical Press.

Yu, K. L., & Peter, R. E. (1990). Dopaminergic regulation of brain gonadotropin-releasing hormone in male goldfish during spawning behavior. Neuroendocrinology, 52, 276-283.

Zhang, A.-Z., & Pasternak, G. W. (1980). μ- and δ-opiate receptors: Correlation with high and low affinity opiate binding sites. European Journal of Pharmacology, 67, 323-324.

Zito, K. A., Vickers, G., & Roberts, D. C. S. (1985). Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. Pharmacology Biochemistry & Behavior, 23, 1029-1036.