The Neuroanatomical Substrate of Opiate Reward in the Rat

Michael A. Bozarth

A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

November 1982

ABSTRACT

THE NEUROANATOMICAL SUBSTRATE OF OPIATE REWARD IN THE RAT

Michael A. Bozarth, Ph.D.
Concordia University, 1982

An intracranial self-administration procedure was developed to test animals for the acquisition of a lever-pressing response to deliver morphine infusions into discrete brain regions. Subjects rapidly learned to self-administer morphine into the ventral tegmental area but did not learn to self-administer morphine into other opiate receptor fields. Further studies determined that the rostral-caudal boundaries of this system correspond to those of the A10 dopamine cell group.

Evidence was reviewed suggesting that opiates enhance dopaminergic neurotransmission. Behavioral indices observed during intracranial morphine self administration further support this notion. The effect of dopaminergic receptor-blockade on heroin reward was tested using two conditioning procedures. The conditioned reinforcement and the conditioned place preference

produced by systemic heroin injections were blocked by neuroleptic pretreatment. Both of these procedures tested animals in the drug-free condition eliminating motor sedation as a possible explanation of this effect.

A number of brain regions were mapped for their ability to produce physical dependence on morphine following chronic infusions. Specific attention was focused on the ventral tegmental area to determine the relationship of the site of morphine reward to the development of physical dependence. Although a moderate degree of physical dependence was demonstrable after chronic morphine infusions into this brain region, this effect was eliminated by the use of cannulae that were angled to avoid penetration of the periventricular gray substance. Infusions into the periventricular gray region produced the most severe physical dependence with withdrawal jumping emanating primarily from the rostral region while wet-dog shakes were more pronounced after infusions into the caudal region. Chronic morphine infusions into other brain regions produced little or no physical dependence.

The results of these and other studies suggest that opiate reward is produced by a drug action in the ventral tegmental area which is dependent on a dopaminergic mechanism. It is likely that the A10 dopamine system is involved in this rewarding action. Physical dependence

is produced by a drug action primarily in the periventricular gray region. These data are concordant with the notion that opiate reward is another instance of appetitive motivation and may pharmacologically activate the neural substrate of natural rewards.

ACKNOWLEDGEMENTS

A number of people have made important contributions to the development of the ideas outlined in this thesis and to the practical aspects of data collection. Dr. Larry D. Reid is thanked for the early training and for the discussion of ideas which helped form the basis of much of this research. Lydia Alessi, Martha Asselin, and William Mundl are thanked for technical assistance and for a great deal of moral support during various phases of this research project. Kali Sakell and other members of the Department of Psychology are thanked for keeping the "wolves at bay" during times when I neglected many of the bureaucratic aspects of graduate school.

I wish to express my sincere appreciation to Dr. Roy A. Wise for the support, mentorship, and friendship which have made this research possible. The environment you provided nurtured the development of these ideas and of specific techniques capable of testing the experimental hypotheses derived from them. Without such support, both professional and personal, this research project would have died in the early stages of its conceptualization.

Finally, I wish to thank Valarie for her love and support that has made the realization of my goals possible. Thanks for encouraging me to make the best professional

choice which was moving to Montreal, even though it has brought inconveniences in our personal lives. Thanks for tolerating (and even fostering) my seemingly unitary commitment to my research and the development of my ideas. Thanks for making all of this not only bearable, but enjoyable as well.

This research was supported by grants from the Natural Science and Engineering Research Council of Canada and from the National Institute on Drug Abuse (DA 2285).

This thesis is dedicated

to the memory

of my father.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

FRAME OF REFERENCE

With the discovery of brain stimulation reward (J. Olds & Milner, 1954), it has become widely accepted that the neural mechanisms subserving reward are part of a specialized reward substrate (e.g., J. Olds, 1962, 1977; Wise, 1981) and not a ubiquitous property of the nervous system: not all neurons are directly involved in reward and those that are may comprise a relatively small portion of the nervous system. Delineation of the neural basis of motivation and reward has been achieved largely by electrical brain stimulation experiments which study both the ability of electrical stimulation to induce various "motivated" behaviors[1] and the ability of electrical stimulation to directly reinforce arbitrary behavioral responses. Some of the neuroanatomy and neurochemistry of this reward system has been determined (see Hall, Bloom, & J. Olds, 1977; Wise, 1978a). The relationship of this neural substrate to natural reward has been debated, but

[1]Whether any behavior more complex than the patellar reflex is "unmotivated" could be debated to great lengths. Maslow (1954) suggests, however, that some forms of self expression may be essentially unmotivated; that is, they are "ends" in themselves and not directed toward achieving any other goal.

it appears that this system may be involved in the incentive motivational properties of conventional rewards (see J. Olds, 1962, 1977; Reid, 1967; Wise, 1974).

The brain also contains specialized receptors for opiates; again, these receptors are not distributed uniformly throughout the brain but, rather, they are clustered in numerous regions (Atweh & Kuhar, 1977ab; Pert, Kuhar, & Snyder, 1975, 1976; Snyder & Matthysse, 1975) forming what might be termed opiate-receptor fields. In the past decade endogenous substances have been isolated and identified that specifically bind to these opiate receptors (Cox, Opheim, Teschemacher, & Goldstein, 1975; Hughes, 1975; Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975; Pasternak, Goodman, & Snyder, 1975; Terenius & Wahlstrom, 1975; Teschemacher, Opheim, Cox, & Goldstein, 1975) and the role of these endorphins[2] in physiology and behavior has become the subject of considerable research. Some of the processes advanced as being influenced by endorphinergic systems include social behavior (Kavaliers, 1981; Panksepp, Herman, Vilberg, Bishop, & DeEskinazi, 1978), feeding and drinking (Holtzman, 1975; Jalowiec, Panksepp, Zolovick, Najam, & Herman, 1981; Morley,

---

[2]Endorphin is used as the generic term to refer to endogenous opioid-like peptides. This is in agreement with the policy of many researchers in the field (e.g., Goldstein & Cox, 1978; Simon & Hiller, 1978; Smith & Simon, 1981), although Adler (1980) has suggested that endogenous opioid peptides would better describe the class.

1980; Ostrowski, Rowland, Foley, Nelson, & Reid, 1981; Sanger, 1981; Siviy, Calcagnetti, & Reid, 1982); learning and memory (Izquierdo, 1979; Rigter, 1978; Riley, Zellner, & Duncan, 1980; L. Stein & Belluzzi, 1979), pain (Amir, Brown, & Amit, 1980; Fields, 1981; Lewis, Caldecott-Hazard, Cannon, & Liebeskind, 1981; Terenius, 1978) psychopathology (Berger, Watson, Akil, Elliot, Rubin, Pfefferbaum, Davis, Barchas, & Li, 1980; Davis & Bunney, 1980; Watson, Akil, Berger, & Barchas, 1979), copulation (Gessa, Paglietti, & Quarantotti, 1979; Murphy, 1981; Myers & Baum, 1980), and opiate addiction (Herz, 1981; Herz, Hollt, & Przewtocki, 1980). (For general reviews of endorphins and behavior, see Barchas, Akil, Elliot, Holman, & Watson, 1978; Bolles & Fanselow, 1982; Kosterlitz, 1980; Olson, Olson, Kastin, & Coy, 1980; Smith & Simon, 1981). Narcotic antagonists, which are assumed to block the actions of endorphins (cf. Hill, 1981; Sawynok, Pinsky, & LeBella, 1979), have been shown to produce dysphoria and mental depression in humans (File & Silverstone, 1981; Hollister, Johnson, Boukhabza, & Gillespie, 1981; Mendelson, Ellingboe, Keuhnle, & Mello, 1979). Thus endorphins appear to influence many aspects of behavior and have been postulated to have a special role in motivation and reward (Belluzzi, & L. Stein, 1977; L. Stein, 1978; L. Stein & Belluzzi, 1978, 1979). These effects can potentially be revealed by studying the action of drugs which activate or block the activation of opiate

receptors[3] (i.e., opiates and narcotic antagonists, respectively).

One of the most dramatic pharmacological actions of opiates is their ability to reward or "reinforce" behaviors associated with their self-administration. In fact, drug addiction is currently defined in behavioral terms emphasizing a preoccupation with the acquisition and assimilation of the drug (Jaffe, 1975, Martin & Sloan, 1977). Most biological properties, such as physical dependence and analgesia, are not considered necessary attributes of a compound to qualify it as an addictive agent. Rather, the determination that a drug is an addictive agent relies on a demonstration of the behavioral aspects of addiction since the relevant biological properties of these drugs have not yet been identified.

There is no pre-existing need to ingest opiates, and considerable experience is necessary to establish opiate assimilation with the characteristic vigor seen in chronic drug addiction. Once established, however, this behavior may result in the diminution of the efficacy of natural rewards. This ability of opiates and other addictive agents to reinforce their own ingestion while some-

---

[3]Endorphins have been considered as endogenous ligands for opiate receptors and this practice reflects the chronology of discoveries made in this field (i.e., opiate receptors were discovered before endorphins). However, it might be more appropriate to consider opiates as exogenous ligands for endorphin receptors.

times blunting the rewarding impact of other reinforcers suggests that opiates may have profound effects on the neural mechanisms underlying conventional motivation and reward.

Most theories of drug addiction have focused on psychodynamic or sociological models of drug abuse (see Hoch & Zubin, 1958; Lettieri, Sayers, & Pearson, 1980). One of the most popular theories of drug addiction that has been directly related to biological processes involves the physical dependence-producing properties of opiates. The repeated use of large quantities of these drugs produces a physical dependence on their continued intake. When opiate administration is discontinued, withdrawal symptoms emerge that can be readily suppressed by opiate assimilation. It has been suggested that the discomfort produced by withdrawal from opiates in the physically dependent person provides the motivation for the continued ingestion of opiates (e.g., Wikler, Martin, Pescor, & Eades, 1963; Wikler & Pescor, 1967; Wikler, Pescor, Miller, & Porrell, 1971). This model dominated much of the early preclinical screening of new compounds for addiction liability (e.g., Committee on Problems of Drug Dependence, 1970).

Although tension-reduction models[4] of opiate addic-

[4]The hypothesis that opiates are ingested to avoid withdrawal distress is basically a variant of the tension reduction model. Essentially, some aversive event (e.g.,

tion have remained popular explanations of drug addiction (e.g., Dole, 1980; Lindesmith, 1980; Wikler, 1980; see also Lettieri et al., 1980), current views have shifted in favor of positive-reinforcement models of drug addiction. Initial assimilation of opiates produces pronounced "feelings of well being" (see Martin & Sloan, 1977; McAuliffe, 1975; McAuliffe & Gordon, 1974) which have been termed positive-euphoria[5] (Kolb, 1925). These mood elevating properties of opiates have been recognized for many years by some researchers (e.g., Eddy, Halback, & Braenden, 1957; Martin, 1966) even though psychosocial and tension-reduction theories continued to dominate the field of drug addiction (e.g., Lindesmith, 1938, 1970, 1980; Prout, White, & Charry, 1958; Rado, 1958; Rasor, 1958; deRopp, 1957).

The physical-dependence withdrawal syndrome associated with opiates may contribute to the long-term maintenance of opiate addiction, but acquisition and early maintenance of opiate assimilation appear to be motivated

---

withdrawal discomfort, psychological tension) is terminated by the ingestion of an opiate. This might also be viewed as negative reinforcement (see Chapter 7).

[5]In common usage euphoria connotes a state of bliss or ecstasy. The mood elevation experienced by opiate addicts is probably not well described by this term; the effect of opiates may be more subtle producing what is better described as a feeling of well being or contentment. Nonetheless, euphoria is frequently used to refer to this alteration in mood and the reader is cautioned about the specialized meaning of this term.

by positive reinforcement processes.[6] For this reason attention has focused on the effects of opiates on the neural mechanisms of reward and attempts to identify an action of opiates on the substrate of natural rewards have become the subject of considerable interest.

The study of opiate addiction and the delineation of the mechanisms involved in the acquisition and maintenance of addiction have implications that transcend the treatment and prevention of drug abuse. The fact that these drugs have profound effects on motivated behavior make them potentially useful tools for the study of brain mechanisms subserving reward and motivation. Seldom do conventional rewards seem to control and direct behavior as strongly as addictive drugs reinforce the behaviors associated with their acquisition and assimilation. Thus the study of opiate addiction may be viewed as a study of the neural substrate of basic motivational processes using opiates as exogenous ligands to determine the role of endorphins in the biological mediation of motivated behavior.

---

[6]The role of physical dependence in opiate addiction will be addressed in more detail in Chapter 7. In general, the evidence argues against a central role of physical dependence in the genesis of drug addiction (see Eddy et al., 1957; Jaffe, 1975; Martin & Sloan, 1977).

CHAPTER 2

TECHNIQUES USED TO STUDY THE NEURAL MECHANISMS OF OPIATE REWARD[1]

2.1 Methods of Localizing Opiate Reward

The interest in opiates as positive reinforcers has prompted a search for the identification of the neural mechanisms subserving opiate reward and its relationship to the neural substrate mediating the effects of conventional rewards. Several techniques have been used to study the rewarding properties of abused drugs. Each offers a unique contribution to the study of drug reward, but each is also limited in the type of question that it can answer. The strongest support for any relationship is provided by convergent evidence across several different techniques.

2.1.1 Brain Stimulation Reward

One approach has been to examine the interaction of abused drugs with rewarding brain stimulation (Adams, Lorens, & Mitchell, 1972; Blake & Halpern, 1971; Miller, 1957; J.

---

[1]Portions of this chapter have appeared in M. A. Bozarth, Opiate reward mechanisms mapped by intracranial self-administration. In J. E. Smith and J. D. Lane (Eds.), The Neurobiology of Opiate Reward Processes. Amsterdam: Elsevier Biomedical Press, 1983 (in press) and M. A. Bozarth and R. A. Wise, Electrolytic microinfusion transducer system: An alternative method of intracranial drug application. Journal of Neuroscience Methods, 1980, 2, 273-275.

Olds & Travis 1960). Opiates, as well as many other drugs of abuse, can facilitate brain stimulation reward (Esposito & Kornetsky, 1978; Reid & Bozarth, 1978; Wise, 1980). This facilitation has been demonstrated as both a lowering of thresholds (Kelly & Reid, 1977; Marcus & Kornetsky, 1974) and an increase in rates of responding for a fixed intensity of brain stimulation reward (Adams, et al., 1972; Bush, Bush, Miller, & Reid, 1976; Lorens & Mitchell, 1973). In fact, the effects of various opiates on self-stimulation seem to be a better indicant of their relative addiction liability than is their ability to produce physical dependence (Reid & Bozarth, 1978). Although the data gathered from this technique are promising, studies of brain stimulaton reward have not received much attention from drug addiction specialists. This is probably because this proposed model of drug reward makes several important assumptions which have not been empirically validated (see Wise, 1980). As the critical predictions derived from this model are systematically explored (e.g., Bozarth, Gerber, & Wise, 1980; Gerber, Bozarth, & Wise, 1981), the potential usefulness of this technique should become increasingly appreciated.

If the facilitation of brain stimulation reward reflects the intrinsically rewarding properties of abused drugs, Broekkamp and his coworkers (e.g., Broekkamp, 1976; Broekkamp, Van Den Bogaard, Heynen, Rops, Cools, & Van Rossum, 1976) appear to have localized the site of action for morphine's rewarding effect. In an elegant series of

experiments, it was found that morphine produced facilitation of brain stimulation reward when microinjected into several brain sites. The shortest latency to onset and the strongest magnitude of effect were associated with microinjections into the ventral tegmental area. It was concluded that the facilitations resulting from morphine injections into other brain regions (e.g., posterior lateral hypothalamus; lateral cerebral ventricle) were probably the result of drug diffusion to this area. While this finding has been replicated with both morphine and D-ala^2-met^5-enkephalinamide (Broekkamp, Phillips, & Cools, 1979), the conclusions drawn from this work seem to have been largely ignored. Broekkamp (1976) used response rates as the dependent measure of brain stimulation reward and this metric has been criticized by workers within the field (Kornetsky & Esposito, 1979; Valenstein, 1964). The use of response rates and the lack of widespread acceptance of this model of opiate reward have severely blunted the impact of this potentially important work. Ironically, the independent confirmation of the ventral tegmental area as the site of morphine's rewarding action would strengthen the proposed relationship between a drug's intrinsically rewarding properties and its effect on brain stimulation reward. Broekkamp's (Broekkamp, 1976; Broekkamp et al., 1976; Broekkamp et al., 1979) suggestion that the ventral tegmental area is the site of action for morphine reward remains an unsupported speculation, and the localization of the brain

site responsible for morphine's rewarding action remains a problem for other techniques to determine.

2.1.2 Intravenous Self-Administration

The intravenous self-administration paradigm is probably the most widely accepted method of assessing the rewarding properties of abused drugs.[2] There are direct and obvious parallels between human drug abuse and animal self-administration. With the exception of hallucinogenic substances, drugs which are abused by humans are generally self-administered by laboratory animals and drugs which are not self-administered by animals are usually not abused by humans (see Schuster, 1973; Seiden & Dykstra, 1977; Woods, 1978). This has helped to make the intravenous self-administration paradigm the preparation of choice for studying the rewarding properties of many abused drugs.

A wealth of information regarding how drug rewards can control operant behavior has been gained using the

---

[2]Oral models of opiate self-administration have also yielded useful information regarding the nature of opiate reward (e.g., Alexander, Beyerstein, Hadaway, & Coambs, 1981; Alexander, Coambs, & Hadaway, 1978; Amit, Corcoran, Amir, & Urca, 1973; Carroll & Meisch, 1978; Collaer, Magnuson, & Reid, 1977; Gorman, DeObaldia, Scott, & Reid, 1978; Khavari & Risner, 1973). This method offers several advantages such as (i) simplification of the preparation making the testing of large numbers of subjects feasible, (ii) long preparation life making chronic intake studies practical, and (iii) continuous access to opiate providing a true ad libitum assessment of the pattern and quantity of opiate intake. This method, however, lacks the advantages of the intravenous model such as immediacy of the rewarding effect and regulated patterns of intake. A discussion of the relative advantages of each method can be found in Altshuler (1978) and Seiden and Dykstra (1977).

intravenous self-administration paradigm (see Thompson & Pickens, 1971). Opiates and psychomotor stimulants have been shown to control behavior in much the same way as do conventional rewards such as food and water (Spealman & Goldberg, 1978; Woods, 1978). With experience, stable patterns of responding for drug emerge and changes in the response-reinforcement contingencies, such as the dose of drug delivered per infusion, lead to predictable changes in the rate of self-administration (Woods & Schuster, 1968). Likewise, alterations in the pattern of drug intake have been interpreted in terms of changes in the reinforcing efficacy of the drug infusions (e.g., Yokel & Wise, 1975, 1976). While this paradigm provides an excellent method of assessing the effects of some manipulations on drug reward, attempts to localize the brain site of rewarding drug action have been plagued by serious limitations of this technique.

The most common method of studying the neuroanatomical substrate of drug reward has been to lesion various brain regions and to compare drug self-administration before and after lesioning (e.g., Glick & Cox, 1977, 1978; Glick, Cox, & Crane, 1975; Lyness, Friedle, & Moore, 1979; Pozuelo & Kerr, 1972; Roberts, Corcoran, & Fibiger, 1977; Roberts, Koob, Klonoff, & Fibiger, 1980). Although this technique can be useful, a variety of nonspecified lesion effects can confound behavioral measures of drug reward. Most notable are the profound changes in locomotor activity, feeding, and

drinking that accompany lesions of some of the more interesting brain regions such as the medial forebrain bundle or ventral tegmental area (Teitelbaum & Epstein, 1962; Ungerstedt, 1971d). Such side-effects make the consequences of lesioning very difficult to interpret since behavioral indices of drug reward are easily confounded by treatments that alter motor performance. Furthermore, some neural systems show dynamic changes in functioning after denervation (Cannon & Rosenblueth, 1937). The best documented of these systems is the dopamine-containing nigro-striatal system which shows increased neurotransmitter release and receptor binding after the partial destruction of its synaptic terminals (Creese & Snyder, 1978; Hefti, Melamed, & Wurtman, 1980; Mishra, Marshall, & Varmuza, 1980). This compensatory activity can lead to a restoration of function (Stricker & Zigmond, 1976; Teitelbaum & Epstein, 1962; Ungerstedt, 1971cd) and a normalization of responding for drug (see Roberts et al., 1980) that could mask the primary effect of lesioning. Even when the motor debilitating effects and functional recovery can be controlled, lesions that disrupt drug intake would suggest that the lesioned element is critical for drug reward but would not suggest that the drug is acting directly at the lesioned site. Lesions at any point along the system activated by a drug might disrupt self-administration, despite the fact that the lesioned element may be several synapses removed from the initial target of drug action (see Figure 2.1; see Adler & Geller (1978) for

an informative discussion of the problems associated with the interpretation of lesion data).

Another method that has been used to localize the site of various opiate effects involves selectively blocking the action of systemically injected opiate at a restricted brain site (e.g., Laschka, Teschemacher, Mehraein, & Herz, 1976b; Wei, Loh, & Way, 1973; Wilcox & Levitt, 1978). After systemic injection of an opiate, animals receive a micro-injection of a narcotic antagonist directly into a specific brain region. This procedure allows opiate action at all

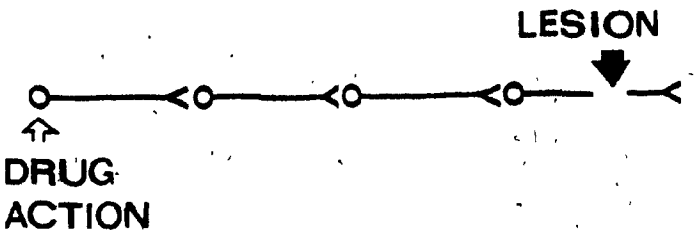

Fig. 2.1. Lesions anywhere in a series of neuronal elements can eliminate drug reward, even if the drug is not acting directly at the lesioned element. For this reason, lesions do not identify the site where a drug initiates the sequence of events that lead to reward but only identifies individual elements which mediate this reward.

brain sites except the one that received the microinjected narcotic antagonist. The ability of the centrally delivered antagonist to reverse the systemically induced opiate effect suggests the location of the opiate-receptor field mediating the behavior under study.

Using this approach, the intravenous self-administration of an opiate can be challenged with central microinjections of a narcotic antagonist, and changes in the pattern of drug intake can be interpreted to reflect the importance of a given brain site in opiate reward. There are, however, three serious limitations to this approach. The first problem with this technique is that the most commonly used narcotic antagonists (i.e., naloxone, naltrexone) have a high lipid solubility; drugs that possess this property diffuse rapidly throughout the brain making localization of the effect impossible (Schubert, Teschemacher, Kreutzberg, & Herz, 1970). New drugs have been developed that are hydrophilic and thus avoid this problem (see Britt & Wise, 198la), but difficulties in interpreting the results of these studies can emerge if drugs of the mixed agonist-antagonist type are used (e.g., diallyl-normorphinium bromide).[3] A second problem associated with the regional

[3]Diallyl-nor-morphinium bromide is a quaternary derivative of nalorphine (Laschka, Herz, Blasig, 1976a). Like its parent compound, nalorphine, it is a mixed agonist-antagonist. Drugs in this class (e.g., cyclazocine, levallorphanol, nalorphine, pentazocine) have both narcotic agonist and antagonist properties (Houde, 1979; Jaffe & Martin, 1975; Martin, 1967).

application of narcotic antagonist is related to the role of endorphins in behavior. Drugs that block the effects of opiates also block the effects of endogenously released endorphins. This may lead to the production of behaviors that are incompatible with opiate self-administration but are not directly related to the reinforcing efficacy of the systemically injected drug. These effects include (i) changes in motor behavior caused by the administration of narcotic antagonists (Amir, Galina, Blair, Brown, & Amit, 1980; Pert, DeWald, Liao, & Sivit, 1979), (ii) deficits in the regulation of food and water intake (Holtzman, 1975, 1979; Ostrowski, Foley, Lind, & Reid, 1980; Siviy, Calcagnetti, & Reid, 1982), and (iii) alterations in schedule-controlled behavior (Downs & Woods, 1976; Kelleher & Goldberg, 1979; McMillan & Morse, 1967). The third and most serious problem with the use of centrally delivered narcotic antagonists to determine brain sites involved in opiate reward involves the physical dependence-producing properties of opiates. While it has been suggested that opiate self-administration is independent of physical dependence (Thompson, 1968; Woods & Schuster, 1968), animals will lever press to avoid withdrawal reactions (Goldberg, Hoffmeister, Schlichting, & Wuttke, 1971b; Goldberg, Woods, & Schuster, 1971c; Weeks, 1962; Weeks & Collins, 1979). If a narcotic antagonist were microinjected into a brain region involved in physical dependence, it might be expected that the animals would elevate their opiate intake to compensate

for the displacement of opiate at this receptor site. This pattern of responding could easily be mistaken to indicate that opiate reward had been attenuated by the microinjected narcotic antagonist although the animal is simply attempting to avoid withdrawal discomfort. A total cessation of responding might also be caused by withdrawal stress even though no overt signs of abstinence are noted.[4]

Recently, neurochemical techniques have been used to determine changes in regional brain activity (Glick, Cox, & Meibach, 1980) and neurotransmitter release (Smith, Co, Freeman, & Lane, 1982; Smith, Co, Freeman, Sands, & Lane, 1980) during intravenous morphine self-administration. These studies can provide valuable information regarding the neurochemistry of opiate reward, but caution must be exercised in the interpretation of such data. This type of correlative neurochemical approach[5] fails to discriminate

---

[4]Goldberg et al. (1971ab) have shown that monkeys increase their intake of morphine when challenged with small doses of a narcotic antagonist but show a dose-dependent suppression of responding at higher doses. They have suggested that this is caused by the precipitation of withdrawal reactions since overt signs of physical dependence were seen. Similarly, rats have been observed to abruptly stop self-administration of heroin when challenged with narcotic antagonists without the compensatory increase in drug intake usually thought to occur in this situation (G. Gerber, personal communication). In the latter case, withdrawal signs were not noted.

[5]Although it has been argued that all data are correlational in nature (Hume, 1739), the designation "correlative approach" is frequently applied to what might more appropriately be termed an observational approach (Campbell & Stanley, 1966; Sheridan, 1979; Wood, 1974); others have referred to this method as natural accretion measures (Webb, Campbell, Schwartz, & Sechrest, 1966) and ex post facto research (Kerlinger, 1973).

evoked activity that is relevant to a given behavior from evoked activity that is unrelated. Such neurochemical studies can be very informative, but they need direction from other types of experiments that clearly show the importance of the brain region or neurotransmitter under study. Otherwise, it is impossible to discern whether the brain system in question is involved in the locomotor, thermoregulatory, analgesic, rewarding, or dependence-producing properties of opiates. Indeed, correlative neurochemical evidence alone cannot demonstrate that these actions are causally related to any behavioral effect of opiates.

2.1.3 Conditioned Place Preference

Another method that has been proposed to study the rewarding properties of abused drugs is the conditioned place preference paradigm (Rossi & Reid, 1976; Schwartz & Marchok, 1974; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980a).[6] In this paradigm, animals are confined to a normally non-preferred portion of a test chamber following injections of a drug. They are subsequently tested under drug-free conditions to determine if a learned preference or

---

[6]The term conditioned place preference is a misnomer. The animals rarely spend over half of the total period on the conditioning side of the test apparatus. The use of this term is retained, however, since it has become widely accepted by those working in the field (e.g., Bozarth & Wise, 1981b; Mucha, Vand Der Kooy, O'Shaughnessy, & Bucenieks, 1982; Phillips & LePiane, 1980; Phillips, Spyraki, & Fibiger, 1982; Stewart & Grupp, 1981; Van Der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982).

aversion has developed to the place where they had experienced the drug effect. This technique has been proposed to reveal the affective valance of the drug experience; if the animals show a conditioned place preference for the side where the drug effect was experienced, it is frequently inferred that the drug state has positive affective consequences (Rossi & Reid, 1976). This paradigm offers the advantage of not making any response demands on the animal in the drugged condition, so it avoids the problem of sedative side-effects of some drug treatments. The influence of motor debilitating brain lesions is also decreased since the response requirement necessary to assess place preference is minimal.

Phillips and LePiane (1980) have shown that morphine injected into the ventral tegmental area produces a conditioned place preference similar to that caused by systemic morphine injections (Rossi & Reid, 1976; Sherman et al., 1980a; Stapleton, Lind, Merriman, Bozarth, & Reid, 1979). This finding is concordant with Broekkamp (1976) and supports the notion that the ventral tegmental area is the site of morphine's rewarding action. The potential limitations of this technique have not been explored, but it would appear that either anxiolytic or physical dependence-producing properties of a drug might influence the place preference. Habituation to the side of putative conditioning during anxiolytic drug action or drug-seeking associated with the relief of withdrawal distress could also increase the prefer-

ence for the side where the drug effect was experienced. In the case of morphine microinjections into brain regions not associated with physical dependence, this latter possibility is unlikely. Nonetheless, the fact that the conditioned place preference paradigm does not appear to yield graded dose-response effects (Phillips & LePiane, 1980) and is not sensitive to the number of conditioning trials (see Phillips & LePiane, 1980; Stapleton et al., 1979) questions the basis of this conditioned response.

2.1.4 Intracranial Self-Administration

Perhaps the most powerful method to demonstrate that a drug has its rewarding action at a particular receptor field is to show that the drug is self-administered directly into that brain region. Self-administration procedures can be modified to allow the infusion of drug directly into discrete brain regions, and responding for central drug can be interpreted in much the same way as responding for intravenous injections. Furthermore, by restricting drug delivery to the reward-relevant receptor field, the intracranial self-administration paradigm can potentially minimize many of the side-effects caused by systemic drug injections.

Intracranial self-administration offers a novel method of assessing the rewarding impact of drug activation of specific brain regions. It should be noted, however, that this technique identifies brain regions where drug action is sufficient for reward, but it does not determine

if that action is necessary for reward. This can only be accomplished by selectively blocking a drug's action in a specific brain area and by assessing the rewarding impact of systemic drug injections. Brain sites identified by intracranial self-administration studies should be tested using central receptor-blockade challenge of either intravenous self-administration or conditioned place preference. This can determine if the brain regions that are sufficient to produce reward are also necessary for reward from systemically delivered drug. Other problems with the interpretation of intracranial self-administration data will be discussed in later sections.

2.2 Methodological Considerations

Many of the methods of studying brain mechanisms of opiate reward rely on drug microinjection techniques. These methods are subject to the limitations of microinjection technology which have been reviewed in detail elsewhere (Myers, 1972, 1974; Routtenberg, 1972). The study of intracranial self-administration adds two new dimensions to the problems of drug microinjections: (i) the need for smaller infusion volumes necessitated by repeated drug administration and (2) the requirement of response-contingent drug infusions in freely moving animals. These considerations are of minor importance in other applications of microinjection technology where the experimenter controls a single, large infusion of drug, frequently into a restrained

animal. The inability of existing methods to provide response contingent, small volume drug delivery has been the most obvious obstacle to the study of intracranial self-administration.

2.2.1 Technical Aspects of Microinjection

Work with single infusions into brain tissue has shown that the volume of drug injected is a critical determinant of the area of drug spread throughout the brain (Myers, 1974; Myers & Hoch, 1978; Routtenberg, 1972).[7] For localization of drug action, it has been suggested that the infusion volume be 0.5 µl or less; larger volumes are likely to lead to excessive drug spread, making definition of the relevant brain site difficult. Even with an infusion volume of 1 µl, however, drug has been reported to spread only about 0.6 mm from the injection cannula (Lomax, 1966). Similar estimates of drug spread have been derived from both physical dispersion kinetics (Lomax, 1966; Myers & Hoch, 1978; but see also Schubert et al., 1970) and functional tests of drug diffusion (Lomax, 1967; Lotti, Lomax, & George, 1965). These reports are surprising since a 1 µl volume of drug would occupy 1 mm^3 in vitro.

---

[7]Other important variables include the physicochemical properties of the substance, the concentration of the injected substance, and the proximity of intracerebral vascular supply to the infusion site. Diffusion of drug up the cannula shaft has also been a persistent problem, especially with rapid infusion rates. See Jacquet (1975), Myers (1972, 1974), and Routtenberg (1972) for a discussion of these and other factors affecting drug diffusion after intracranial application. Furthermore, the radius of drug spread may vary as a function of time after the injection (Schubert et al., 1970).

Unfortunately, little is known about the rate of drug dispersion following an infusion, and one cannot equate acute infusion volumes with the cumulative volumes reached with repeated infusions. A single injection of 1 µl is not equivalent to 10 injections of 0.1 µl since the tissue can accommodate a series of small microinjections more readily than a single bolus infusion. The interval between microinjections thus becomes a critical variable in determining the range of drug spread after repeated infusions. In vivo estimates must be derived to determine the maximum allowable volume for chronic infusion experiments. Albert and Madryga (1980) have reported data that suggest that repeated drug infusions can be accommodated surprisingly rapidly. In a study of the functionally effective spread of 4 µl of lidocaine infused over 15 minutes, they estimated the range of effective drug spread to be 0.25 to 0.6 mm from the injection cannula. This range is much smaller than would be predicted from the physical spread of the same volume injected over a shorter period of time (Myers, 1974; Myers & Hoch, 1978). The study of Albert and Madryga (1980) illustrates the necessity of determining drug spread estimates for repeated infusions in vivo and not relying on estimates of similar volumes injected in a single bolus.

Confirmation of drug delivery is a primary consideration in studies employing microinjection techniques. It should be noted that calibration, not computation of drug flow, is the critical factor. The relationship between the

volume of drug displaced in the injection apparatus (e.g., microsyringe) and the amount actually delivered to the brain is seldom obvious. It cannot be assumed that a 1 mm^3 displacement at the microsyringe will produce a 1 µl infusion volume to a subject some distance away. The volume of drug injected needs to be determined empirically with a technique similar to that actually used during behavioral testing. This will take into account some of the error produced by the introduction of various elements between the infusion transducer and the subject (e.g., tubing, fluid swivel). Bench tests at atmospheric pressure offer a first approximation to the amount of drug delivered, but accurate measurement of the injected volume requires in vivo determination of the amount of drug actually delivered into the behaving animal. This can be done by using radiolabelled tracers and by determining the amount of radioactive material recoverable from the brain tissue after microinjection (e.g., Lomax, 1966; Myers & Hoch, 1978; Routtenberg, 1972).

It is also important that normal drug flow be visually confirmed at the injection cannula before and after behavior testing. It cannot be safely assumed that the infusion system is functioning properly during behavioral testing. Frequently, the injection cannula becomes obstructed as it is placed in the guide cannula because tissue or blood has accumulated in the guide shaft. High pressure microinjection systems tend to minimize the influence of small tissue obstructions, but low pressure systems

are particularly sensitive to this problem.

There are several variables that must be considered when selecting a method of drug delivery. The sizes of the guide and the injection cannulae partially determine the resolution of the anatomical mapping which can be accomplished with a given system. Dispersion of drug up the cannula shaft is facilitated by the use of a large diameter cannula (Routtenberg, 1972), but problems with obstruction are augmented with smaller cannulae as are the difficulties in construction and utilization. The pressure generated at the tip of the injection cannula is a function of the flow rate and of the diameter of the injection cannula. For a given rate of infusion, small diameter injection cannulae will achieve a higher injection pressure and hence more tissue damage than will large diameter cannulae. This can also alter the dispersion of drug during microinjections. The use of a 28 gauge injection cannula combined with a 22 gauge guide cannula seems to offer a reasonable compromise between anatomical resolution and practical considerations (cf. Jacquet, 1975; Routtenberg, 1972).

2.2.2 Drug Delivery Systems

The most important factor that intracranial self-administration studies add to the existing problems of microinjection technology is the requirement of small volume, response contingent drug delivery. If infusions are delayed or prolonged in most behavioral tests involving microinjec-

tions, usually few problems are associated with response measurement. Alterations in the time course and in dose-response relationship may be produced, but little chance exists of missing the main effect associated with the drug infusion. Intracranial self-administration studies, however, necessitate that drug infusions be contiguous with some operant response such as lever pressing: that is, microinjections must occur immediately after lever pressing and drug must not be infused when the subject is not making the appropriate response. Otherwise, the animal is unlikely to learn the lever-pressing response since a temporal delay of reward severely impairs response acquisition (Renner, 1964; Tarpy & Sawabini, 1974). The problem of contiguous drug application would appear to be even more important than the reliability of the infusion volume since the animal can behaviorally compensate for slight variations in the amount of drug delivered by making additional drug requests. There is, however, no substitute for contiguity, except perhaps in the well trained animal capable of performing on a partial reinforcement schedule.

Conventional microinjection systems are very similar to their counterparts used for intravenous self-administration (e.g., Myers, 1974; J. Olds, 1962; M. Olds, 1979). A motor driven syringe pump is used to advance the plunger of a microsyringe displacing a controlled amount of drug solution. This solution is forced by hydraulic pressure through a length of flexible tubing connected to a cannula implanted

in the subject. Lever presses activate the infusion pump for a predetermined duration, and the infusion volume is assumed to be controlled by the amount of drug solution displaced in the microsyringe.

While the microsyringe may dispense reliable volumes of drug, the elasticity inherent in the flexible tubing leads to variability in the injected volume. This problem is exacerbated by movement of the animal producing additional stretching and compression of the flexible tubing, thus resulting in uncontrolled (i.e., noncontingent) drug delivery. The introduction of a fluid swivel, permitting rotation of the animal, can compound the problem of reliable drug delivery by creating leaks and "dead space" in the delivery system. Therefore, control over both the amount and contiguity of drug infusions is sacrificed by allowing the subject unrestrained movement. Attempts to relieve the stress on the connecting tubing by the use of a spring-covered infusion line may decrease the delivery of noncontingent drug but probably does not assure accuracy in the low microliter range. In an early attempt to demonstrate intracranial self-administration of morphine, it was noted that animals implanted with cannulae in the periventricular gray substance showed strong analgesia after being allowed to explore the test chamber, even though no microinjections were given (M. Bozarth, unpublished observations). This was probably the result of the noncontingent infusion of several microliters of the morphine

solution.[8]

As an alternative to the microsyringe method, J. Olds, Yuwiler, M. Olds, and Yun (1964) and later E. Stein and Rodd (1980) have developed a microinjection system that eliminates the need for a fluid swivel. Recognizing the problem associated with the introduction of a swivel, this system used a series of rollers to compress the flexible tubing and to deliver drug to the subject. The rollers are mounted on an electrical commutator maintaining unrestricted movement of the subject during testing. Bench tests of the infusion volume suggest promising results (E. Stein & Rodd, 1980), but close inspection of the data shows that this system also produces large variations in the infused volume of drug. This lack of reliability regarding the volume of drug infused would be expected to be even greater in the freely moving animal since this system retains the flexible tubing to connect the animal to the infusion apparatus.[9]

---

[8]The infusion of 1 to 5 µg of morphine into the periventricular gray has been shown to produce analgesia (Mohrland & Gebhard, 1980; Sharpe, Garnett, & Cicero, 1974; Yaksh, Yeung, & Rudy, 1976). Since the concentration of the test solution was 0.25 µg/µl, it appears that an amount in excess of 4 µl was delivered to produce the level of analgesia observed in these subjects.

[9]E. Stein and Rodd's (1980) presentation of their data is somewhat misleading. First, if the volume actually injected, calculated from the counts per minute in their Table 1 (i.e., counts obtained in the sample divided by the standard of 8140 counts per minute per microliter), is compared to the volume they attempted to inject, it is apparent that the infusion volumes ranged from 63 to 143% of the intended volumes. Second, the smallest volume that they verified is 10 times the volume they claim to be using on a routine basis. Third, the system is not adequately tested

Another approach to this problem has been to eliminate both the fluid swivel and flexible tubing. This has been accomplished by using an electrolytic microinfusion transducer (EMIT) system that was adapted from a method originally described by Criswell (1977). With the EMIT method, a gas-tight drug reservoir is filled with drug dissolved in Ringer's solution and attached to an injection cannula positioned in the target area through an implanted guide cannula (Bozarth & Wise, 1980a). Infusions are controlled by applying a direct current across two electrodes contained in the gas-tight drug reservoir (see Figure 2.2). The current flow across the electrodes produces hydrogen gas and the pressure that is generated forces drug through the injection cannula. The amount of drug delivered is controlled by the current intensity and duration. The EMIT assembly is mounted directly on the animal's head. Light flexible wires are used to connect this unit to a constant current source (e.g., Mundl, 1981) and an electrical commutator assures unrestricted movement of the subject during testing.

---

in freely moving animals. Even the diffusion controls are not sufficient since the labeled 2-deoxyglucose could have gone through several half-lives during the 20 hours allowed for passive diffusion (Sokoloff, 1981); it should be noted that (i) maximum noncontingent drug delivery, resulting from stretching the flexible tubing, would probably occur during the first few minutes of testing and (ii) retention of 2-deoxyglucose in brain tissue is a function of glucose utilization rates and a large central injection would probably be followed by appreciable transport of this substance out of the brain.

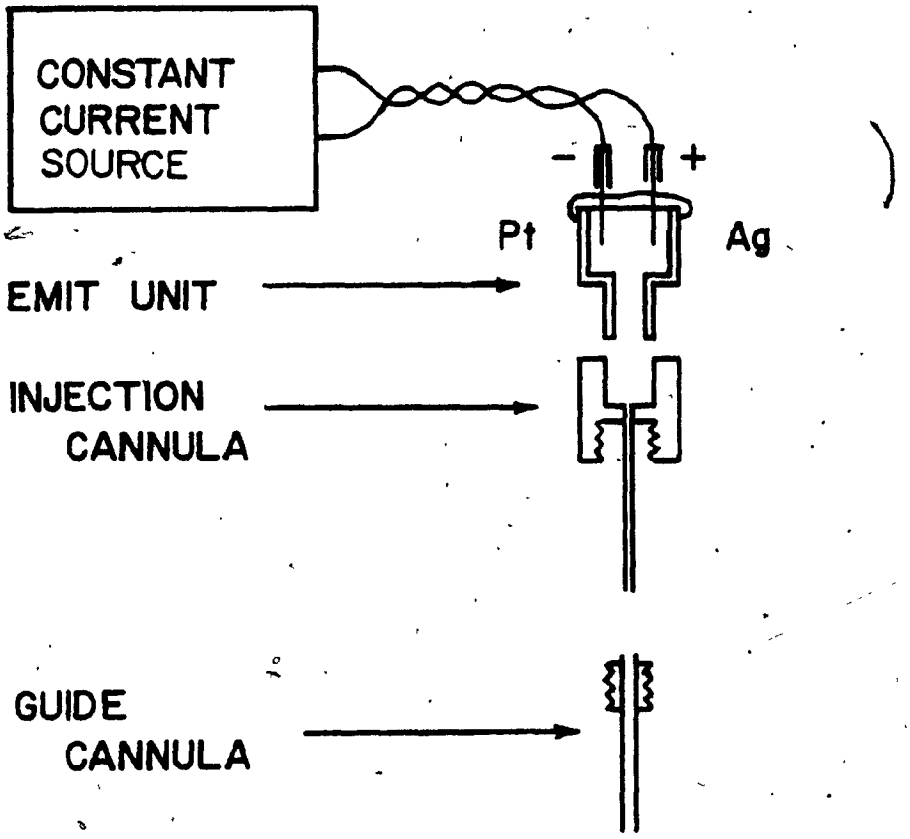

Fig. 2.2. The volume of solution infused can be calculated by the formula µl=0.12 x mA x seconds. Since choloride ions may be produced during the application of current, care must be exercised in the selection of electrode materials. A platinum (Pt) cathode is used while the anode is silver (Ag) wire. Free chloride will combine with the silver anode forming silver chloride on the electrode surface and preventing the mixing of chloride ions with the drug solution.

With the elimination of the fluid swivel and flexible tubing, the major sources of error in small volume drug delivery are circumvented. Movements of the subject during testing can no longer affect the operation of the microinjection system and contiguity of response and drug delivery is achieved. The lower limit of the volume that can be reliably delivered with EMIT system has not been assessed but probably extends into the mid nanoliter range.[10] Smaller volumes than this are probably ineffective since passive drug diffusion from the injection cannula may proceed at a rate higher than the actual volume infused (see Routtenberg, 1972). The EMIT method is routinely used to deliver a 100 nl infusion volume during tests of intracranial self-administration (e.g., Bozarth & Wise, 1980bc, 1981ab). This represents a practical trade-off between a volume giving reasonable anatomical resolution and a volume that can be visualized to check the patency of the drug delivery system before and after behavioral testing.

---

[10] Preliminary tests of the volume delivered with the EMIT system have shown that a 200 μA current applied for five seconds produces a 100±5 nl infusion. These bench tests monitored the movement of an air bubble through a microsyringe across a series of 50 infusions (M. Bozarth, unpublished observation). Similar tests suggest accuracy with 50 nl volumes, although this method of measurement does not permit detection of discrete infusions below 100 nl. Independent tests have confirmed the reliability of this system at 100 nl volumes using radiolabeled infusions in vitro (N. Goeders, personal communication). Subsequent references in the text to the volume of drug delivered (e.g., Sections 3.3.1, 4.2.1) are based on these estimates since the actual volume delivered has not been determined for this preparation. It is unlikely, however, that the volume infused in vivo would exceed that obtained in the bench tests where the effect of tissue resistance is absent.

CHAPTER 3

INTRACRANIAL SELF-ADMINISTRATION OF MORPHINE IN RATS[1]

3.1 Rationale for the study of Intracranial Self-Administration

Just as the discovery of electrical brain stimulation reward by J. Olds and Milner (1954) opened a new avenue of research in the study of brain reward mechanisms, the development of intracranial self-administration can add an important new dimension to this same field. Electrical stimulation is grossly nonspecific activating most cells within a given radius of the stimulating electrode.[2] Chemical stimulation provides a method of activating only certain neurons within a given region of stimulation and relating this effect to specific neurotransmitter systems (Fisher,

---

[1]Portions of this chapter have appeared in M. A. Bozarth and R. A. Wise, Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sciences, 1981, 28, 551-555, and in M. A. Bozarth and R. A. Wise, Localization of the reward-relevant opiate receptors. In L. S. Harris (Ed.), Problems of Drug Dependence, 1981. Washington, D. C.: NIDA Research Monograph Series, 1982.

[2]Ranck (1975; 1981) has reviewed evidence that some units (e.g., large myelinated fibers) are more easily excited than others (e.g., cell bodies), but in general there is no selectivity produced by electrical stimulation in regard to the neurochemical coding of the elements activated or the direction of impulse conduction.

1956; Grossman, 1962; Miller, 1965; Myers, 1974). While this technique may be preferable to electrical stimulation in many circumstances, attempts to develop a paradigm to demonstrate the reliable self-administration of chemicals into the brain has a documented history of failure (see J. Olds, 1962). Early reports from several laboratories provided encouraging results (Morgane, 1962; Myers, 1963; J. Olds & M. Olds, 1958; J. Olds, Yuwiler, M. Olds, & Yun, 1964), but this work has remained obscure with virtually no mention of it in the current literature (cf. M. Olds, 1979; Wise, 1980). This is probably because chemical brain stimulation was floundering while the field of electrical brain stimulation was rapidly developing.

The rationale for the study of intracranial morphine self-administration is three-fold. First, this procedure may circumvent some of the problems encountered with other methods of localizing the reward-relevant opiate receptor population. Second, if some of the effects of opiates (e.g., analgesia, sedation) were initiated at brain sites other than those supporting intracranial self-administration, then these effects of systemic opiate self-administration could be minimized and the rewarding properties of opiates studied without the potentially confounding influence of these other effects. Third, the development of a paradigm that demonstrates intracranial self-administration of any substance opens the possibility of using this same technique to study

reward from other chemical injections. Identification of the chemical coding of reward neurons might then be possible as well as the study of the central sites of action of other addictive agents.

3.2 Criteria for Establishing the Validity of Intracranial Self-Administration

The field of intracranial self-administration is relatively new and, thus far, few guidelines have been established for assessing the conclusions based on data from this paradigm. There are several criteria that must be fulfilled before such data can be expected to contribute meaningfully to our understanding of the brain mechanisms of motivation and reward. Attempts to delineate the mechanism of action for central self-administration of a drug are useless unless they are preceded by a firm empirical basis establishing the validity of drug self-administration, qua drug self-administration.

3.2.1 Behavioral Specificity

The first criterion for establishing the validity of intracranial self-administration studies is the demonstration that the animals are working for the rewarding properties of the drug. Microinjections of morphine into brain tissue can produce increases in locomotor activity and stereotypy (Joyce & Iversen, 1979; Pert & Sivit, 1977) that could artifactually elevate lever-press scores by causing accidental lever contacts. It must be clearly

established that the lever-press response is dependent on the rewarding effects of the drug infusions and not the result of nonspecific behavioral activation.

Procedures that have been employed in the study of intravenous self-administration can be used to determine the behavioral specificity of the lever-pressing response (see Pickens & Thompson, 1971). One such procedure is the yoked control where one animal lever presses for response contingent infusions of drug while another passively receives infusions. Each experimental animal is paired with a yoked control partner such that lever presses by the experimental animal produce concurrent infusions in both animals. Lever presses of the yoked control subject are recorded but do not produce infusions. With this procedure the reinforcing properties of the drug can be inferred from differences between the response rates of the experimental and yoked control subjects. Another method of determining the degree of nonspecific lever-pressing is the two lever choice test. In this procedure each animal is tested in a two-lever box. Responses on one lever produce response contingent drug infusions while responses on the other are recorded but do not produce infusions. Increases in responding on the "inactive"lever are interpreted as nonspecific behavioral activation or arousal.

A potential problem with each of these procedures is that increased responding on an "inactive" lever (i.e.,

yoked control or "inactive" lever in the two-lever choice test) may reflect nonspecific behavioral arousal, but it does not preclude the possibility that the drug infusions are rewarding. This limitation is probably more pronounced in the two-lever choice test where the subject is required to discriminate between an active and inactive lever. In this case the animal may continue to press the inactive lever because of (i) response generalization or (ii) superstitious behavior resulting from a delayed rewarding impact of the drug infusions. For these reasons the yoked control procedure may be preferable to the two-lever choice test although positive findings from both techniques are more definitive. In either case responding on the inactive lever does not prove that the drug infusions are not rewarding, but, rather, it suggests that more rigid control procedures are necessary to assess the effect.[3]

3.2.2 Pharmacological Specificity

Once behavioral specificity has been established,

---

[3] If a cue light associated with rewarding drug infusions were located directly over the lever, autoshaping may occur and lead to increases in lever pressing of the yoked control animals. This phenomenon is well established in procedures showing that animals display increased activity and will approach and manipulate a stimulus correlated with reward (Bindra & Palfai, 1967; Epstein & Skinner, 1980; Hall, Channel, & Pearce, 1981; Leslie, Boakes, Linaza, & Ridgers, 1979; see also Bindra 1972, 1974; Bolles, 1975). Thus the behaviorally arousing effects of rewarding stimuli present another potential source of "inflated" lever-press scores of control animals.

it must be shown that the rewarding effects of the central drug injections are dependent on the same mechanism as mediates systemic drug reward. The rewarding effects of microinjections that result from nonspecific changes in cell function are of limited interest to the study of drug reward. Intracranial self-administration must be shown to depend on the same neural mechanisms as reward from systemic drug injections if this technique be used for the localization of the reward-relevant receptor population.[4] In the case of opiate reward, this pharmacological specificity can easily be established by challenging intracranial self administration with a narcotic antagonist such as naloxone. If this pharmacological challenge blocks the lever-pressing response, then it can safely be concluded that morphine self administration is not due to changes in cell osmolarity, calcium chelation, or other nonspecific factors associated with the microinjections. Active and inactive stereoisomers[5]

---

[4]This is important if conclusions be drawn from these experiments regarding the nature of systemic drug reward. If intravenous amphetamine injections were rewarding through an action mediated at dopaminergic systems (e.g., release of dopamine, reuptake blockade), then treatments that block the effects of this action should also block the intracranial self-administration of amphetamine. For drug actions that are not initiated at specific receptor sites, this type of control procedure becomes much more difficult. This is likely to be the case for barbiturates (Ho & Harris, 1981) and ethanol (Deitrich & Erwin, 1980; Goldstein, 1979; Goldstein & Chin, 1981; Sun & Seaman, 1980; Wayner, Ono, & Nolley, 1975). The criterion of pharmacological specificity might have to be suspended until the primary action of the agent can be identified and procedures developed to block this action at the level of the cell membrane.

[5]It is important that stereoisomers and not geometric

of an opiate can also be compared to determine if the effect be mediated by opiate receptors.

In a review by J. Olds (1962), the importance of demonstrating pharmacological specificity was dramatically illustrated when it was shown that the intracranial self administration of norepinephrine was an artifact of its calcium chelating properties. It appears that this nonspecific activation of neurons in the lateral hypothalamic area produces reward in much the same way as does electrical stimulation of these neurons. Thus the demonstration of reward from chemical stimulation of this area was of little interest since it merely mimicked the nonspecific activation caused by electrical stimulation.

Unfortunately, the early work of J. Olds (1962) seems to have been neglected. Cytawa and Jurkowlaniec (1978, 1979) and Cytawa, Jurkowlaniec, & Bialowas (1980) have again reported that noradrenergic stimulation of the lateral hypothalamus produces positive reinforcement. While these studies may not suffer from the problem encountered with the early work of J. Olds (1962), the new reports fail to demonstrate that this rewarding action of norepinephrine shows pharmacological specificity. Without establishing that this effect is (i) blocked by noradrenergic synthesis inhibition, (ii) blocked by drugs that selectively.

---

isomers of a drug be used in this type of control procedure. Stereoisomers retain the physico-chemical properties of the parent compound while geometric isomers frequently do not (Beckett, 1959).

block noradrenergic receptors, or (iii) produced only by the biologically active stereoisomer of norepinephrine, these studies cannot be meaningfully interpreted. Similar problems in interpretation may arise from studies employing other agents. Recently, cocaine self-administration has been reported into the frontal cortex (Goeders & Smith, 1982). Unfortunately, no control procedures were used to determine the pharmacological specificity of this response; the potent local anesthetic properties of cocaine (Ritchie & Cohen, 1975) make nonspecific effects on neural functioning a viable explanation of this intracranial self administration response.

3.2.3 Anatomical Specificity

The single most important contribution that intracranial self-administration studies can make to the understanding of the neural mechanisms of opiate reward is the localization of the reward-relevant receptor field(s). Once a suitable preparation has been developed (i.e., behavioral and pharmacological specificity established), the brain can be mapped for sites which support self administration. If it were found that a large number of brain areas engendered lever pressing for intracranial morphine injections, then this paradigm might be of limited utility in the characterization of opiate reward mechanisms. If intracranial self-administration of morphine occurred at only a few brain sites, then this technique could be used

to restrict drug delivery to the reward-relevant receptor sites. This would raise the possibility of minimizing the influence of reward-independent effects (e.g., analgesia? physical dependence? sedation?) that are associated with the whole-brain drug delivery that results from intravenous self-administration.

There are two aspects of anatomical specificity to be considered. The first is a comparison across various brain sites to determine if multiple brain regions support self-administration. Using a standard protocol, the brain can be neuroanatomically mapped for sites that support this behavior. Negative findings are difficult to interpret since a larger drug dose or infusion volume could yield conflicting results. Nonetheless, the relative sensitivity of various brain regions to morphine reward can be determined using a reasonably effective dose range and an infusion volume that minimizes the influence of drug diffusion. Those brain regions that are the most sensitive to the rewarding properties of a drug are likely to be closest to the target of rewarding drug action and the most important regions involved in the mediation of reward from systemically administered drug.

The second aspect of anatomical specificity involves the assessment of drug spread within a given brain region. The purpose of this procedure is two-fold: (1) to determine if the rewarding effects of the microinjections were due to a local action or diffusion to a distal site of action, and

(2) to define the anatomical boundaries of the receptor field mediating drug reward within a given brain region. The dispersion kinetics of microinjected drug can be determined by using autoradiography (see Routtenberg, 1972) or by combining the micro-punch assay technique of Palkovits (1973) with liquid scintillation counting (e.g., Myers & Hoch, 1978). These methods provide a quantitative estimate of the physical spread of drug produced by a given microinjection procedure. Another approach is to assess the functional effective spread of drug by systematically varying the cannula placements within a given brain region. This method has been used to determine the anatomical boundaries of other opiate effects (e.g., Lomax, 1967; Yaksh, Yeung, & Rudy, 1976). The latter approach has the advantage of showing the limits of spread for behaviorally relevant concentrations of drug but is more laborious. Only whole-brain autoradiography, however, can detect the spread of drug to distal sites of action that can be produced by ventricular diffusion or microinjection into intracerebral circulatory systems. A combination of these techniques would obviously be advantageous.

3.3 Intracranial Self-Administration of Morphine into the Ventral Tegmental Area

Broekkamp's (1976) study of the facilitatory effect of central morphine injections on brain stimulation reward suggested that the ventral tegmental area might be the site of morphine's rewarding action. The present study was

designed to determine if naive rats would learn to press a lever for morphine infusions into the ventral tegmental area. To control for accidental lever contacts, a yoked control procedure was used. Each experimental rat was paired with a yoked control subject such that lever presses by the experimental rat produced concurrent infusions in both the experimental and yoked control subjects. Lever presses of the yoked control rat were recorded but did not produce an infusion. Animals were also tested for the self administration of the drug vehicle solution. The results of these two control procedures were then used to determine the behavioral specificity of the intracranial self administration response.

3.3.1 Method

Subjects: Fifteen experimentally naive, male, Long-Evans rats, weighing 300 to 350 g at the time of surgery, were unilaterally implanted with 22 gauge guide cannulae stereotaxically aimed at the ventral tegmental area. With the upper incisor bar 5 mm above the interaural line, the coordinates for the guide cannulae were 3.8 mm posterior to bregma, 0.6 mm lateral to the mid-sagittal suture, and 7.8 mm ventral to dura. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic and a single injection of penicillin G (30,000 units, i.m.) was administered prophylactically following surgery. Obturators were fitted at a depth of 0.5 mm beyond the guide cannulae imme-

diately after surgery and remained in place except during behavioral testing. Food and water were available ad libitum.

Apparatus: Rats were tested in a 27 x 38 x 39 cm box housed in a dimly illuminated, sound attenuating chamber. A small exhaust fan provided ventilation and additional masking of peripheral noise. On one end of the test chamber, a 4 x 6 cm lever was located 5 cm above the floor.

The microinfusions were delivered by an electrolytic microinfusion transducer (EMIT) system mounted directly on the rat's head during testing. Infusion was accomplished by passing a direct current between two electrodes contained in the gas-tight reservoir. The production of hydrogen gas forced a controlled amount of solution through the injection cannula while a small quiescent current prevented the redissolution of hydrogen gas evolved during previous infusions. The use of EMIT method minimized the problems of uncontrollable drug infusions inherent in systems which rely on flexible tubing and fluid swivels to permit unrestrained movement of the subject during testing. (For details of the EMIT method, see Bozarth & Wise, 1980a, and Section 2.2.2.)

The EMIT unit was connected to a constant current source (Mundl, 1981) which produced a 200 µA infusion current and a 10 µA quiescent current. Depression of the lever resulted in a 100 nl infusion delivered over five seconds. The EMIT unit was attached to a 28 gauge hypodermic needle

precut to penetrate 1 mm beyond the guide cannula. A light flexible lead was used to connect the EMIT unit to a mercury commutator allowing unrestrained movement of the rat during testing.

Procedure: After at least five days recovery from surgery, rats were randomly assigned to either the experimental, yoked control, or Ringer's control group. For the experimental group, depression of the lever resulted in a 100 ng infusion of morphine sulfate (300 pmoles/infusion) dissolved in 100 nl of Ringer's solution. Yoked control rats were placed in an identical test chamber and infused concurrently with their experimental partners. The lever presses of the yoked control group were recorded but did not produce infusions. Five rats were tested for intracranial self-administration of Ringer's solution under conditions otherwise identical to the experimental group. The house light was extinguished and a cue light was activated during infusions for all groups.

The rats were tested every other day for three four hour sessions. Four rats from the experimental group received intraperitoneal injections of naloxone hydrochloride (10 mg/kg) one hour into a fourth test session.

3.3.2 Results

The mean number of responses per hour during the first three sessions is depicted in Figure 3.1. The experimental group showed a rapid acquisition of the lever-pressing

response while the responding of the yoked control group was similar to that of the Ringer's control group. An analysis of variance (ANOVA) with repeated measures on one factor (Winer, 1971) revealed a significant effect for the factors of Groups {$F(2,13)=17.95$, $p<.001$} and Hours of Testing {$F(3,36)=16.63$, $p<.001$}. Planned comparisons using a Neuman-Keuls' test (Winer, 1971) demonstrated significant differences between the experimental and other groups during

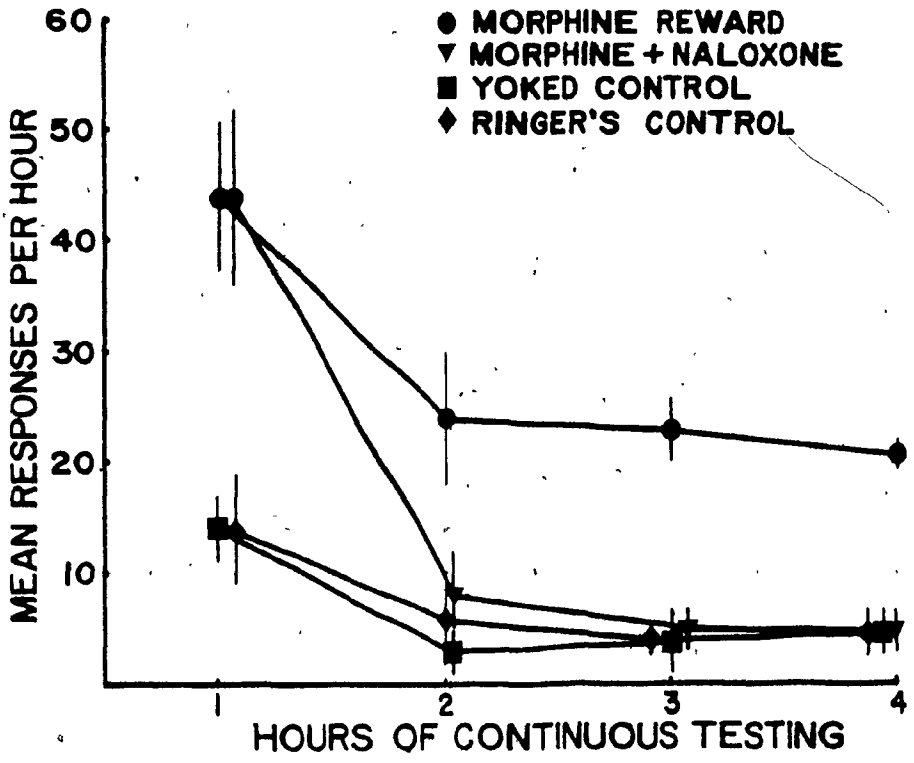

Fig. 3.1. A comparison of the responding of the experimental (morphine reward), yoked control, and Ringer's control groups (n=5/group). The figure depicts the mean (±SEM) number of responses per hour averaged across the three sessions of testing. The experimental group received an injection of naloxone one hour into their fourth test session (morphine + naloxone).

all hours of testing ($p's<.01$). The responding of the experimental group during the first hour of testing was significantly greater than their response rates during each of the subsequent three one hour periods ($p's<.01$).

Given one hour into the fourth test session, naloxone effectively blocked intracranial self-administration (see Figure 3.1). A t-test for correlated measures did not show any differences between sessions for the one hour prior to naloxone treatment {$t(3)=2.35$, $p>.3$}, but an ANOVA with repeated measures on both factors (Winer, 1971) revealed a significant difference between treatments following naloxone {$F(1,3)=21.81$, $p<.025$}.

Histological analysis confirmed that cannula placements were in the ventral tegmental area. Most cannulae were just lateral to the border of the interpeduncular nucleus (see Figure 3.2). Cell damage was similar to that seen after discrete infusions of 0.5 µl or less (M. Bozarth, unpublished observations). Although the dispersion kinetics of this infusion regimen have not been determined, the proximal location of opiate receptors in the A9 and A10 dopaminergic cell bodies makes this a likely site of action (Lindvall & Björklund, 1974; Pollard, Llorens, Bonnet, Costentin, & Schwartz, 1977; Pollard, Llorens, & Schwartz, 1977). Other sites of action cannot be eliminated until discrete localization of these infusions has been demonstrated.

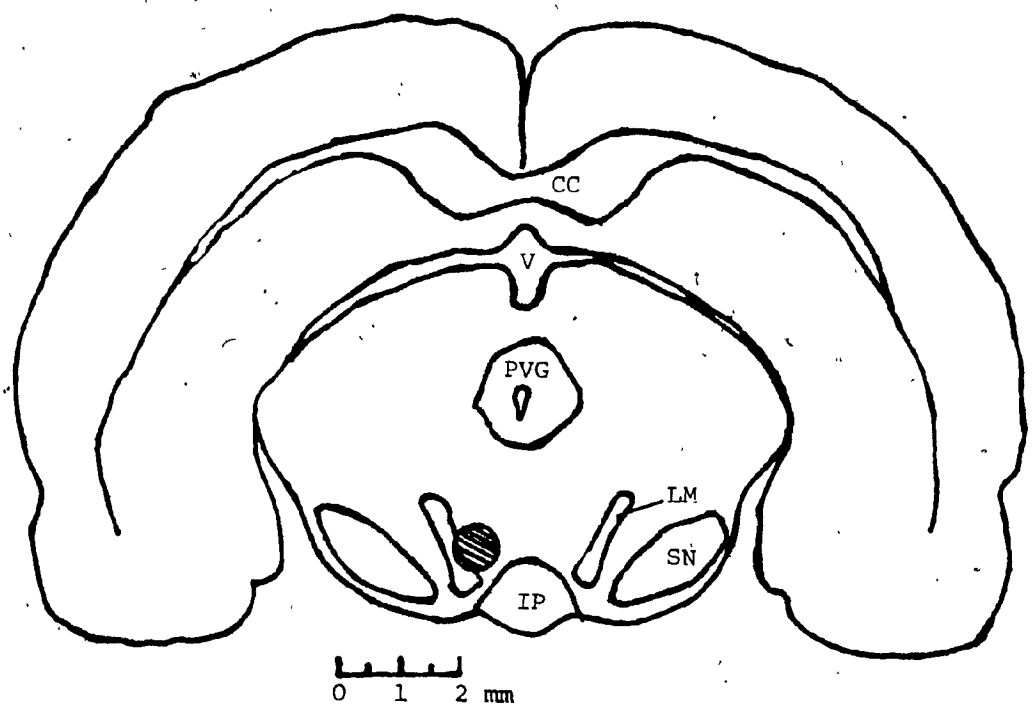

Fig. 3.2. The extent of tissue damage seen in animals self-administering morphine is indicated by the striped zone. Abbreviations: CC, corpus callosum; IP, interpeduncular nucleus; LM, medial lemniscus; PVG, periventricular gray substance; SN, substantia nigra; V, ventricle.

3.3.3 Discussion

The present data suggest that intracranial morphine injections can serve as a true reinforcer, establishing increased levels of lever pressing in a response-contingent manner. Support for this conclusion is derived from the facts that intracranial self-administration was demonstrated in experimentally naive rats and that animals receiving the drug as a consequence of lever pressing responded more than animals passively receiving the same pattern of injections in a yoked control condition. Since naloxone effectively blocked lever pressing, it would appear that the intracranial self-administration of morphine is mediated through opiate receptor mechanisms (see Martin, 1967). This eliminates the possibility that self-administration was the result of mechanical trauma or of changes in osmolarity or pH factors, since these effects would not be sensitive to opiate receptor blockade.

The rapid acquisition of responding seen in experimentally naive subjects suggests that the rewarding effects of microinjected morphine occur soon after the infusions. Pronounced delays in reward from these injections would not be expected to lead to such rapid acquisition of the lever pressing response (Renner, 1964; Tarpy & Sawabini, 1974). The fact that the animals space their responding, rather than responding in erratic bursts seen with lateral hypo-

thalamic injections (M. Olds, 1979), also suggests that the rewarding consequences of the lever pressing occur soon after the response. Thus a significant portion of the critical receptor population would seem to lie proximal to the injection site in the ventral tegmental area.

The rate of responding during the first hour was significantly higher than that of each subsequent hour of testing. There are a variety of factors which could be involved in this effect including reverse tolerance, progressive effects of learning, and drug dispersion. Perhaps the most interesting possibility is that the changes in response rates are related to the differential requirements of establishing and maintaining satiating (i.e., maximally rewarding) drug concentrations at the reward-relevant population of receptors. The elevated period of responding (as little as ten minutes in some cases) may reflect the time required for morphine to occupy the proportion of these receptors necessary to produce reward satiation. Once this concentration is reached, further injections should not have additional rewarding impact until some fraction of the drug occupying these receptors is released and metabolized. A lower rate of drug intake should then maintain this concentration equilibrium. In the case of an injection directly into the reward-relevant receptor population, the duration of the initial response burst may give an indication of the size of that population.

A detailed analysis of the dispersion kinetics of

morphine produced by this infusion regimen is necessary before a firm statement regarding drug spread can be made. The small hourly intake of morphine, however, makes it extremely unlikely that the drug acts by re-entering the peripheral circulatory system or the cerebral ventricles. If the intracranial injections used in this paradigm can be localized to restricted brain regions, such localization would go far toward defining the population of opiate receptors responsible for the rewarding effects of systemically delivered opioids.

3.4 Intracranial Self-Administration of Morphine: An Anatomical Mapping Study

The next step in the investigation of the intracranial self-administration of morphine is the determination of the number and location of brain sites that can support this behavior. If it were found that the intracranial self administration of a particular drug were demonstrable throughout the brain, this paradigm might be of limited utility in identifying and in studying the brain regions which initiate the rewarding properties of that drug. It is in the localization of discrete brain regions mediating drug reward that this technique enjoys one of its most profound advantages over other paradigms. Furthermore, if the neural substrates mediating the rewarding, analgesic, sedative, and physical dependence-producing properties of opiates can be neuroanatomically dissociated, this finding would have important implications regarding the neural basis of

opiate addiction.

An early study by E. Stein and J. Olds (1977) reported intracranial self-administration of morphine into a variety of brain regions. They suggested that any site that supported brain stimulation reward might also support morphine self-administration. Following along these same lines, M. Olds (1979) has reported morphine self administration into the lateral hypothalamic area. Both of these reports, however, involved subjects that were previously trained to lever press for brain stimulation reward. This raises the possibility that the observed responding for morphine may be related to the rats' history of training. Although this possibility seems unlikely, the erratic pattern of responding for morphine injected into the lateral hypothalamic area and the apparent failure of naloxone to sustain its blockade of this putative opiate reward[6] (cf. M. Olds, 1979) point to a potential problem in using lever trained animals. Furthermore, since reinforcement by definition involves not only the maintenance but also the acquisition of a response (Bolles, 1975), the test of response acquisition in experimentally naive subjects is very important.

In this experiment, experimentally naive rats were

---

[6] See Figure 3, M. Olds (1979). Since the naloxone is delivered with the morphine (i.e., mixed with the morphine solution in the infusion apparatus), the antagonist action is present throughout the testing session. Note that responding reappears after about 11 hours of testing.

tested for the acquisition of a lever-pressing response that would deliver 100 nq of morphine into various brain regions. Using the same procedure as in the first experiment, the rats were tested for three four-hour sessions. Although this protocol does not provide a definitive assessment of the rewarding properties of morphine injected into these brain areas, it does assess the relative strength of morphine reward across the various brain regions tested. The rate of acquisition of a response has been shown to increase with increments in the magnitude of reinforcement for a variety of rewards (see Bolles, 1975). Therefore, brain regions where morphine self-administration is rapidly acquired (e.g., ventral tegmental area) would seem to be regions where morphine injections are more rewarding than those which require extensive training to establish this response.

3.4.1 Method

Subjects: Male, Long-Evans rats (weighing 325 to 375 g) were unilaterally implanted with 22 gauge guide cannulae stereotaxically aimed at one of the brain areas listed in Table 3.1. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic and a single injection of penicillin G (30,000 units, i.m.) was administered prophylactically following surgery. Obturators were fitted at a depth of 0.2 to 0.5 mm beyond the guide cannulae immediately after surgery and were removed only during behavioral testing. Food and water were available ad libitum. The animals were

housed in a 12 hour light-dark cycle of illumination and all testing occurred during the light phase of this cycle.

TABLE 3.1

STEREOTAXIC COORDINATES USED FOR THE GUIDE CANNULAE

Placement	anterior-posterior[a]	lateral[b]	ventral[c]	subjects[d]
VTA	-3.8	±0.6	7.3	6
LHA	-3.3	±1.5	6.5	6
PVG	-3.8	±0.6	5.0	5
ACC	+3.5	±1.5	5.7	5
CAUD	+2.0	±3.0	5.0	5

NOTE: The upper incisor bar was 5 mm above the interaural line for all placements except the LHA when it was 2.5 mm below the interaural line. Abbreviations: VTA, ventral tegmental area; LHA, lateral hypothalamic area; PVG, periventricular gray substance; ACC, nucleus accumbens; CAUD, caudate nucleus. The coordinates were adapted from Pellegrino, Pellegrino, and Cushman (1979).

[a]mm from bregma

[b]mm from the midline

[c]mm from dura

[d]number of subjects tested

Apparatus and Procedure: After at least five days recovery from surgery, rats were tested for the acquisition of a lever-pressing response using the same procedure as in the first experiment. Each lever press resulted in a 100 ng infusion of morphine dissolved in 100 nl of Ringer's solution. The rats were tested every four hours per day for three sessions.

housed in a 12 hour light-dark cycle of illumination and all testing occurred during the light phase of this cycle.

TABLE 3.1

STEREOTAXIC COORDINATES USED FOR THE GUIDE CANNULAE

Placement	anterior-posterior[a]	lateral[b]	ventral[c]	subjects[d]
VTA	-3.8	±0.6	7.3	6
LHA	-3.3	±1.5	6.5	6
PVG	-3.8	±0.6	5.0	5
ACC	+3.5	±1.5	5.7	5
CAUD	+2.0	±3.0	5.0	5

NOTE: The upper incisor bar was 5 mm above the interaural line for all placements except the LHA when it was 2.5 mm below the interaural line. Abbreviations: VTA, ventral tegmental area; LHA, lateral hypothalamic area; PVG, periventricular gray substance; ACC, nucleus accumbens; CAUD, caudate nucleus. The coordinates were adapted from Pellegrino, Pellegrino, and Cushman (1979).

[a]mm from bregma

[b]mm from the midline

[c]mm from dura

[d]number of subjects tested

Apparatus and Procedure: After at least five days recovery from surgery, rats were tested for the acquisition of a lever-pressing response using the same procedure as in the first experiment. Each lever press resulted in a 100 ng infusion of morphine dissolved in 100 nl of Ringer's solution. The rats were tested every four hours per day for three sessions.

3.4.2 Results

The number of infusions delivered per hour during the three sessions of testing were averaged across rats for each infusion site (see Figure 3.3). Rats with cannulae in the ventral tegmental area quickly learned the lever pressing response as in the first experiment. Rats with cannula placements in the other brain regions failed to learn this response. An ANOVA (Winer, 1971) showed a

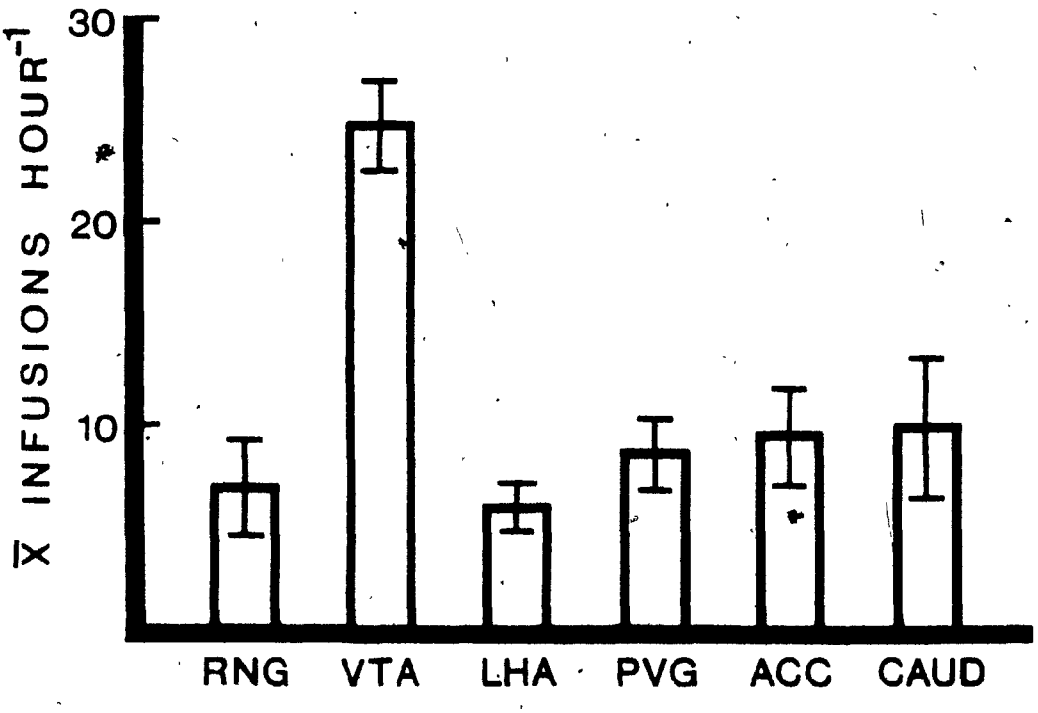

Fig. 3.3. Mean (±SEM) number of infusions per hour averaged across the three sessions of testing. RNG=Ringer's control group; the data are from the first experiment. (See Table 3.1 for abbreviations.)

significant effect for the factor of Groups {F(5,6)=10.79, p<.01}. Planned comparisons using a Tukey's (a) test (Winer, 1971) demonstrated that the ventral tegmental group was reliably different from all other groups (p's<.01). Cannula placements were histologically verified using 40 µm, thionon stained sections (Pellegrino et al., 1979) and representative placements are illustrated in Figure 3.4.

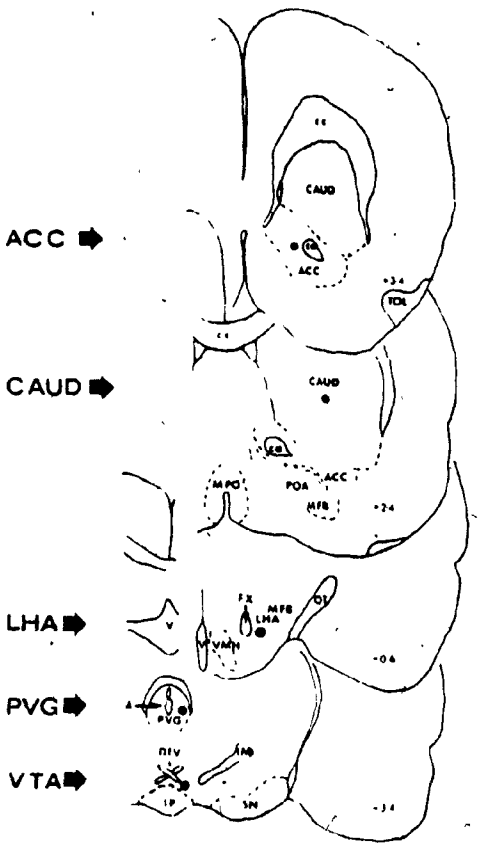

Fig. 3.4. Nominal cannula placements are indicated by the black circles. Brain sections were adapted from Pellegrino et al. (1979). Abbreviations: A, aqueduct; ACC, nucleus accumbens; ca, anterior commissure; CAUD, caudate nucleus; cc, corpus callosum; DTV, decussation of the ventral tegmentum; Fx, fornix; IP, interpeduncular nucleus; LHA, lateral hypothalamic area; MFB, medial forebrain bundle; OT, optic tract; POA, lateral preoptic area; PVG, periventricular gray substance; SN, substantia nigra; TOL, lateral olfactory tract; V, ventricle; VMH, ventromedial nucleus of the hypothalamus.

Some of the rats that received infusions into the nucleus accumbens, caudate nucleus, or periventricular gray regions showed pronounced locomotor excitement during the first 30 minutes of each session. This resulted in a burst of lever contacts, but this behavioral activation was not maintained throughout the four hours of testing. The stimulatory effect of morphine injected into the nucleus accumbens has been previously reported (Pert & Sivit, 1977), and it seems likely that the locomotor excitement produced in some animals by infusions into the caudate nucleus was the result of morphine diffusion to the accumbens. The hyperactivity seen after periventricular gray infusions of morphine has also been reported (Amir, Blair, Shizgal, & Amit, 1979; Blair, Cytrynaik, Shizgal, & Amit, 1980; Jacquet, 1978) although that seen in the present study was far less pronounced than the explosive hyperactivity produced by the higher doses previously studied.[7]

Microinjections of morphine into the ventral tegmental area also produced an increase in locomotor activity. The behavior of these animals, however, was much different than that of rats injected into the nucleus accumbens, caudate nucleus, or periventricular gray area. The locomotor excitement of the latter groups tended to be expressed as erratic bursts of activity lasting less than one hour

---

[7]Explosive motor behavior (i.e., very intense hyperactivity) is produced by the injection of 10 to 250 µg of drug into this region (Blair, Liran, Cytryniak, Shizgal, & Amit, 1978; Jacquet & Lajtha, 1973, 1974; Sharpe, Garnett, & Cicero, 1974).

after the beginning of a session. The ventral tegmental rats showed a sustained increase in activity throughout the four hours of testing. This increase in activity was less pronounced than that of the other groups, and previous tests have shown that it leads to few accidental lever contacts (see Section 3.3). Furthermore, since the microinjections into the ventral tegmental area were unilateral, the motor activity was asymmetrical resulting in circling. As previously reported, the circling was contralateral to the side of injections[8] suggesting that these morphine infusions produced an increase in dopamine release (Iwamoto & Way, 1977; Pert, DeWald, Liao, & Sivit, 1979).

3.4.3 Discussion

The failure to find morphine self-administration into the lateral hypothalamic area is in direct contrast to previous reports (M. Olds, 1979; E. Stein & J. Olds, 1977). These discrepant finds are probably the result of methodological differences. First, the reports showing self administration of morphine into the lateral hypothalamic area used guide cannulae that were considerably larger than those used in this study.[9] Routtenberg (1972) has reviewed

---

[8]The rate of rotation was measured in several animals and found to be around 4 to 5 rotations per minute when measured approximately 1 hour into the test session. Rotational behavior was also observed during the first experiment, but no attempt was made to determine the proportion of animals showing this behavior.

[9]The outside diameter of various gauges of tubing varies somewhat depending on the system of measuring that

evidence suggesting that the use of large diameter cannulae facilitates the diffusion of drug into the cerebral ventricles. The possibility exists that the reported self administration of morphine into the lateral hypothalamus is the result of such ventricular diffusion.[10] A second procedural difference is that the studies showing lateral hypothalamic self-administration used rats that were previously trained to lever-press for rewarding brain stimulation. Since M. Olds (1970) has shown that some drugs can prolong lever pressing for brain stimulation reward even when the electrical stimulation has been discontinued, it is possible that the reported self-administration represents a type of extinction responding. A similar effect has been reported for animals trained to intravenously self-administer various drugs including morphine (de Wit & Stewart, 1981; Gerber & Stretch, 1975; Stretch & Gerber, 1973). Another possibility is that lateral hypothalamic morphine is rewarding but less so than reward from ventral tegmental microinjections. Rats may maintain a previously learned lever-pressing response but fail to acquire a new response because this reinforcement

---

is used (see Hodgman, Weast, Shankland, & Selby, 1962). The specifications for commercially available hypodermic tubing show that 18 gauge tubing is around 0.7 mm in diameter while 22 gauge tubing is approximately 1.2 mm in diameter. Hence, the outside diameter of the 18 gauge cannulae is almost twice that of the 22 gauge cannulae used in the present study.

[10] The fact that the periventricular gray rats do not self-administer morphine eliminates this possibility in the present experiment since the periventricular gray placement is just 2 mm dorsal to the ventral tegmental area.

has a poor efficacy. In any case, it is obvious that reward from ventral tegmental morphine infusions is far more potent than reward from microinjections of morphine into the other brain regions tested in this study.

The fact that morphine was self-administered into ventral tegmental area but not the periventricular gray substance suggests an anatomical separation of the rewarding, analgesic, and sedative properties of opiates. The periventricular gray region has been implicated by other microinjection studies in opiate-induced analgesia (Pert & Yaksh, 1974; Sharpe et al., 1974) and sedation (Broekkamp, Van Den Bogaard, Heynen, Rops, Cools, & Van Rossum, 1976; Pert et al., 1979) while the rewarding properties of morphine appear to be mediated by receptors in the ventral tegmentum and not in the periventricular gray area. The region mediating opiate-induced physical dependence is less clearly defined, but it too seems to involve opiate receptors in the periventricular gray region and perhaps some thalamic regions as well (Wei, 1981; Wei, Loh & Way, 1973). While direct tests of the physical dependence-producing properties of ventral tegmental morphine injections have not yet been made, the present data suggest the possibility that the rewarding properties of opiates may be dissociable from their dependence-producing properties as well as the analgesic and sedative effects of these drugs. Such a dissociation of the rewarding and physical dependence-producing properties of opiates would

have a major significance for theories of addiction and direct tests will be reported in Chapter 7.

3.5 Further Investigation of Intracranial Self-Administration of Morphine into the Lateral Hypothalamic Area

The discordant findings of the present study and those of M. Olds (1979) and E. Stein and J. Olds (1977) are particularly important because they reflect directly conflicting results using the same general paradigm. One of the differences in the procedure used by M. Olds (1979) from that used in the current study concerns the size of the guide cannula implanted in the animals. M. Olds (1979) used 18 gauge cannulae while the experiments reported in Sections 3.3 and 3.4 used 22 gauge cannulae. The diameters of these cannulae are approximately 1.2 and 0.7 mm, respectively. Since the size of the cannulae used in chemical stimulation studies can affect the amount of diffusion up the cannula shaft and into the cerebral ventricles (Routtenberg, 1972), the possibility exists that animals in the M. Olds' (1979) report are self-administering morphine because the rewarding action is dependent on ventricular diffusion to another site of action. To test this hypothesis, the results of the previous study (i.e., Section 3.4) were compared with those obtained in animals implanted with cannulae having a much larger diameter.

3.5.1 Method

Subjects: Male, Long-Evans rats (weighing 350 to

400 g) were unilaterally implanted with chemitrodes[11] stereotaxically aimed at the lateral hypothalamic area using the coordinates listed in Table 3.1. Surgical anesthesia was accomplished using sodium pentobarbital (60 mg/kg, i.p.) and penicillin G (30,000 units, i.m.) was given after surgery. Obturators were fitted 0.5 mm beyond the tip of the cannula portion of the chemitrodes which remained in place except during behavioral testing. The animals had free access to food and water in their home cages and were placed on a 12 hour light-12 hour dark cycle of illumination. All testing occurred during the light cycle.

Apparatus and Procedure: Chemitrodes were made from bipolar electrodes wrapped around 22 gauge guide cannulae. Epoxy cement was used to retain the electrodes to the cannula shafts. The diameters of the chemitrodes ranged from about 1.1 to 1.5 mm and thus were similar in size to the 18 gauge cannulae used by M. Olds (1979). The animals were allowed 6 to 7 days to recover from the surgical procedure. Next, they were tested using the same procedure as described in Section 3.4: all parameters of testing

[11] Chemitrodes were originally implanted in these subjects to test another possible explanation of M. Olds' (1979) report of lateral hypothalamic self-administration. The initial protocol entailed training the subjects to press for brain stimulation reward in the lateral hypothalamus and then testing them for intracranial self-administration of morphine. A control procedure consisting of testing chemitrode animals for intracranial self-administration before experience with brain stimulation reward revealed, however, that these experimentally naive rats would self-administer morphine into the lateral hypothalamus. The results of this testing forms the basis of this section.

were identical to those used for the initial anatomical mapping study. The experimentally naive rats were tested for four hours per day every other day for a total of three sessions. Each lever press resulted in the delivery of 100 ng of morphine sulfate dissolved in Ringer's solution. The infusions were delivered over 5 seconds in a volume of 100 nl using the EMIT method described in Section 3.3.1.

3.5.2 Results

Figure 3.5 illustrates the mean number of infusions earned per hour across the three hours of testing. Rats implanted with chemitrodes in the lateral hypothalamic area quickly learned the lever-pressing response. The mean hourly intake of animals self-administering morphine into the ventral tegmental area and animals with cannulae implanted in the lateral hypothalamic area are included for comparison. An ANOVA showed a significant difference among the three groups of animals represented in the figure $\{F(2,12)=77.91, p<.001\}$. A Tukey's (a) test revealed that the rate of lever pressing in chemitrode implanted subjects was significantly higher than that seen when identical placements were tested using cannulae ($p<.01$). Also, the rate of responding was even higher than seen with cannulae implanted into the ventral tegmental area ($p<.01$).

3.5.3 Disucssion

The data presented in this section are preliminary since additional testing is required to draw definitive

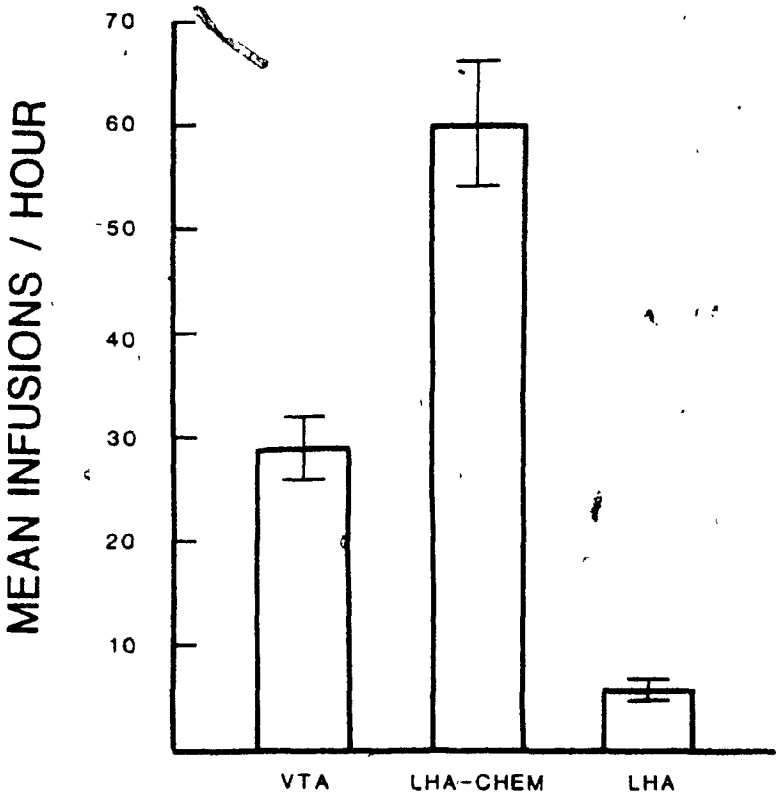

Fig. 3.5. Mean (±SEM) number of infusions per hour averaged across the three sessions of testing. VTA and LHA data are from Figure 3.3 (n=6/group). LHA-chemitrode=lateral hypothalamic placements with chemitrodes, n=3. (See Table 3.1 for other abbreviations.)

conclusions regarding the basis of lateral hypothalamic self-administration. They do suggest, however, that morphine infusions into the lateral hypothalamus are rewarding because ventricular diffusion occurs to a distal site of action. To clearly establish this, one of several procedures must be used. First, the ability of infusions dorsal to this placement to support intracranial self-administration could be evaluated. If the tissue at the tip of the cannulae were responsible for the initiation of the rewarding

effects of morphine infusions into the lateral hypothalamus, then dorsal placements should be ineffective. If ventricular diffusion were responsible for this behavior, then dorsal placements would be expected to be even more effective in establishing and maintaining self-administration. Similarly, self-administration of morphine directly into the cerebral ventricles might be tested to determine if the same pattern of self-administration occurs. Alternatively, kainic acid might be used to selectively destroy the cell bodies in the lateral hypothalamic area (Britt & Wise, 1981bc; Jonsson, 1980; McGeer & McGeer, 1981, 1982; Peterson & Moore, 1980). Since it is likely that the opiate receptors are located on the cell bodies of these neurons and not on the axons of passage (Criado, Aguilar, & De Robertis, 1981; Perry, Mullis, Oie, & Sadee, 1980; Pert, Snowman, & Snyder, 1974; see also Snyder & Matthysse, 1975), this procedure should eliminate intracranial self-administration into the lateral hypothalamic area if this behavior were dependent on the cells in this region. Self-administration which survives kainic acid lesions is likely to be dependent on drug diffusion to a distal site of action outside the lateral hypothalamic area.[12]

The fact that animals self-administer morphine into

---

[12] Another approach would be to use agents that irreversibly inactivate opiate receptors such as the compounds shown to specifically alkalate opiate receptors (Caruso, Larson, Portoghese, & Takemori, 1980; Craviso & Musacchio, 1976; Hazum, Chang, Cuatrecasas, & Pasternak, 1981; Pasternak, Childers, & Snyder, 1980). The application of this approach, however, is likely to be limited by the availability of these agents and their suitability for use in vivo.

the lateral hypothalamus at a higher rate than they self administer it into the ventral tegmentum may be indicative of a diminished potency of lateral hypothalamic morphine infusions. If these infusions of morphine were diffusing to a distal site of action such as the ventral tegmentum, the drug concentration would be appreciably diminished by the time it reached this site. Thus, it would require a larger quantity of morphine injected into the lateral hypothalamus to support self-administration behavior. On the other hand, the rapid acquisition of the lever-pressing response seen in animals with chemitrodes implanted in the lateral hypothalamus suggests that the rewarding effects of these infusions occur soon after injections. It might still be viable that this is the result of ventricular diffusion since ventricular morphine quickly penetrates into many brain regions. The rapid learning of the intracranial self administration response does, however, make the conclusions drawn from this study more tenuous. Plainly, additional testing is required to clearly establish the ability of lateral hypothalamic morphine to support intracranial self administration. The fact that this behavior is seen (in the present study) only in subjects implanted with chemitrodes is suggestive of a ventricular diffusion basis of this behavior.

3.6 General Discussion

The demonstration of behavioral and pharmacological

specificity of intracranial morphine self-administration substantiates this approach as a viable alternative for studying the neural mechanisms of opiate reward. Tests of behavioral specificity (i.e., yoked and Ringer's controls) clearly establish that the animals are working for the rewarding properties of the drug infusions. Since naloxone blocked the self-administration response, it is also apparent that lever-pressing is dependent on a drug action at opiate receptors since narcotic antagonists would not be expected to alter the physico-chemical properties of the morphine solution. Further tests showing that morphine is not self administered at most opiate receptor fields suggest that opiate reward is not the result of an opiate action common to all receptor fields. This observation implies specialization of the various opiate receptor fields for different functions; thus, opiate reward is not a ubiquitous property of these receptor fields, but rather it is associated with the activation of a specific opiate receptor field.

The fact that morphine was self-administered into the ventral tegmental area, but not other brain regions associated with other opiate effects, suggests an anatomical separation of opiate reward and other opiate-induced behaviors. This opens the possibility that use of the intracranial self-administration paradigm might minimize the side-effects caused by whole-brain drug delivery (e.g., intravenous self-administration). Thus, the sedative, analgesic, and physical dependence-producing properties of

opiates may be eliminated as potential sources of confounding during tests of opiate reward.

There are several qualifications which must be considered when generalizing the results of these studies to opiate reward produced by systemic drug injections. First, the anatomical mapping study was based on a protocol proven effective in establishing intracranial self administration into the ventral tegmental area. It is possible that increasing the dose of morphine delivered per infusion, the volume infused, or the number of trials of testing would result in acquisition of the intracranial self administration response for morphine infused into the other brain regions tested in Section 3.4. The present study provided a test only of the relative potency of morphine infused into these brain regions and not a definitive assessment of their ability to support intracranial self administration. Second, although several major opiate receptor fields were tested in this study, the possibility remains that other brain sites not yet studied will support self-administration. Third, the experiments in this chapter revolve around acquisition and different mechanisms may be responsible for the acquisition and maintenance of opiate intake. If this were the case, it might be that morphine infused into one of the other brain regions is actually more rewarding than when delivered into the ventral tegmentum. Fourth, the demonstration of intracranial self administration shows that opiate action at a given brain

region is sufficient for reward, but it does not establish that such opiate action is necessary for reward. This can only be done using tests of the rewarding properties of systemically applied opiate while blocking opiate action at specific receptor fields. It could be that concurrent activation at several opiate receptor fields (e.g., nucleus accumbens, lateral hypothalamus, periventricular gray substance) is equivalent in its rewarding effect to discrete activation of the ventral tegmentum. Thus, opiate action at the ventral tegmental area may not be necessary for this rewarding action to occur when the drug is delivered systemically and thus reaches a number of opiate receptor fields.

With the demonstration of behavioral, pharmacological, and anatomical specificity, the intracranial self administration paradigm is established as a viable method for studying the neural mechanisms of drug reward. To fully evaluate the usefulness of this technique, however, there are four additional aspects of this phenomenon which need to be studied. First, the chronicity of the preparation needs to be evaluated. The studies reported in this chapter reveal minimal tissue damage from these infusions, but there is no indication about how long the intracranial self administration response can be maintained. Second, the effects of changes in unit-dose need to be evaluated. Increasing the dose of drug delivered with each infusion might cause a decrease in response rate, but it is also

possible response rates will increase.[13] It is necessary to document the effects of unit-dose manipulations on response rates before conclusions can be drawn regarding the meaning of increased or decreased rates of responding. Third, the identification of the opiate receptor population mediating the intracranial self-administration response might suggest specific ligands lacking the addictive but retaining the analgesic properties of opiates. Special attention should be focused on evaluating the potency of mu, sigma, kappa, and delta opiate-receptor ligands. Fourth, the degree that intracranial morphine infusions control behavior in a fashion similar to intravenous drug and conventional rewards needs to be investigated. This would reveal whether this method of studying opiate reward is governed by the same principles as other rewards and would suggest the degree of generality of these data to intravenous drug reward and conventional rewards.

---

[13] With continuous reinforcement schedules, increases in unit dose generally lead to decreases in the rate of intravenous opiate self-administration; testing with low doses or partial reinforcement schedules has suggested that there may also be an ascending aspect to the dose-response curve (see Jones & Prada, 1981; Weeks & Collins, 1968, 1979; Young, Swain, & Woods, 1981; Young & Woods, 1980). Thus it is difficult to predict the influence of manipulations of unit dose on intracranial self-administration.

CHAPTER 4

THE NEUROANATOMICAL BOUNDARIES OF THE REWARD-RELEVANT OPIATE RECEPTOR FIELD[1]

4.1 Conditioned Place Preference Paradigm

The conditioned place preference paradigm can make several important contributions to the study of drug reward. First, it offers an independent method of assessing a drug's rewarding properties with a rate-free measure. This is especially useful when assessing the effects of brain lesions that can severely disrupt normal sensory-motor integration. Second, conditioning variables have been implicated in the maintenance of drug-seeking behavior (Crowder, Smith, Davis, Noel, & Cossens, 1972; Schuster & Woods, 1968). The study of conditioned place preference allows a direct comparison of such conditioning effects across different drugs and parameters of testing. Third, this paradigm is extremely quick and easy to use. It avoids the problems associated with intravenous self-administration, and it is a more direct demonstration of a drug's rewarding proper-

[1] Preliminary versions of this report have appeared in M. A. Bozarth and R. A. Wise, Society for Neuroscience Abstracts, 1981, 7, 50, and M. A. Bozarth and R. A. Wise, Localization of the reward-relevant opiate receptors. In L. S. Harris (Ed.), Problems of Drug Dependence, 1981. Washington, D. C.: NIDA Research Monograph Series, 1982.

ties than are paradigms employing brain stimulation reward. Finally, the conditioned place preference paradigm can be combined with microinjection technology to demarcate the anatomical boundaries of the reward-relevant opiate receptor population within a given brain region.

4.2 Conditioned Place Preference from Central Morphine Infusions

Intracranial self-administration studies are perhaps the most direct demonstration of the rewarding properties of central morphine injections. This technique can be used to directly compare the rewarding effects of morphine injected across various brain regions such as in Chapter 3 where the reward-relevant population of opiate receptors was identified in the ventral tegmental area. There is, however, a serious limitation to the use of this paradigm to determine the neuroanatomical boundaries of the population of opiate receptors that are responsible for opiate reward within a given brain region.

In intracranial self-administration studies, the animal controls the number of infusions and hence the total volume of drug injected into its brain. Animals with a high rate of self-administration have a larger field of effective drug spread than animals with low response rates; even microinjections through cannulae that are distal to the site of drug action may be rewarding when significant concentrations of drug have diffused to that region (see Figure 4.1). Therefore, it is difficult to estimate the

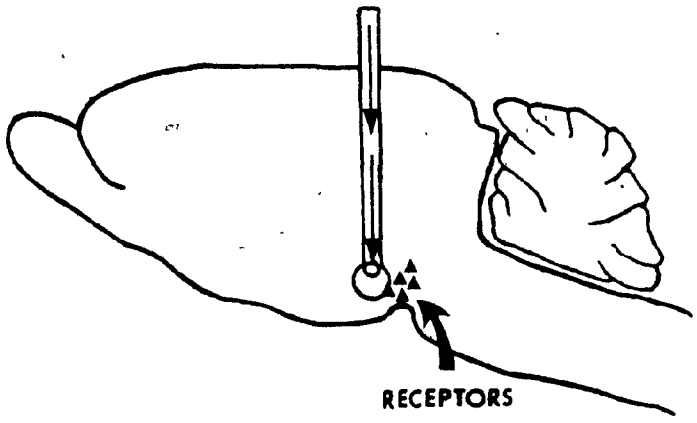

Fig. 4.1. Since changes in the infusion volume produce a concomitant change in the field of effective drug action, attempts to extrapolate the anatomical boundaries of the receptor population mediating reward from cannula placements must use a fixed infusion volume.

distance of the cannula placements to the reward-relevant receptors using intracranial self-administration experiments.

This problem can be overcome by injecting each animal with the same volume of drug and assessing reward using the conditioned place preference paradigm. Cannula placements can then be meaningfully compared to determine the location of the reward-relevant receptors. With this technique, the limits of the brain regions mediating reward from a given drug can be more precisely defined in relation to various microinjection sites.

4.2.1 Method

Subjects: Experimentally naive, male, Long-Evans

rats (weighing 350 to 400 g) were unilaterally implanted with 22 gauge guide cannulae aimed at the ventral tegmental area. With the upper incisor bar 5 mm above the intra-aural line, the coordinates ranged from 2.0 to 4.4. mm posterior to bregma, 0.6 mm lateral to the midsagittal suture, and 7.8 to 8.2 mm ventral from dura. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic with atropine sulfate (.04 mg/kg, s.c.) and penicillin G (30,000 units, i.m.) given prophylactically. Obturators were fitted approximately 0.25 mm beyond the tips of the guide cannulae and remained in place except during infusions. All testing occurred during the light phase of a 12 hour light-12 hour dark cycle of illumination. Rats were individually housed and had food and water available ad libitum in their home cages.

Apparatus and Procedure: Place preference was measured in a shuttle box (25 x 36 x 35 cm) with a plywood floor on one side and a plywood floor covered with wire mesh on the other. The amount of time spent on each side of the box was automatically recorded. Rats were allowed access to the entire shuttle box for 15 minutes per day on five consecutive days; the last day served as an indication of the animals' initial place preference. After these preconditioning trials, they received four daily injections of morphine while being forced to remain on their nonpreferred sides for 30 minutes. Following the four days of conditioning, the rats were injected with vehicle and tested

again for their place preference (15 minutes). An electrolytic microinfusion transducer (EMIT) was used to unilaterally inject morphine sulfate into the ventral tegmental area immediately before each of the four conditioning trials. A 150 μA infusion current delivered 250 ng of morphine sulfate dissolved in 500 nl of Ringer's solution. Infusions were delivered over 28 seconds through a 28 gauge injection cannula that extended 0.5 to 1.0 mm beyond the guide cannula. An additional 30 seconds was allowed for drug diffusion before the injection cannula was removed from the guide cannula. Ringer's solution was injected prior to the test trial.

Histological Analysis: Following completion of the behavioral testing, the rats were deeply anesthetized with sodium pentobarbital (circa 90 mg/kg, i.p.) and perfused intracardially with isotonic saline followed by formalin. After at least three days of fixation in formalin, the brains were sectioned at 40 micron intervals and then stained using formol-thionin. Brain sections were viewed at approximately 10 times magnification and the cannula placements were identified according to the brain atlas of Pellegrino, Pellegrino, and Cushman (1979). Changes in place preference were then plotted as a function of the number of millimeters that the cannulae were posterior to bregma on de Groot's (1959) plane of sectioning.

Since the determination of cannula placements was of central importance to this study, special attention was

focused on the method used to classify placements. The initial groupings were done with knowledge of the place preference scores for some of the subjects. Next, two additional judges blindly rated the placements for 72% of the animals. The reliability coefficient (Kerlinger, 1973) of these ratings was found to be 0.979 indicating a high degree of interjudge reliability.[2]

4.2.2 Results

Figure 4.2 shows the changes in place preference following the conditioning trials. The scores were derived

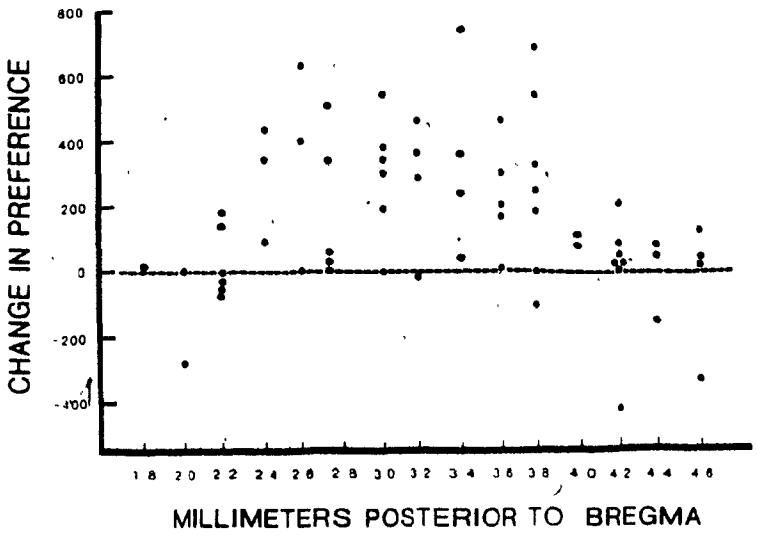

Fig. 4.2. Change in place preference as a function of cannula placement plotted for individual subjects.

[2]The main effect associated with the factor of judges was not significant {$F(2,86)=1.29$, $p>.05$} while the effect associated with differences among rats was reliable {$F(43,86)=50.97$, $p<.001$}. This type of analysis is also discussed in Winer (1971), but the computational formula of Kerlinger (1973) was used.

by subtracting the amount of time spent on the conditioning side during the last preconditioning trial from the time spent on the conditioning side after the conditioning trials. Positive scores indicate an increase in preference for the conditioning side while negative scores show a decrease in the amount of time spent on the conditioning side. The scattergram was used to determine the anatomical intervals for grouping the data for subsequent analysis in Section 4.3.

The nominal cannula placement is shown in Figure 4.3. The amount of tissue damage resulting from these infusions was minimal and probably less than that usually observed after infusions using the microsyringe method of microinjection. Most cannulae were on the lateral border of the interpeduncular nucleus just medial to the substantia nigra. Several subjects with cannulae more dorsal than those illustrated in the figure were eliminated from this study. Also, 10 animals with injection cannulae that were ventral to this region were also tested. These cannulae probably terminated in the ventral cerebral vasculature or cistern as evidenced by the frequent appearance of cerebral spinal fluid flowing up the guide cannulae (placements ranged from 2.6 to 4.0 mm posterior to bregma). The mean change in place preference for this group was 45.7 (SEM=55.8) indicating that infusions into this region were not effective in producing a conditioned place preference. This finding is important, albeit fortuitous, since it eliminates the possibility that morphine

infusions into the ventral tegmental area were rewarding because they had entered the cerebral vasculature or ventral cistern and were transported to a distal site of action.

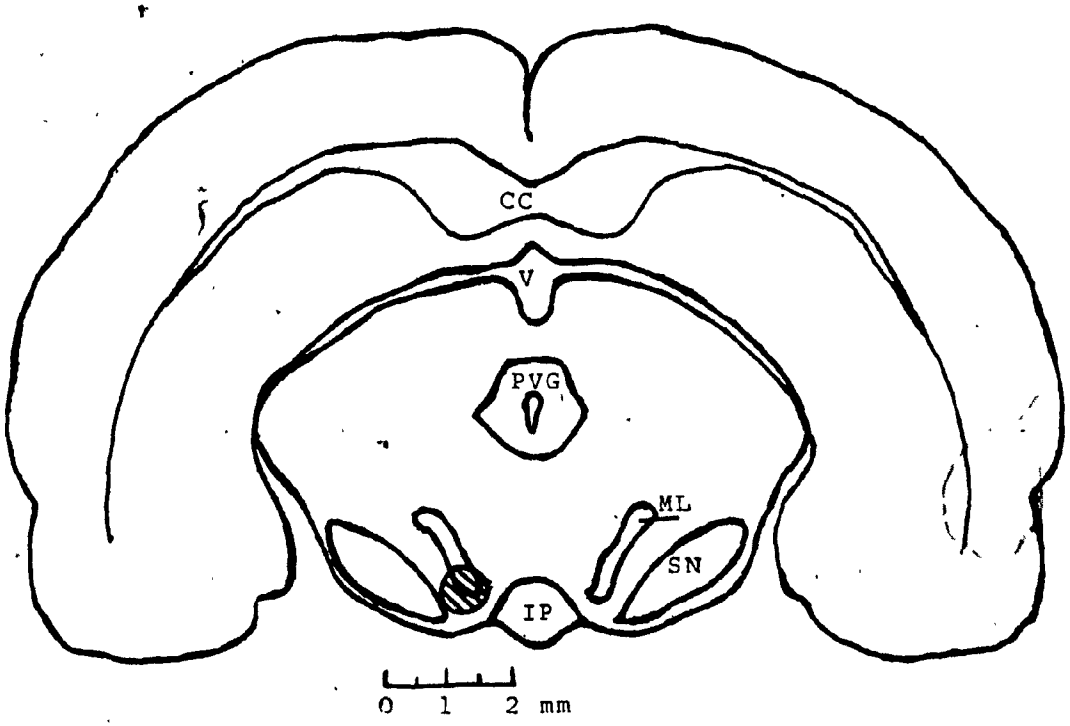

Fig. 4.3. The extent of tissue damage seen following four infusions of 500 nl delivered over 28 seconds is the striped zone. Abbreviations: CC, corpus callosum; IP, interpeduncular nucleus; ML, medial lemniscus; PVG, periventricular gray substance; SN, substantia nigra; V, ventricle.

4.3 Determination of Anatomical Boundaries

To facilitate statistical comparisons of the effects of cannula placement on place preference, the scores illustrated in Figure 4.2 were grouped into anatomical zones at approximately 0.6 mm intervals. This was guided by visual inspection of the scatterplot which suggested that placements rostral to 2.4 mm posterior to bregma and those caudal to 3.8 mm posterior to bregma were ineffective in producing a change in place preference. To decrease the differences in the number of subjects in each zone, the effective range from 2.4 to 3.8 mm posterior to bregma was also divided into two groupings. The results of this analysis is shown in Figure 4.4. An anlysis of variance (Winer, 1971) demonstrated a significant difference among rats implanted with cannulae in the various zones throughout the ventral tegmental area {$F(3,58)=10.267$, $p<.001$}. A Newman-Keuls' test was performed for specific comparisons among the various groups (Winer, 1971). Both the 2.4 to 3.0 mm and the 3.2 to 3.8 mm zones were reliably different from the rostral and caudal placements defined in this procedure ($p's<.01$).

Since the number of subjects tested in each group ranged from 9 to 20, a more conservative approach to data analysis might be to analyze each group separately for

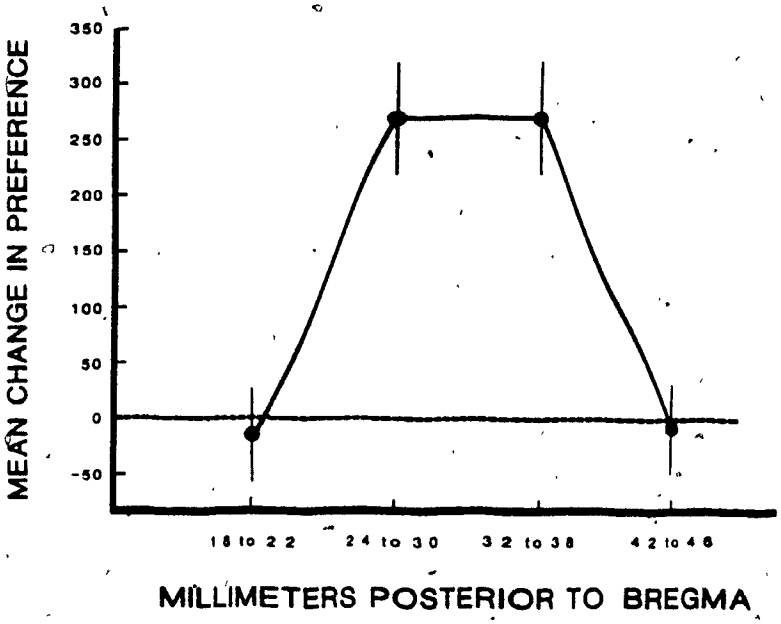

Fig. 4.4. Mean (±SEM) change in place preference for the various anatomical zones.

changes in place preference.[3] This was done by a series of t-tests and by using Fisher's method for specific comparisons (Lindman, 1974) to provide protection against Type I errors. T-tests for correlated measures revealed

[3]The data derived from the analysis by zones are normally distributed and the variances of these groups are not significantly different. Therefore, the effect of unequal sample size is probably negligible (see Lindman, 1974; Winer, 1971). Nonetheless, treating each group as an independent sample and analyzing them separately to assess changes in place preference following morphine infusions would appear to be a somewhat more conservative approach, assuming, of course, that a satisfactory method of holding the alpha level constant is used. The real strength in the conclusions suggested by the analysis of place preference as a function of anatomical zone, however, comes from the fact that both approaches to statistical analysis yield similar results.

The Neuroanatomical Substrate of Opiate Reward in the Rat

Michael A. Bozarth

A Thesis
in
The Department
of
Psychology

Presented in Partial Fulfillment of the Requirements
for the degree of Doctor of Philosophy at
Concordia University
Montréal, Québec, Canada

November 1982

ABSTRACT

THE NEUROANATOMICAL SUBSTRATE OF OPIATE REWARD IN THE RAT

Michael A. Bozarth, Ph.D.
Concordia University, 1982

An intracranial self-administration procedure was developed to test animals for the acquisition of a lever-pressing response to deliver morphine infusions into discrete brain regions. Subjects rapidly learned to self-administer morphine into the ventral tegmental area but did not learn to self-administer morphine into other opiate receptor fields. Further studies determined that the rostral-caudal boundaries of this system correspond to those of the A10 dopamine cell group.

Evidence was reviewed suggesting that opiates enhance dopaminergic neurotransmission. Behavioral indices observed during intracranial morphine self administration further support this notion. The effect of dopaminergic receptor-blockade on heroin reward was tested using two conditioning procedures. The conditioned reinforcement and the conditioned place preference

produced by systemic heroin injections were blocked by neuroleptic pretreatment. Both of these procedures tested animals in the drug-free condition eliminating motor sedation as a possible explanation of this effect.

A number of brain regions were mapped for their ability to produce physical dependence on morphine following chronic infusions. Specific attention was focused on the ventral tegmental area to determine the relationship of the site of morphine reward to the development of physical dependence. Although a moderate degree of physical dependence was demonstrable after chronic morphine infusions into this brain region, this effect was eliminated by the use of cannulae that were angled to avoid penetration of the periventricular gray substance. Infusions into the periventricular gray region produced the most severe physical dependence with withdrawal jumping emanating primarily from the rostral region while wet-dog shakes were more pronounced after infusions into the caudal region. Chronic morphine infusions into other brain regions produced little or no physical dependence.

The results of these and other studies suggest that opiate reward is produced by a drug action in the ventral tegmental area which is dependent on a dopaminergic mechanism. It is likely that the A10 dopamine system is involved in this rewarding action. Physical dependence

is produced by a drug action primarily in the periventricular gray region. These data are concordant with the notion that opiate reward is another instance of appetitive motivation and may pharmacologically activate the neural substrate of natural rewards.

ACKNOWLEDGEMENTS

A number of people have made important contributions to the development of the ideas outlined in this thesis and to the practical aspects of data collection. Dr. Larry D. Reid is thanked for the early training and for the discussion of ideas which helped form the basis of much of this research. Lydia Alessi, Martha Asselin, and William Mundl are thanked for technical assistance and for a great deal of moral support during various phases of this research project. Kali Sakell and other members of the Department of Psychology are thanked for keeping the "wolves at bay" during times when I neglected many of the bureaucratic aspects of graduate school.

I wish to express my sincere appreciation to Dr. Roy A. Wise for the support, mentorship, and friendship which have made this research possible. The environment you provided nurtured the development of these ideas and of specific techniques capable of testing the experimental hypotheses derived from them. Without such support, both professional and personal, this research project would have died in the early stages of its conceptualization.

Finally, I wish to thank Valarie for her love and support that has made the realization of my goals possible. Thanks for encouraging me to make the best professional

choice which was moving to Montreal, even though it has brought inconveniences in our personal lives. Thanks for tolerating (and even fostering) my seemingly unitary commitment to my research and the development of my ideas. Thanks for making all of this not only bearable, but enjoyable as well.

This research was supported by grants from the Natural Science and Engineering Research Council of Canada and from the National Institute on Drug Abuse (DA 2285).

This thesis is dedicated

to the memory

of my father.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

FRAME OF REFERENCE

With the discovery of brain stimulation reward (J. Olds & Milner, 1954), it has become widely accepted that the neural mechanisms subserving reward are part of a specialized reward substrate (e.g., J. Olds, 1962, 1977; Wise, 1981) and not a ubiquitous property of the nervous system: not all neurons are directly involved in reward and those that are may comprise a relatively small portion of the nervous system. Delineation of the neural basis of motivation and reward has been achieved largely by electrical brain stimulation experiments which study both the ability of electrical stimulation to induce various "motivated" behaviors[1] and the ability of electrical stimulation to directly reinforce arbitrary behavioral responses. Some of the neuroanatomy and neurochemistry of this reward system has been determined (see Hall, Bloom, & J. Olds, 1977; Wise, 1978a). The relationship of this neural substrate to natural reward has been debated, but

[1] Whether any behavior more complex than the patellar reflex is "unmotivated" could be debated to great lengths. Maslow (1954) suggests, however, that some forms of self expression may be essentially unmotivated; that is, they are "ends" in themselves and not directed toward achieving any other goal.

it appears that this system may be involved in the incentive motivational properties of conventional rewards (see J. Olds, 1962, 1977; Reid, 1967; Wise, 1974).

The brain also contains specialized receptors for opiates; again, these receptors are not distributed uniformly throughout the brain but, rather, they are clustered in numerous regions (Atweh & Kuhar, 1977ab; Pert, Kuhar, & Snyder, 1975, 1976; Snyder & Matthysse, 1975) forming what might be termed opiate-receptor fields. In the past decade endogenous substances have been isolated and identified that specifically bind to these opiate receptors (Cox, Opheim, Teschemacher, & Goldstein, 1975; Hughes, 1975; Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975; Pasternak, Goodman, & Snyder, 1975; Terenius & Wahlstrom, 1975; Teschemacher, Opheim, Cox, & Goldstein, 1975) and the role of these endorphins[2] in physiology and behavior has become the subject of considerable research. Some of the processes advanced as being influenced by endorphinergic systems include social behavior (Kavaliers, 1981; Panksepp, Herman, Vilberg, Bishop, & DeEskinazi, 1978), feeding and drinking (Holtzman, 1975; Jalowiec, Panksepp, Zolovick, Najam, & Herman, 1981; Morley,

---

[2]Endorphin is used as the generic term to refer to endogenous opioid-like peptides. This is in agreement with the policy of many researchers in the field (e.g., Goldstein & Cox, 1978; Simon & Hiller, 1978; Smith & Simon, 1981), although Adler (1980) has suggested that endogenous opioid peptides would better describe the class.

1980; Ostrowski, Rowland, Foley, Nelson, & Reid, 1981; Sanger, 1981; Siviy, Calcagnetti, & Reid, 1982); learning and memory (Izquierdo, 1979; Rigter, 1978; Riley, Zellner, & Duncan, 1980; L. Stein & Belluzzi, 1979), pain (Amir, Brown, & Amit, 1980; Fields, 1981; Lewis, Caldecott-Hazard, Cannon, & Liebeskind, 1981; Terenius, 1978) psychopathology (Berger, Watson, Akil, Elliot, Rubin, Pfefferbaum, Davis, Barchas, & Li, 1980; Davis & Bunney, 1980; Watson, Akil, Berger, & Barchas, 1979), copulation (Gessa, Paglietti, & Quarantotti, 1979; Murphy, 1981; Myers & Baum, 1980), and opiate addiction (Herz, 1981; Herz, Hollt, & Przewtocki, 1980). (For general reviews of endorphins and behavior, see Barchas, Akil, Elliot, Holman, & Watson, 1978; Bolles & Fanselow, 1982; Kosterlitz, 1980; Olson, Olson, Kastin, & Coy, 1980; Smith & Simon, 1981). Narcotic antagonists, which are assumed to block the actions of endorphins (cf. Hill, 1981; Sawynok, Pinsky, & LeBella, 1979), have been shown to produce dysphoria and mental depression in humans (File & Silverstone, 1981; Hollister, Johnson, Boukhabza, & Gillespie, 1981; Mendelson, Ellingboe, Keuhnle, & Mello, 1979). Thus endorphins appear to influence many aspects of behavior and have been postulated to have a special role in motivation and reward (Belluzzi, & L. Stein, 1977; L. Stein, 1978; L. Stein & Belluzzi, 1978, 1979). These effects can potentially be revealed by studying the action of drugs which activate or block the activation of opiate

receptors[3] (i.e., opiates and narcotic antagonists, respectively).

One of the most dramatic pharmacological actions of opiates is their ability to reward or "reinforce" behaviors associated with their self-administration. In fact, drug addiction is currently defined in behavioral terms emphasizing a preoccupation with the acquisition and assimilation of the drug (Jaffe, 1975, Martin & Sloan, 1977). Most biological properties, such as physical dependence and analgesia, are not considered necessary attributes of a compound to qualify it as an addictive agent. Rather, the determination that a drug is an addictive agent relies on a demonstration of the behavioral aspects of addiction since the relevant biological properties of these drugs have not yet been identified.

There is no pre-existing need to ingest opiates, and considerable experience is necessary to establish opiate assimilation with the characteristic vigor seen in chronic drug addiction. Once established, however, this behavior may result in the diminution of the efficacy of natural rewards. This ability of opiates and other addictive agents to reinforce their own ingestion while some-

---

[3]Endorphins have been considered as endogenous ligands for opiate receptors and this practice reflects the chronology of discoveries made in this field (i.e., opiate receptors were discovered before endorphins). However, it might be more appropriate to consider opiates as exogenous ligands for endorphin receptors.

times blunting the rewarding impact of other reinforcers suggests that opiates may have profound effects on the neural mechanisms underlying conventional motivation and reward.

Most theories of drug addiction have focused on psychodynamic or sociological models of drug abuse (see Hoch & Zubin, 1958; Lettieri, Sayers, & Pearson, 1980). One of the most popular theories of drug addiction that has been directly related to biological processes involves the physical dependence-producing properties of opiates. The repeated use of large quantities of these drugs produces a physical dependence on their continued intake. When opiate administration is discontinued, withdrawal symptoms emerge that can be readily suppressed by opiate assimilation. It has been suggested that the discomfort produced by withdrawal from opiates in the physically dependent person provides the motivation for the continued ingestion of opiates (e.g., Wikler, Martin, Pescor, & Eades, 1963; Wikler & Pescor, 1967; Wikler, Pescor, Miller, & Porrell, 1971). This model dominated much of the early preclinical screening of new compounds for addiction liability (e.g., Committee on Problems of Drug Dependence, 1970).

Although tension-reduction models[4] of opiate addic-

[4]The hypothesis that opiates are ingested to avoid withdrawal distress is basically a variant of the tension reduction model. Essentially, some aversive event (e.g.,

tion have remained popular explanations of drug addiction (e.g., Dole, 1980; Lindesmith, 1980; Wikler, 1980; see also Lettieri et al., 1980), current views have shifted in favor of positive-reinforcement models of drug addiction. Initial assimilation of opiates produces pronounced "feelings of well being" (see Martin & Sloan, 1977; McAuliffe, 1975; McAuliffe & Gordon, 1974) which have been termed positive-euphoria[5] (Kolb, 1925). These mood elevating properties of opiates have been recognized for many years by some researchers (e.g., Eddy, Halback, & Braenden, 1957; Martin, 1966) even though psychosocial and tension-reduction theories continued to dominate the field of drug addiction (e.g., Lindesmith, 1938, 1970, 1980; Prout, White, & Charry, 1958; Rado, 1958; Rasor, 1958; deRopp, 1957).

The physical-dependence withdrawal syndrome associated with opiates may contribute to the long-term maintenance of opiate addiction, but acquisition and early maintenance of opiate assimilation appear to be motivated

---

withdrawal discomfort, psychological tension) is terminated by the ingestion of an opiate. This might also be viewed as negative reinforcement (see Chapter 7).

[5]In common usage euphoria connotes a state of bliss or ecstasy. The mood elevation experienced by opiate addicts is probably not well described by this term; the effect of opiates may be more subtle producing what is better described as a feeling of well being or contentment. Nonetheless, euphoria is frequently used to refer to this alteration in mood and the reader is cautioned about the specialized meaning of this term.

by positive reinforcement processes.[6] For this reason attention has focused on the effects of opiates on the neural mechanisms of reward and attempts to identify an action of opiates on the substrate of natural rewards have become the subject of considerable interest.

The study of opiate addiction and the delineation of the mechanisms involved in the acquisition and maintenance of addiction have implications that transcend the treatment and prevention of drug abuse. The fact that these drugs have profound effects on motivated behavior make them potentially useful tools for the study of brain mechanisms subserving reward and motivation. Seldom do conventional rewards seem to control and direct behavior as strongly as addictive drugs reinforce the behaviors associated with their acquisition and assimilation. Thus the study of opiate addiction may be viewed as a study of the neural substrate of basic motivational processes using opiates as exogenous ligands to determine the role of endorphins in the biological mediation of motivated behavior.

---

[6]The role of physical dependence in opiate addiction will be addressed in more detail in Chapter 7. In general, the evidence argues against a central role of physical dependence in the genesis of drug addiction (see Eddy et al., 1957; Jaffe, 1975; Martin & Sloan, 1977).

CHAPTER 2

TECHNIQUES USED TO STUDY THE NEURAL MECHANISMS OF OPIATE REWARD[1]

2.1 Methods of Localizing Opiate Reward

The interest in opiates as positive reinforcers has prompted a search for the identification of the neural mechanisms subserving opiate reward and its relationship to the neural substrate mediating the effects of conventional rewards. Several techniques have been used to study the rewarding properties of abused drugs. Each offers a unique contribution to the study of drug reward, but each is also limited in the type of question that it can answer. The strongest support for any relationship is provided by convergent evidence across several different techniques.

2.1.1 Brain Stimulation Reward

One approach has been to examine the interaction of abused drugs with rewarding brain stimulation (Adams, Lorens, & Mitchell, 1972; Blake & Halpern, 1971; Miller, 1957; J.

---

[1]Portions of this chapter have appeared in M. A. Bozarth, Opiate reward mechanisms mapped by intracranial self-administration. In J. E. Smith and J. D. Lane (Eds.), The Neurobiology of Opiate Reward Processes. Amsterdam: Elsevier Biomedical Press, 1983 (in press) and M. A. Bozarth and R. A. Wise, Electrolytic microinfusion transducer system: An alternative method of intracranial drug application. Journal of Neuroscience Methods, 1980, 2, 273-275.

Olds & Travis 1960). Opiates, as well as many other drugs of abuse, can facilitate brain stimulation reward (Esposito & Kornetsky, 1978; Reid & Bozarth, 1978; Wise, 1980). This facilitation has been demonstrated as both a lowering of thresholds (Kelly & Reid, 1977; Marcus & Kornetsky, 1974) and an increase in rates of responding for a fixed intensity of brain stimulation reward (Adams, et al., 1972; Bush, Bush, Miller, & Reid, 1976; Lorens & Mitchell, 1973). In fact, the effects of various opiates on self-stimulation seem to be a better indicant of their relative addiction liability than is their ability to produce physical dependence (Reid & Bozarth, 1978). Although the data gathered from this technique are promising, studies of brain stimulaton reward have not received much attention from drug addiction specialists. This is probably because this proposed model of drug reward makes several important assumptions which have not been empirically validated (see Wise, 1980). As the critical predictions derived from this model are systematically explored (e.g., Bozarth, Gerber, & Wise, 1980; Gerber, Bozarth, & Wise, 1981), the potential usefulness of this technique should become increasingly appreciated.

If the facilitation of brain stimulation reward reflects the intrinsically rewarding properties of abused drugs, Broekkamp and his coworkers (e.g., Broekkamp, 1976; Broekkamp, Van Den Bogaard, Heynen, Rops, Cools, & Van Rossum, 1976) appear to have localized the site of action for morphine's rewarding effect. In an elegant series of

experiments, it was found that morphine produced facilitation of brain stimulation reward when microinjected into several brain sites. The shortest latency to onset and the strongest magnitude of effect were associated with microinjections into the ventral tegmental area. It was concluded that the facilitations resulting from morphine injections into other brain regions (e.g., posterior lateral hypothalamus; lateral cerebral ventricle) were probably the result of drug diffusion to this area. While this finding has been replicated with both morphine and D-ala^2-met^5-enkephalinamide (Broekkamp, Phillips, & Cools, 1979), the conclusions drawn from this work seem to have been largely ignored. Broekkamp (1976) used response rates as the dependent measure of brain stimulation reward and this metric has been criticized by workers within the field (Kornetsky & Esposito, 1979; Valenstein, 1964). The use of response rates and the lack of widespread acceptance of this model of opiate reward have severely blunted the impact of this potentially important work. Ironically, the independent confirmation of the ventral tegmental area as the site of morphine's rewarding action would strengthen the proposed relationship between a drug's intrinsically rewarding properties and its effect on brain stimulation reward. Broekkamp's (Broekkamp, 1976; Broekkamp et al., 1976; Broekkamp et al., 1979) suggestion that the ventral tegmental area is the site of action for morphine reward remains an unsupported speculation, and the localization of the brain

site responsible for morphine's rewarding action remains a problem for other techniques to determine.

2.1.2 Intravenous Self-Administration

The intravenous self-administration paradigm is probably the most widely accepted method of assessing the rewarding properties of abused drugs.[2] There are direct and obvious parallels between human drug abuse and animal self-administration. With the exception of hallucinogenic substances, drugs which are abused by humans are generally self-administered by laboratory animals and drugs which are not self-administered by animals are usually not abused by humans (see Schuster, 1973; Seiden & Dykstra, 1977; Woods, 1978). This has helped to make the intravenous self-administration paradigm the preparation of choice for studying the rewarding properties of many abused drugs.

A wealth of information regarding how drug rewards can control operant behavior has been gained using the

---

[2]Oral models of opiate self-administration have also yielded useful information regarding the nature of opiate reward (e.g., Alexander, Beyerstein, Hadaway, & Coambs, 1981; Alexander, Coambs, & Hadaway, 1978; Amit, Corcoran, Amir, & Urca, 1973; Carroll & Meisch, 1978; Collaer, Magnuson, & Reid, 1977; Gorman, DeObaldia, Scott, & Reid, 1978; Khavari & Risner, 1973). This method offers several advantages such as (i) simplification of the preparation making the testing of large numbers of subjects feasible, (ii) long preparation life making chronic intake studies practical, and (iii) continuous access to opiate providing a true ad libitum assessment of the pattern and quantity of opiate intake. This method, however, lacks the advantages of the intravenous model such as immediacy of the rewarding effect and regulated patterns of intake. A discussion of the relative advantages of each method can be found in Altshuler (1978) and Seiden and Dykstra (1977).

intravenous self-administration paradigm (see Thompson & Pickens, 1971). Opiates and psychomotor stimulants have been shown to control behavior in much the same way as do conventional rewards such as food and water (Spealman & Goldberg, 1978; Woods, 1978). With experience, stable patterns of responding for drug emerge and changes in the response-reinforcement contingencies, such as the dose of drug delivered per infusion, lead to predictable changes in the rate of self-administration (Woods & Schuster, 1968). Likewise, alterations in the pattern of drug intake have been interpreted in terms of changes in the reinforcing efficacy of the drug infusions (e.g., Yokel & Wise, 1975, 1976). While this paradigm provides an excellent method of assessing the effects of some manipulations on drug reward, attempts to localize the brain site of rewarding drug action have been plagued by serious limitations of this technique.

The most common method of studying the neuroanatomical substrate of drug reward has been to lesion various brain regions and to compare drug self-administration before and after lesioning (e.g., Glick & Cox, 1977, 1978; Glick, Cox, & Crane, 1975; Lyness, Friedle, & Moore, 1979; Pozuelo & Kerr, 1972; Roberts, Corcoran, & Fibiger, 1977; Roberts, Koob, Klonoff, & Fibiger, 1980). Although this technique can be useful, a variety of nonspecified lesion effects can confound behavioral measures of drug reward. Most notable are the profound changes in locomotor activity, feeding, and

drinking that accompany lesions of some of the more interesting brain regions such as the medial forebrain bundle or ventral tegmental area (Teitelbaum & Epstein, 1962; Ungerstedt, 1971d). Such side-effects make the consequences of lesioning very difficult to interpret since behavioral indices of drug reward are easily confounded by treatments that alter motor performance. Furthermore, some neural systems show dynamic changes in functioning after denervation (Cannon & Rosenblueth, 1937). The best documented of these systems is the dopamine-containing nigro-striatal system which shows increased neurotransmitter release and receptor binding after the partial destruction of its synaptic terminals (Creese & Snyder, 1978; Hefti, Melamed, & Wurtman, 1980; Mishra, Marshall, & Varmuza, 1980). This compensatory activity can lead to a restoration of function (Stricker & Zigmond, 1976; Teitelbaum & Epstein, 1962; Ungerstedt, 1971cd) and a normalization of responding for drug (see Roberts et al., 1980) that could mask the primary effect of lesioning. Even when the motor debilitating effects and functional recovery can be controlled, lesions that disrupt drug intake would suggest that the lesioned element is critical for drug reward but would not suggest that the drug is acting directly at the lesioned site. Lesions at any point along the system activated by a drug might disrupt self-administration, despite the fact that the lesioned element may be several synapses removed from the initial target of drug action (see Figure 2.1; see Adler & Geller (1978) for

an informative discussion of the problems associated with the interpretation of lesion data).

Another method that has been used to localize the site of various opiate effects involves selectively blocking the action of systemically injected opiate at a restricted brain site (e.g., Laschka, Teschemacher, Mehraein, & Herz, 1976b; Wei, Loh, & Way, 1973; Wilcox & Levitt, 1978). After systemic injection of an opiate, animals receive a micro-injection of a narcotic antagonist directly into a specific brain region. This procedure allows opiate action at all

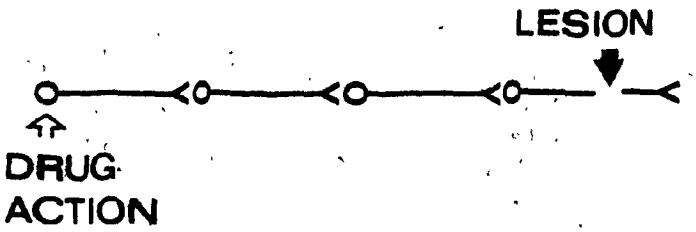

Fig. 2.1. Lesions anywhere in a series of neuronal elements can eliminate drug reward, even if the drug is not acting directly at the lesioned element. For this reason, lesions do not identify the site where a drug initiates the sequence of events that lead to reward but only identifies individual elements which mediate this reward.

brain sites except the one that received the microinjected narcotic antagonist. The ability of the centrally delivered antagonist to reverse the systemically induced opiate effect suggests the location of the opiate-receptor field mediating the behavior under study.

Using this approach, the intravenous self-administration of an opiate can be challenged with central microinjections of a narcotic antagonist, and changes in the pattern of drug intake can be interpreted to reflect the importance of a given brain site in opiate reward. There are, however, three serious limitations to this approach. The first problem with this technique is that the most commonly used narcotic antagonists (i.e., naloxone, naltrexone) have a high lipid solubility; drugs that possess this property diffuse rapidly throughout the brain making localization of the effect impossible (Schubert, Teschemacher, Kreutzberg, & Herz, 1970). New drugs have been developed that are hydrophilic and thus avoid this problem (see Britt & Wise, 1981a), but difficulties in interpreting the results of these studies can emerge if drugs of the mixed agonist-antagonist type are used (e.g., diallyl-normorphinium bromide).[3] A second problem associated with the regional

---

[3]Diallyl-nor-morphinium bromide is a quaternary derivative of nalorphine (Laschka, Herz, Blasig, 1976a). Like its parent compound, nalorphine, it is a mixed agonist-antagonist. Drugs in this class (e.g., cyclazocine, levallorphanol, nalorphine, pentazocine) have both narcotic agonist and antagonist properties (Houde, 1979; Jaffe & Martin, 1975; Martin, 1967).

application of narcotic antagonist is related to the role of endorphins in behavior. Drugs that block the effects of opiates also block the effects of endogenously released endorphins. This may lead to the production of behaviors that are incompatible with opiate self-administration but are not directly related to the reinforcing efficacy of the systemically injected drug. These effects include (i) changes in motor behavior caused by the administration of narcotic antagonists (Amir, Galina, Blair, Brown, & Amit, 1980; Pert, DeWald, Liao, & Sivit, 1979), (ii) deficits in the regulation of food and water intake (Holtzman, 1975, 1979; Ostrowski, Foley, Lind, & Reid, 1980; Siviy, Calcagnetti, & Reid, 1982), and (iii) alterations in schedule-controlled behavior (Downs & Woods, 1976; Kelleher & Goldberg, 1979; McMillan & Morse, 1967). The third and most serious problem with the use of centrally delivered narcotic antagonists to determine brain sites involved in opiate reward involves the physical dependence-producing properties of opiates. While it has been suggested that opiate self-administration is independent of physical dependence (Thompson, 1968; Woods & Schuster, 1968), animals will lever press to avoid withdrawal reactions (Goldberg, Hoffmeister, Schlichting, & Wuttke, 1971b; Goldberg, Woods, & Schuster, 1971c; Weeks, 1962; Weeks & Collins, 1979). If a narcotic antagonist were microinjected into a brain region involved in physical dependence, it might be expected that the animals would elevate their opiate intake to compensate

for the displacement of opiate at this receptor site. This pattern of responding could easily be mistaken to indicate that opiate reward had been attenuated by the microinjected narcotic antagonist although the animal is simply attempting to avoid withdrawal discomfort. A total cessation of responding might also be caused by withdrawal stress even though no overt signs of abstinence are noted.[4]

Recently, neurochemical techniques have been used to determine changes in regional brain activity (Glick, Cox, & Meibach, 1980) and neurotransmitter release (Smith, Co, Freeman, & Lane, 1982; Smith, Co, Freeman, Sands, & Lane, 1980) during intravenous morphine self-administration. These studies can provide valuable information regarding the neurochemistry of opiate reward, but caution must be exercised in the interpretation of such data. This type of correlative neurochemical approach[5] fails to discriminate

---

[4]Goldberg et al. (1971ab) have shown that monkeys increase their intake of morphine when challenged with small doses of a narcotic antagonist but show a dose-dependent suppression of responding at higher doses. They have suggested that this is caused by the precipitation of withdrawal reactions since overt signs of physical dependence were seen. Similarly, rats have been observed to abruptly stop self-administration of heroin when challenged with narcotic antagonists without the compensatory increase in drug intake usually thought to occur in this situation (G. Gerber, personal communication). In the latter case, withdrawal signs were not noted.

[5]Although it has been argued that all data are correlational in nature (Hume, 1739), the designation "correlative approach" is frequently applied to what might more appropriately be termed an observational approach (Campbell & Stanley, 1966; Sheridan, 1979; Wood, 1974); others have referred to this method as natural accretion measures (Webb, Campbell, Schwartz, & Sechrest, 1966) and ex post facto research (Kerlinger, 1973).

evoked activity that is relevant to a given behavior from evoked activity that is unrelated. Such neurochemical studies can be very informative, but they need direction from other types of experiments that clearly show the importance of the brain region or neurotransmitter under study. Otherwise, it is impossible to discern whether the brain system in question is involved in the locomotor, thermoregulatory, analgesic, rewarding, or dependence-producing properties of opiates. Indeed, correlative neurochemical evidence alone cannot demonstrate that these actions are causally related to any behavioral effect of opiates.

2.1.3 Conditioned Place Preference

Another method that has been proposed to study the rewarding properties of abused drugs is the conditioned place preference paradigm (Rossi & Reid, 1976; Schwartz & Marchok, 1974; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980a).[6] In this paradigm, animals are confined to a normally non-preferred portion of a test chamber following injections of a drug. They are subsequently tested under drug-free conditions to determine if a learned preference or

---

[6]The term conditioned place preference is a misnomer. The animals rarely spend over half of the total period on the conditioning side of the test apparatus. The use of this term is retained, however, since it has become widely accepted by those working in the field (e.g., Bozarth & Wise, 1981b; Mucha, Vand Der Kooy, O'Shaughnessy, & Bucenieks, 1982; Phillips & LePiane, 1980; Phillips, Spyraki, & Fibiger, 1982; Stewart & Grupp, 1981; Van Der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982).

aversion has developed to the place where they had experienced the drug effect. This technique has been proposed to reveal the affective valance of the drug experience; if the animals show a conditioned place preference for the side where the drug effect was experienced, it is frequently inferred that the drug state has positive affective consequences (Rossi & Reid, 1976). This paradigm offers the advantage of not making any response demands on the animal in the drugged condition, so it avoids the problem of sedative side-effects of some drug treatments. The influence of motor debilitating brain lesions is also decreased since the response requirement necessary to assess place preference is minimal.

Phillips and LePiane (1980) have shown that morphine injected into the ventral tegmental area produces a conditioned place preference similar to that caused by systemic morphine injections (Rossi & Reid, 1976; Sherman et al., 1980a; Stapleton, Lind, Merriman, Bozarth, & Reid, 1979). This finding is concordant with Broekkamp (1976) and supports the notion that the ventral tegmental area is the site of morphine's rewarding action. The potential limitations of this technique have not been explored, but it would appear that either anxiolytic or physical dependence-producing properties of a drug might influence the place preference. Habituation to the side of putative conditioning during anxiolytic drug action or drug-seeking associated with the relief of withdrawal distress could also increase the prefer-

ence for the side where the drug effect was experienced. In the case of morphine microinjections into brain regions not associated with physical dependence, this latter possibility is unlikely. Nonetheless, the fact that the conditioned place preference paradigm does not appear to yield graded dose-response effects (Phillips & LePiane, 1980) and is not sensitive to the number of conditioning trials (see Phillips & LePiane, 1980; Stapleton et al., 1979) questions the basis of this conditioned response.

2.1.4 Intracranial Self-Administration

Perhaps the most powerful method to demonstrate that a drug has its rewarding action at a particular receptor field is to show that the drug is self-administered directly into that brain region. Self-administration procedures can be modified to allow the infusion of drug directly into discrete brain regions, and responding for central drug can be interpreted in much the same way as responding for intravenous injections. Furthermore, by restricting drug delivery to the reward-relevant receptor field, the intracranial self-administration paradigm can potentially minimize many of the side-effects caused by systemic drug injections.

Intracranial self-administration offers a novel method of assessing the rewarding impact of drug activation of specific brain regions. It should be noted, however, that this technique identifies brain regions where drug action is sufficient for reward, but it does not determine

if that action is necessary for reward. This can only be accomplished by selectively blocking a drug's action in a specific brain area and by assessing the rewarding impact of systemic drug injections. Brain sites identified by intracranial self-administration studies should be tested using central receptor-blockade challenge of either intravenous self-administration or conditioned place preference. This can determine if the brain regions that are sufficient to produce reward are also necessary for reward from systemically delivered drug. Other problems with the interpretation of intracranial self-administration data will be discussed in later sections.

2.2 Methodological Considerations

Many of the methods of studying brain mechanisms of opiate reward rely on drug microinjection techniques. These methods are subject to the limitations of microinjection technology which have been reviewed in detail elsewhere (Myers, 1972, 1974; Routtenberg, 1972). The study of intracranial self-administration adds two new dimensions to the problems of drug microinjections: (i) the need for smaller infusion volumes necessitated by repeated drug administration and (2) the requirement of response-contingent drug infusions in freely moving animals. These considerations are of minor importance in other applications of microinjection technology where the experimenter controls a single, large infusion of drug, frequently into a restrained

animal. The inability of existing methods to provide response contingent, small volume drug delivery has been the most obvious obstacle to the study of intracranial self-administration.

2.2.1 Technical Aspects of Microinjection

Work with single infusions into brain tissue has shown that the volume of drug injected is a critical determinant of the area of drug spread throughout the brain (Myers, 1974; Myers & Hoch, 1978; Routtenberg, 1972).[7] For localization of drug action, it has been suggested that the infusion volume be 0.5 µl or less; larger volumes are likely to lead to excessive drug spread, making definition of the relevant brain site difficult. Even with an infusion volume of 1 µl, however, drug has been reported to spread only about 0.6 mm from the injection cannula (Lomax, 1966). Similar estimates of drug spread have been derived from both physical dispersion kinetics (Lomax, 1966; Myers & Hoch, 1978; but see also Schubert et al., 1970) and functional tests of drug diffusion (Lomax, 1967; Lotti, Lomax, & George, 1965). These reports are surprising since a 1 µl volume of drug would occupy 1 mm^3 in vitro.

---

[7] Other important variables include the physicochemical properties of the substance, the concentration of the injected substance, and the proximity of intracerebral vascular supply to the infusion site. Diffusion of drug up the cannula shaft has also been a persistent problem, especially with rapid infusion rates. See Jacquet (1975), Myers (1972, 1974), and Routtenberg (1972) for a discussion of these and other factors affecting drug diffusion after intracranial application. Furthermore, the radius of drug spread may vary as a function of time after the injection (Schubert et al., 1970).

Unfortunately, little is known about the rate of drug dispersion following an infusion, and one cannot equate acute infusion volumes with the cumulative volumes reached with repeated infusions. A single injection of 1 µl is not equivalent to 10 injections of 0.1 µl since the tissue can accommodate a series of small microinjections more readily than a single bolus infusion. The interval between microinjections thus becomes a critical variable in determining the range of drug spread after repeated infusions. In vivo estimates must be derived to determine the maximum allowable volume for chronic infusion experiments. Albert and Madryga (1980) have reported data that suggest that repeated drug infusions can be accommodated surprisingly rapidly. In a study of the functionally effective spread of 4 µl of lidocaine infused over 15 minutes, they estimated the range of effective drug spread to be 0.25 to 0.6 mm from the injection cannula. This range is much smaller than would be predicted from the physical spread of the same volume injected over a shorter period of time (Myers, 1974; Myers & Hoch, 1978). The study of Albert and Madryga (1980) illustrates the necessity of determining drug spread estimates for repeated infusions in vivo and not relying on estimates of similar volumes injected in a single bolus.

Confirmation of drug delivery is a primary consideration in studies employing microinjection techniques. It should be noted that calibration, not computation of drug flow, is the critical factor. The relationship between the

volume of drug displaced in the injection apparatus (e.g., microsyringe) and the amount actually delivered to the brain is seldom obvious. It cannot be assumed that a 1 mm^3 displacement at the microsyringe will produce a 1 µl infusion volume to a subject some distance away. The volume of drug injected needs to be determined empirically with a technique similar to that actually used during behavioral testing. This will take into account some of the error produced by the introduction of various elements between the infusion transducer and the subject (e.g., tubing, fluid swivel). Bench tests at atmospheric pressure offer a first approximation to the amount of drug delivered, but accurate measurement of the injected volume requires in vivo determination of the amount of drug actually delivered into the behaving animal. This can be done by using radiolabelled tracers and by determining the amount of radioactive material recoverable from the brain tissue after microinjection (e.g., Lomax, 1966; Myers & Hoch, 1978; Routtenberg, 1972).

It is also important that normal drug flow be visually confirmed at the injection cannula before and after behavior testing. It cannot be safely assumed that the infusion system is functioning properly during behavioral testing. Frequently, the injection cannula becomes obstructed as it is placed in the guide cannula because tissue or blood has accumulated in the guide shaft. High pressure microinjection systems tend to minimize the influence of small tissue obstructions, but low pressure systems

are particularly sensitive to this problem.

There are several variables that must be considered when selecting a method of drug delivery. The sizes of the guide and the injection cannulae partially determine the resolution of the anatomical mapping which can be accomplished with a given system. Dispersion of drug up the cannula shaft is facilitated by the use of a large diameter cannula (Routtenberg, 1972), but problems with obstruction are augmented with smaller cannulae as are the difficulties in construction and utilization. The pressure generated at the tip of the injection cannula is a function of the flow rate and of the diameter of the injection cannula. For a given rate of infusion, small diameter injection cannulae will achieve a higher injection pressure and hence more tissue damage than will large diameter cannulae. This can also alter the dispersion of drug during microinjections. The use of a 28 gauge injection cannula combined with a 22 gauge guide cannula seems to offer a reasonable compromise between anatomical resolution and practical considerations (cf. Jacquet, 1975; Routtenberg, 1972).

2.2.2 Drug Delivery Systems

The most important factor that intracranial self-administration studies add to the existing problems of microinjection technology is the requirement of small volume, response contingent drug delivery. If infusions are delayed or prolonged in most behavioral tests involving microinjec-

tions, usually few problems are associated with response measurement. Alterations in the time course and in dose-response relationship may be produced, but little chance exists of missing the main effect associated with the drug infusion. Intracranial self-administration studies, however, necessitate that drug infusions be contiguous with some operant response such as lever pressing: that is, microinjections must occur immediately after lever pressing and drug must not be infused when the subject is not making the appropriate response. Otherwise, the animal is unlikely to learn the lever-pressing response since a temporal delay of reward severely impairs response acquisition (Renner, 1964; Tarpy & Sawabini, 1974). The problem of contiguous drug application would appear to be even more important than the reliability of the infusion volume since the animal can behaviorally compensate for slight variations in the amount of drug delivered by making additional drug requests. There is, however, no substitute for contiguity, except perhaps in the well trained animal capable of performing on a partial reinforcement schedule.

Conventional microinjection systems are very similar to their counterparts used for intravenous self-administration (e.g., Myers, 1974; J. Olds, 1962; M. Olds, 1979). A motor driven syringe pump is used to advance the plunger of a microsyringe displacing a controlled amount of drug solution. This solution is forced by hydraulic pressure through a length of flexible tubing connected to a cannula implanted

in the subject. Lever presses activate the infusion pump for a predetermined duration, and the infusion volume is assumed to be controlled by the amount of drug solution displaced in the microsyringe.

While the microsyringe may dispense reliable volumes of drug, the elasticity inherent in the flexible tubing leads to variability in the injected volume. This problem is exacerbated by movement of the animal producing additional stretching and compression of the flexible tubing, thus resulting in uncontrolled (i.e., noncontingent) drug delivery. The introduction of a fluid swivel, permitting rotation of the animal, can compound the problem of reliable drug delivery by creating leaks and "dead space" in the delivery system. Therefore, control over both the amount and contiguity of drug infusions is sacrificed by allowing the subject unrestrained movement. Attempts to relieve the stress on the connecting tubing by the use of a spring-covered infusion line may decrease the delivery of noncontingent drug but probably does not assure accuracy in the low microliter range. In an early attempt to demonstrate intracranial self-administration of morphine, it was noted that animals implanted with cannulae in the periventricular gray substance showed strong analgesia after being allowed to explore the test chamber, even though no microinjections were given (M. Bozarth, unpublished observations). This was probably the result of the noncontingent infusion of several microliters of the morphine

solution.[8]

As an alternative to the microsyringe method, J. Olds, Yuwiler, M. Olds, and Yun (1964) and later E. Stein and Rodd (1980) have developed a microinjection system that eliminates the need for a fluid swivel. Recognizing the problem associated with the introduction of a swivel, this system used a series of rollers to compress the flexible tubing and to deliver drug to the subject. The rollers are mounted on an electrical commutator maintaining unrestricted movement of the subject during testing. Bench tests of the infusion volume suggest promising results (E. Stein & Rodd, 1980), but close inspection of the data shows that this system also produces large variations in the infused volume of drug. This lack of reliability regarding the volume of drug infused would be expected to be even greater in the freely moving animal since this system retains the flexible tubing to connect the animal to the infusion apparatus.[9]

---

[8]The infusion of 1 to 5 µg of morphine into the periventricular gray has been shown to produce analgesia (Mohrland & Gebhard, 1980; Sharpe, Garnett, & Cicero, 1974; Yaksh, Yeung, & Rudy, 1976). Since the concentration of the test solution was 0.25 µg/µl, it appears that an amount in excess of 4 µl was delivered to produce the level of analgesia observed in these subjects.

[9]E. Stein and Rodd's (1980) presentation of their data is somewhat misleading. First, if the volume actually injected, calculated from the counts per minute in their Table 1 (i.e., counts obtained in the sample divided by the standard of 8140 counts per minute per microliter), is compared to the volume they attempted to inject, it is apparent that the infusion volumes ranged from 63 to 143% of the intended volumes. Second, the smallest volume that they verified is 10 times the volume they claim to be using on a routine basis. Third, the system is not adequately tested

Another approach to this problem has been to eliminate both the fluid swivel and flexible tubing. This has been accomplished by using an electrolytic microinfusion transducer (EMIT) system that was adapted from a method originally described by Criswell (1977). With the EMIT method, a gas-tight drug reservoir is filled with drug dissolved in Ringer's solution and attached to an injection cannula positioned in the target area through an implanted guide cannula (Bozarth & Wise, 1980a). Infusions are controlled by applying a direct current across two electrodes contained in the gas-tight drug reservoir (see Figure 2.2). The current flow across the electrodes produces hydrogen gas and the pressure that is generated forces drug through the injection cannula. The amount of drug delivered is controlled by the current intensity and duration. The EMIT assembly is mounted directly on the animal's head. Light flexible wires are used to connect this unit to a constant current source (e.g., Mundl, 1981) and an electrical commutator assures unrestricted movement of the subject during testing.

---

in freely moving animals. Even the diffusion controls are not sufficient since the labeled 2-deoxyglucose could have gone through several half-lives during the 20 hours allowed for passive diffusion (Sokoloff, 1981); it should be noted that (i) maximum noncontingent drug delivery, resulting from stretching the flexible tubing, would probably occur during the first few minutes of testing and (ii) retention of 2-deoxyglucose in brain tissue is a function of glucose utilization rates and a large central injection would probably be followed by appreciable transport of this substance out of the brain.

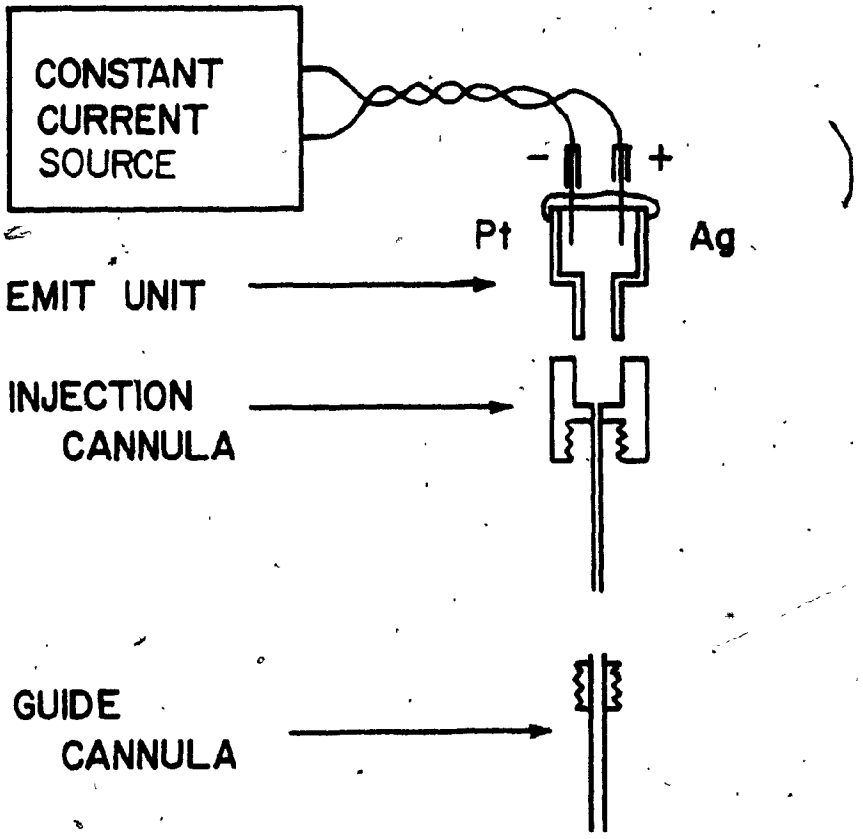

Fig. 2.2. The volume of solution infused can be calculated by the formula µl=0.12 x mA x seconds. Since choloride ions may be produced during the application of current, care must be exercised in the selection of electrode materials. A platinum (Pt) cathode is used while the anode is silver (Ag) wire. Free chloride will combine with the silver anode forming silver chloride on the electrode surface and preventing the mixing of chloride ions with the drug solution.

With the elimination of the fluid swivel and flexible tubing, the major sources of error in small volume drug delivery are circumvented. Movements of the subject during testing can no longer affect the operation of the microinjection system and contiguity of response and drug delivery is achieved. The lower limit of the volume that can be reliably delivered with EMIT system has not been assessed but probably extends into the mid nanoliter range.[10] Smaller volumes than this are probably ineffective since passive drug diffusion from the injection cannula may proceed at a rate higher than the actual volume infused (see Routtenberg, 1972). The EMIT method is routinely used to deliver a 100 nl infusion volume during tests of intracranial self-administration (e.g., Bozarth & Wise, 1980bc, 1981ab). This represents a practical trade-off between a volume giving reasonable anatomical resolution and a volume that can be visualized to check the patency of the drug delivery system before and after behavioral testing.

---

[10] Preliminary tests of the volume delivered with the EMIT system have shown that a 200 μA current applied for five seconds produces a 100±5 nl infusion. These bench tests monitored the movement of an air bubble through a microsyringe across a series of 50 infusions (M. Bozarth, unpublished observation). Similar tests suggest accuracy with 50 nl volumes, although this method of measurement does not permit detection of discrete infusions below 100 nl. Independent tests have confirmed the reliability of this system at 100 nl volumes using radiolabeled infusions in vitro (N. Goeders, personal communication). Subsequent references in the text to the volume of drug delivered (e.g., Sections 3.3.1, 4.2.1) are based on these estimates since the actual volume delivered has not been determined for this preparation. It is unlikely, however, that the volume infused in vivo would exceed that obtained in the bench tests where the effect of tissue resistance is absent.

CHAPTER 3

INTRACRANIAL SELF-ADMINISTRATION OF MORPHINE IN RATS[1]

3.1 Rationale for the study of Intracranial Self-Administration

Just as the discovery of electrical brain stimulation reward by J. Olds and Milner (1954) opened a new avenue of research in the study of brain reward mechanisms, the development of intracranial self-administration can add an important new dimension to this same field. Electrical stimulation is grossly nonspecific activating most cells within a given radius of the stimulating electrode.[2] Chemical stimulation provides a method of activating only certain neurons within a given region of stimulation and relating this effect to specific neurotransmitter systems (Fisher,

---

[1]Portions of this chapter have appeared in M. A. Bozarth and R. A. Wise, Intracranial self-administration of morphine into the ventral tegmental area in rats. Life Sciences, 1981, 28, 551-555, and in M. A. Bozarth and R. A. Wise, Localization of the reward-relevant opiate receptors. In L. S. Harris (Ed.), Problems of Drug Dependence, 1981. Washington, D. C.: NIDA Research Monograph Series, 1982.

[2]Ranck (1975; 1981) has reviewed evidence that some units (e.g., large myelinated fibers) are more easily excited than others (e.g., cell bodies), but in general there is no selectivity produced by electrical stimulation in regard to the neurochemical coding of the elements activated or the direction of impulse conduction.

1956; Grossman, 1962; Miller, 1965; Myers, 1974). While this technique may be preferable to electrical stimulation in many circumstances, attempts to develop a paradigm to demonstrate the reliable self-administration of chemicals into the brain has a documented history of failure (see J. Olds, 1962). Early reports from several laboratories provided encouraging results (Morgane, 1962; Myers, 1963; J. Olds & M. Olds, 1958; J. Olds, Yuwiler, M. Olds, & Yun, 1964), but this work has remained obscure with virtually no mention of it in the current literature (cf. M. Olds, 1979; Wise, 1980). This is probably because chemical brain stimulation was floundering while the field of electrical brain stimulation was rapidly developing.

The rationale for the study of intracranial morphine self-administration is three-fold. First, this procedure may circumvent some of the problems encountered with other methods of localizing the reward-relevant opiate receptor population. Second, if some of the effects of opiates (e.g., analgesia, sedation) were initiated at brain sites other than those supporting intracranial self-administration, then these effects of systemic opiate self-administration could be minimized and the rewarding properties of opiates studied without the potentially confounding influence of these other effects. Third, the development of a paradigm that demonstrates intracranial self-administration of any substance opens the possibility of using this same technique to study

reward from other chemical injections. Identification of the chemical coding of reward neurons might then be possible as well as the study of the central sites of action of other addictive agents.

3.2 Criteria for Establishing the Validity of Intracranial Self-Administration

The field of intracranial self-administration is relatively new and, thus far, few guidelines have been established for assessing the conclusions based on data from this paradigm. There are several criteria that must be fulfilled before such data can be expected to contribute meaningfully to our understanding of the brain mechanisms of motivation and reward. Attempts to delineate the mechanism of action for central self-administration of a drug are useless unless they are preceded by a firm empirical basis establishing the validity of drug self-administration, qua drug self-administration.

3.2.1 Behavioral Specificity

The first criterion for establishing the validity of intracranial self-administration studies is the demonstration that the animals are working for the rewarding properties of the drug. Microinjections of morphine into brain tissue can produce increases in locomotor activity and stereotypy (Joyce & Iversen, 1979; Pert & Sivit, 1977) that could artifactually elevate lever-press scores by causing accidental lever contacts. It must be clearly

established that the lever-press response is dependent on the rewarding effects of the drug infusions and not the result of nonspecific behavioral activation.

Procedures that have been employed in the study of intravenous self-administration can be used to determine the behavioral specificity of the lever-pressing response (see Pickens & Thompson, 1971). One such procedure is the yoked control where one animal lever presses for response contingent infusions of drug while another passively receives infusions. Each experimental animal is paired with a yoked control partner such that lever presses by the experimental animal produce concurrent infusions in both animals. Lever presses of the yoked control subject are recorded but do not produce infusions. With this procedure the reinforcing properties of the drug can be inferred from differences between the response rates of the experimental and yoked control subjects. Another method of determining the degree of nonspecific lever-pressing is the two lever choice test. In this procedure each animal is tested in a two-lever box. Responses on one lever produce response contingent drug infusions while responses on the other are recorded but do not produce infusions. Increases in responding on the "inactive"lever are interpreted as nonspecific behavioral activation or arousal.

A potential problem with each of these procedures is that increased responding on an "inactive" lever (i.e.,

yoked control or "inactive" lever in the two-lever choice test) may reflect nonspecific behavioral arousal, but it does not preclude the possibility that the drug infusions are rewarding. This limitation is probably more pronounced in the two-lever choice test where the subject is required to discriminate between an active and inactive lever. In this case the animal may continue to press the inactive lever because of (i) response generalization or (ii) superstitious behavior resulting from a delayed rewarding impact of the drug infusions. For these reasons the yoked control procedure may be preferable to the two-lever choice test although positive findings from both techniques are more definitive. In either case responding on the inactive lever does not prove that the drug infusions are not rewarding, but, rather, it suggests that more rigid control procedures are necessary to assess the effect.[3]

3.2.2 Pharmacological Specificity

Once behavioral specificity has been established,

---

[3] If a cue light associated with rewarding drug infusions were located directly over the lever, autoshaping may occur and lead to increases in lever pressing of the yoked control animals. This phenomenon is well established in procedures showing that animals display increased activity and will approach and manipulate a stimulus correlated with reward (Bindra & Palfai, 1967; Epstein & Skinner, 1980; Hall, Channel, & Pearce, 1981; Leslie, Boakes, Linaza, & Ridgers, 1979; see also Bindra 1972, 1974; Bolles, 1975). Thus the behaviorally arousing effects of rewarding stimuli present another potential source of "inflated" lever-press scores of control animals.

it must be shown that the rewarding effects of the central drug injections are dependent on the same mechanism as mediates systemic drug reward. The rewarding effects of microinjections that result from nonspecific changes in cell function are of limited interest to the study of drug reward. Intracranial self-administration must be shown to depend on the same neural mechanisms as reward from systemic drug injections if this technique be used for the localization of the reward-relevant receptor population.[4] In the case of opiate reward, this pharmacological specificity can easily be established by challenging intracranial self administration with a narcotic antagonist such as naloxone. If this pharmacological challenge blocks the lever-pressing response, then it can safely be concluded that morphine self administration is not due to changes in cell osmolarity, calcium chelation, or other nonspecific factors associated with the microinjections. Active and inactive stereoisomers[5]

---

[4]This is important if conclusions be drawn from these experiments regarding the nature of systemic drug reward. If intravenous amphetamine injections were rewarding through an action mediated at dopaminergic systems (e.g., release of dopamine, reuptake blockade), then treatments that block the effects of this action should also block the intracranial self-administration of amphetamine. For drug actions that are not initiated at specific receptor sites, this type of control procedure becomes much more difficult. This is likely to be the case for barbiturates (Ho & Harris, 1981) and ethanol (Deitrich & Erwin, 1980; Goldstein, 1979; Goldstein & Chin, 1981; Sun & Seaman, 1980; Wayner, Ono, & Nolley, 1975). The criterion of pharmacological specificity might have to be suspended until the primary action of the agent can be identified and procedures developed to block this action at the level of the cell membrane.

[5]It is important that stereoisomers and not geometric

of an opiate can also be compared to determine if the effect be mediated by opiate receptors.

In a review by J. Olds (1962), the importance of demonstrating pharmacological specificity was dramatically illustrated when it was shown that the intracranial self administration of norepinephrine was an artifact of its calcium chelating properties. It appears that this nonspecific activation of neurons in the lateral hypothalamic area produces reward in much the same way as does electrical stimulation of these neurons. Thus the demonstration of reward from chemical stimulation of this area was of little interest since it merely mimicked the nonspecific activation caused by electrical stimulation.

Unfortunately, the early work of J. Olds (1962) seems to have been neglected. Cytawa and Jurkowlaniec (1978, 1979) and Cytawa, Jurkowlaniec, & Bialowas (1980) have again reported that noradrenergic stimulation of the lateral hypothalamus produces positive reinforcement. While these studies may not suffer from the problem encountered with the early work of J. Olds (1962), the new reports fail to demonstrate that this rewarding action of norepinephrine shows pharmacological specificity. Without establishing that this effect is (i) blocked by noradrenergic synthesis inhibition, (ii) blocked by drugs that selectively.

---

isomers of a drug be used in this type of control procedure. Stereoisomers retain the physico-chemical properties of the parent compound while geometric isomers frequently do not (Beckett, 1959).

block noradrenergic receptors, or (iii) produced only by the biologically active stereoisomer of norepinephrine, these studies cannot be meaningfully interpreted. Similar problems in interpretation may arise from studies employing other agents. Recently, cocaine self-administration has been reported into the frontal cortex (Goeders & Smith, 1982). Unfortunately, no control procedures were used to determine the pharmacological specificity of this response; the potent local anesthetic properties of cocaine (Ritchie & Cohen, 1975) make nonspecific effects on neural functioning a viable explanation of this intracranial self administration response.

3.2.3 Anatomical Specificity

The single most important contribution that intracranial self-administration studies can make to the understanding of the neural mechanisms of opiate reward is the localization of the reward-relevant receptor field(s). Once a suitable preparation has been developed (i.e., behavioral and pharmacological specificity established), the brain can be mapped for sites which support self administration. If it were found that a large number of brain areas engendered lever pressing for intracranial morphine injections, then this paradigm might be of limited utility in the characterization of opiate reward mechanisms. If intracranial self-administration of morphine occurred at only a few brain sites, then this technique could be used

to restrict drug delivery to the reward-relevant receptor sites. This would raise the possibility of minimizing the influence of reward-independent effects (e.g., analgesia? physical dependence? sedation?) that are associated with the whole-brain drug delivery that results from intravenous self-administration.

There are two aspects of anatomical specificity to be considered. The first is a comparison across various brain sites to determine if multiple brain regions support self-administration. Using a standard protocol, the brain can be neuroanatomically mapped for sites that support this behavior. Negative findings are difficult to interpret since a larger drug dose or infusion volume could yield conflicting results. Nonetheless, the relative sensitivity of various brain regions to morphine reward can be determined using a reasonably effective dose range and an infusion volume that minimizes the influence of drug diffusion. Those brain regions that are the most sensitive to the rewarding properties of a drug are likely to be closest to the target of rewarding drug action and the most important regions involved in the mediation of reward from systemically administered drug.

The second aspect of anatomical specificity involves the assessment of drug spread within a given brain region. The purpose of this procedure is two-fold: (1) to determine if the rewarding effects of the microinjections were due to a local action or diffusion to a distal site of action, and

(2) to define the anatomical boundaries of the receptor field mediating drug reward within a given brain region. The dispersion kinetics of microinjected drug can be determined by using autoradiography (see Routtenberg, 1972) or by combining the micro-punch assay technique of Palkovits (1973) with liquid scintillation counting (e.g., Myers & Hoch, 1978). These methods provide a quantitative estimate of the physical spread of drug produced by a given microinjection procedure. Another approach is to assess the functional effective spread of drug by systematically varying the cannula placements within a given brain region. This method has been used to determine the anatomical boundaries of other opiate effects (e.g., Lomax, 1967; Yaksh, Yeung, & Rudy, 1976). The latter approach has the advantage of showing the limits of spread for behaviorally relevant concentrations of drug but is more laborious. Only whole-brain autoradiography, however, can detect the spread of drug to distal sites of action that can be produced by ventricular diffusion or microinjection into intracerebral circulatory systems. A combination of these techniques would obviously be advantageous.

3.3 Intracranial Self-Administration of Morphine into the Ventral Tegmental Area

Broekkamp's (1976) study of the facilitatory effect of central morphine injections on brain stimulation reward suggested that the ventral tegmental area might be the site of morphine's rewarding action. The present study was

designed to determine if naive rats would learn to press a lever for morphine infusions into the ventral tegmental area. To control for accidental lever contacts, a yoked control procedure was used. Each experimental rat was paired with a yoked control subject such that lever presses by the experimental rat produced concurrent infusions in both the experimental and yoked control subjects. Lever presses of the yoked control rat were recorded but did not produce an infusion. Animals were also tested for the self administration of the drug vehicle solution. The results of these two control procedures were then used to determine the behavioral specificity of the intracranial self administration response.

3.3.1 Method

Subjects: Fifteen experimentally naive, male, Long-Evans rats, weighing 300 to 350 g at the time of surgery, were unilaterally implanted with 22 gauge guide cannulae stereotaxically aimed at the ventral tegmental area. With the upper incisor bar 5 mm above the interaural line, the coordinates for the guide cannulae were 3.8 mm posterior to bregma, 0.6 mm lateral to the mid-sagittal suture, and 7.8 mm ventral to dura. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic and a single injection of penicillin G (30,000 units, i.m.) was administered prophylactically following surgery. Obturators were fitted at a depth of 0.5 mm beyond the guide cannulae imme-

diately after surgery and remained in place except during behavioral testing. Food and water were available ad libitum.

Apparatus: Rats were tested in a 27 x 38 x 39 cm box housed in a dimly illuminated, sound attenuating chamber. A small exhaust fan provided ventilation and additional masking of peripheral noise. On one end of the test chamber, a 4 x 6 cm lever was located 5 cm above the floor.

The microinfusions were delivered by an electrolytic microinfusion transducer (EMIT) system mounted directly on the rat's head during testing. Infusion was accomplished by passing a direct current between two electrodes contained in the gas-tight reservoir. The production of hydrogen gas forced a controlled amount of solution through the injection cannula while a small quiescent current prevented the redissolution of hydrogen gas evolved during previous infusions. The use of EMIT method minimized the problems of uncontrollable drug infusions inherent in systems which rely on flexible tubing and fluid swivels to permit unrestrained movement of the subject during testing. (For details of the EMIT method, see Bozarth & Wise, 1980a, and Section 2.2.2.)

The EMIT unit was connected to a constant current source (Mundl, 1981) which produced a 200 µA infusion current and a 10 µA quiescent current. Depression of the lever resulted in a 100 nl infusion delivered over five seconds. The EMIT unit was attached to a 28 gauge hypodermic needle

precut to penetrate 1 mm beyond the guide cannula. A light flexible lead was used to connect the EMIT unit to a mercury commutator allowing unrestrained movement of the rat during testing.

Procedure: After at least five days recovery from surgery, rats were randomly assigned to either the experimental, yoked control, or Ringer's control group. For the experimental group, depression of the lever resulted in a 100 ng infusion of morphine sulfate (300 pmoles/infusion) dissolved in 100 nl of Ringer's solution. Yoked control rats were placed in an identical test chamber and infused concurrently with their experimental partners. The lever presses of the yoked control group were recorded but did not produce infusions. Five rats were tested for intracranial self-administration of Ringer's solution under conditions otherwise identical to the experimental group. The house light was extinguished and a cue light was activated during infusions for all groups.

The rats were tested every other day for three four hour sessions. Four rats from the experimental group received intraperitoneal injections of naloxone hydrochloride (10 mg/kg) one hour into a fourth test session.

3.3.2 Results

The mean number of responses per hour during the first three sessions is depicted in Figure 3.1. The experimental group showed a rapid acquisition of the lever-pressing

response while the responding of the yoked control group was similar to that of the Ringer's control group. An analysis of variance (ANOVA) with repeated measures on one factor (Winer, 1971) revealed a significant effect for the factors of Groups {$F(2,13)=17.95$, $p<.001$} and Hours of Testing {$F(3,36)=16.63$, $p<.001$}. Planned comparisons using a Neuman-Keuls' test (Winer, 1971) demonstrated significant differences between the experimental and other groups during

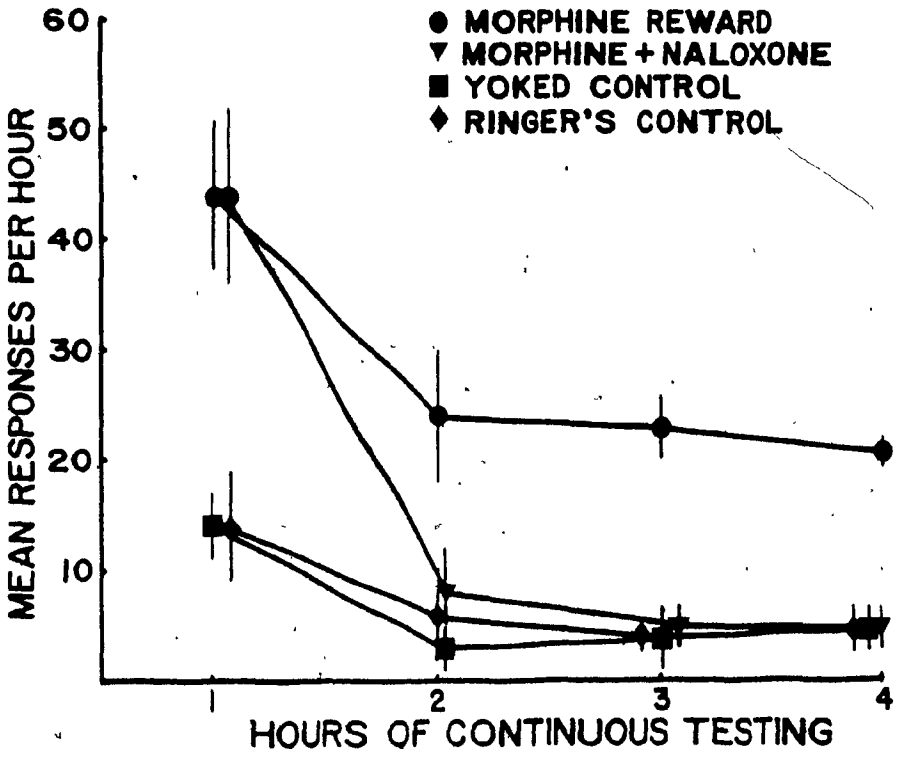

Fig. 3.1. A comparison of the responding of the experimental (morphine reward), yoked control, and Ringer's control groups (n=5/group). The figure depicts the mean (±SEM) number of responses per hour averaged across the three sessions of testing. The experimental group received an injection of naloxone one hour into their fourth test session (morphine + naloxone).

all hours of testing (p's<.01). The responding of the experimental group during the first hour of testing was significantly greater than their response rates during each of the subsequent three one hour periods (p's<.01).

Given one hour into the fourth test session, naloxone effectively blocked intracranial self-administration (see Figure 3.1). A t-test for correlated measures did not show any differences between sessions for the one hour prior to naloxone treatment {t(3)=2.35, p>.3}, but an ANOVA with repeated measures on both factors (Winer, 1971) revealed a significant difference between treatments following naloxone {F(1,3)=21.81, p<.025}.

Histological analysis confirmed that cannula placements were in the ventral tegmental area. Most cannulae were just lateral to the border of the interpeduncular nucleus (see Figure 3.2). Cell damage was similar to that seen after discrete infusions of 0.5 µl or less (M. Bozarth, unpublished observations). Although the dispersion kinetics of this infusion regimen have not been determined, the proximal location of opiate receptors in the A9 and A10 dopaminergic cell bodies makes this a likely site of action (Lindvall & Björklund, 1974; Pollard, Llorens, Bonnet, Costentin, & Schwartz, 1977; Pollard, Llorens, & Schwartz, 1977). Other sites of action cannot be eliminated until discrete localization of these infusions has been demonstrated.

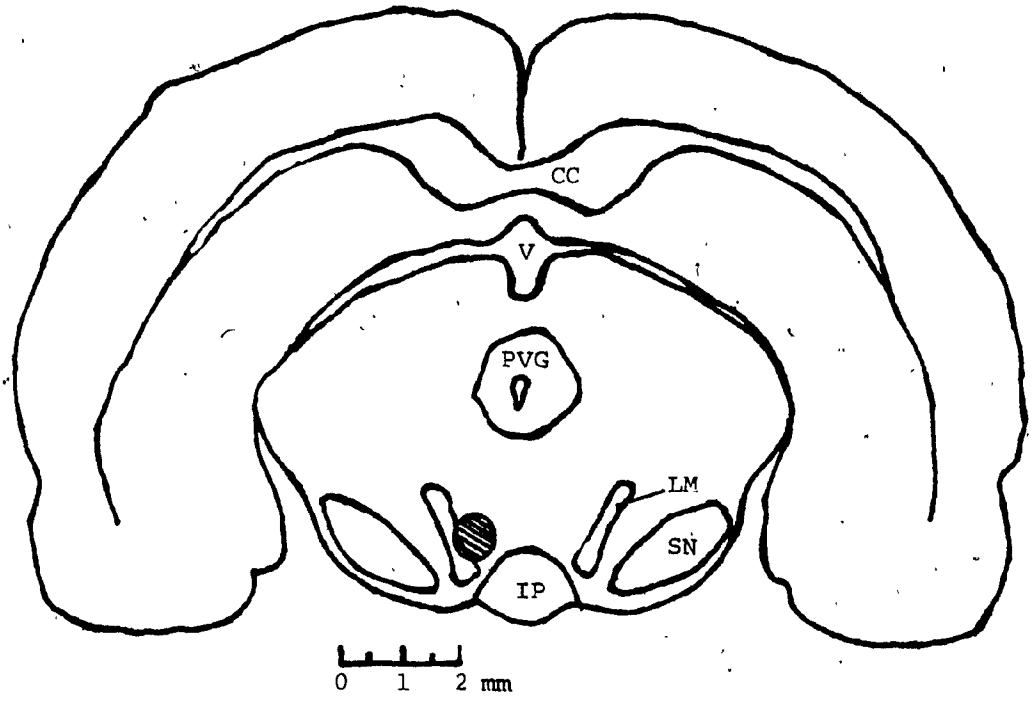

Fig. 3.2. The extent of tissue damage seen in animals self-administering morphine is indicated by the striped zone. Abbreviations: CC, corpus callosum; IP, interpeduncular nucleus; LM, medial lemniscus; PVG, periventricular gray substance; SN, substantia nigra; V, ventricle.

3.3.3 Discussion

The present data suggest that intracranial morphine injections can serve as a true reinforcer, establishing increased levels of lever pressing in a response-contingent manner. Support for this conclusion is derived from the facts that intracranial self-administration was demonstrated in experimentally naive rats and that animals receiving the drug as a consequence of lever pressing responded more than animals passively receiving the same pattern of injections in a yoked control condition. Since naloxone effectively blocked lever pressing, it would appear that the intracranial self-administration of morphine is mediated through opiate receptor mechanisms (see Martin, 1967). This eliminates the possibility that self-administration was the result of mechanical trauma or of changes in osmolarity or pH factors, since these effects would not be sensitive to opiate receptor blockade.

The rapid acquisition of responding seen in experimentally naive subjects suggests that the rewarding effects of microinjected morphine occur soon after the infusions. Pronounced delays in reward from these injections would not be expected to lead to such rapid acquisition of the lever pressing response (Renner, 1964; Tarpy & Sawabini, 1974). The fact that the animals space their responding, rather than responding in erratic bursts seen with lateral hypo-

thalamic injections (M. Olds, 1979), also suggests that the rewarding consequences of the lever pressing occur soon after the response. Thus a significant portion of the critical receptor population would seem to lie proximal to the injection site in the ventral tegmental area.

The rate of responding during the first hour was significantly higher than that of each subsequent hour of testing. There are a variety of factors which could be involved in this effect including reverse tolerance, progressive effects of learning, and drug dispersion. Perhaps the most interesting possibility is that the changes in response rates are related to the differential requirements of establishing and maintaining satiating (i.e., maximally rewarding) drug concentrations at the reward-relevant population of receptors. The elevated period of responding (as little as ten minutes in some cases) may reflect the time required for morphine to occupy the proportion of these receptors necessary to produce reward satiation. Once this concentration is reached, further injections should not have additional rewarding impact until some fraction of the drug occupying these receptors is released and metabolized. A lower rate of drug intake should then maintain this concentration equilibrium. In the case of an injection directly into the reward-relevant receptor population, the duration of the initial response burst may give an indication of the size of that population.

A detailed analysis of the dispersion kinetics of

morphine produced by this infusion regimen is necessary before a firm statement regarding drug spread can be made. The small hourly intake of morphine, however, makes it extremely unlikely that the drug acts by re-entering the peripheral circulatory system or the cerebral ventricles. If the intracranial injections used in this paradigm can be localized to restricted brain regions, such localization would go far toward defining the population of opiate receptors responsible for the rewarding effects of systemically delivered opioids.

3.4 Intracranial Self-Administration of Morphine: An Anatomical Mapping Study

The next step in the investigation of the intracranial self-administration of morphine is the determination of the number and location of brain sites that can support this behavior. If it were found that the intracranial self administration of a particular drug were demonstrable throughout the brain, this paradigm might be of limited utility in identifying and in studying the brain regions which initiate the rewarding properties of that drug. It is in the localization of discrete brain regions mediating drug reward that this technique enjoys one of its most profound advantages over other paradigms. Furthermore, if the neural substrates mediating the rewarding, analgesic, sedative, and physical dependence-producing properties of opiates can be neuroanatomically dissociated, this finding would have important implications regarding the neural basis of

opiate addiction.

An early study by E. Stein and J. Olds (1977) reported intracranial self-administration of morphine into a variety of brain regions. They suggested that any site that supported brain stimulation reward might also support morphine self-administration. Following along these same lines, M. Olds (1979) has reported morphine self administration into the lateral hypothalamic area. Both of these reports, however, involved subjects that were previously trained to lever press for brain stimulation reward. This raises the possibility that the observed responding for morphine may be related to the rats' history of training. Although this possibility seems unlikely, the erratic pattern of responding for morphine injected into the lateral hypothalamic area and the apparent failure of naloxone to sustain its blockade of this putative opiate reward[6] (cf. M. Olds, 1979) point to a potential problem in using lever trained animals. Furthermore, since reinforcement by definition involves not only the maintenance but also the acquisition of a response (Bolles, 1975), the test of response acquisition in experimentally naive subjects is very important.

In this experiment, experimentally naive rats were

---

[6]See Figure 3, M. Olds (1979). Since the naloxone is delivered with the morphine (i.e., mixed with the morphine solution in the infusion apparatus), the antagonist action is present throughout the testing session. Note that responding reappears after about 11 hours of testing.

tested for the acquisition of a lever-pressing response that would deliver 100 nq of morphine into various brain regions. Using the same procedure as in the first experiment, the rats were tested for three four-hour sessions. Although this protocol does not provide a definitive assessment of the rewarding properties of morphine injected into these brain areas, it does assess the relative strength of morphine reward across the various brain regions tested. The rate of acquisition of a response has been shown to increase with increments in the magnitude of reinforcement for a variety of rewards (see Bolles, 1975). Therefore, brain regions where morphine self-administration is rapidly acquired (e.g., ventral tegmental area) would seem to be regions where morphine injections are more rewarding than those which require extensive training to establish this response.

3.4.1 Method

Subjects: Male, Long-Evans rats (weighing 325 to 375 g) were unilaterally implanted with 22 gauge guide cannulae stereotaxically aimed at one of the brain areas listed in Table 3.1. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic and a single injection of penicillin G (30,000 units, i.m.) was administered prophylactically following surgery. Obturators were fitted at a depth of 0.2 to 0.5 mm beyond the guide cannulae immediately after surgery and were removed only during behavioral testing. Food and water were available ad libitum. The animals were

housed in a 12 hour light-dark cycle of illumination and all testing occurred during the light phase of this cycle.

TABLE 3.1

STEREOTAXIC COORDINATES USED FOR THE GUIDE CANNULAE

Placement	anterior-posterior[a]	lateral[b]	ventral[c]	subjects[d]
VTA	-3.8	±0.6	7.3	6
LHA	-3.3	±1.5	6.5	6
PVG	-3.8	±0.6	5.0	5
ACC	+3.5	±1.5	5.7	5
CAUD	+2.0	±3.0	5.0	5

NOTE: The upper incisor bar was 5 mm above the interaural line for all placements except the LHA when it was 2.5 mm below the interaural line. Abbreviations: VTA, ventral tegmental area; LHA, lateral hypothalamic area; PVG, periventricular gray substance; ACC, nucleus accumbens; CAUD, caudate nucleus. The coordinates were adapted from Pellegrino, Pellegrino, and Cushman (1979).

[a]mm from bregma

[b]mm from the midline

[c]mm from dura

[d]number of subjects tested

Apparatus and Procedure: After at least five days recovery from surgery, rats were tested for the acquisition of a lever-pressing response using the same procedure as in the first experiment. Each lever press resulted in a 100 ng infusion of morphine dissolved in 100 nl of Ringer's solution. The rats were tested every four hours per day for three sessions.

3.4.2 Results

The number of infusions delivered per hour during the three sessions of testing were averaged across rats for each infusion site (see Figure 3.3). Rats with cannulae in the ventral tegmental area quickly learned the lever pressing response as in the first experiment. Rats with cannula placements in the other brain regions failed to learn this response. An ANOVA (Winer, 1971) showed a

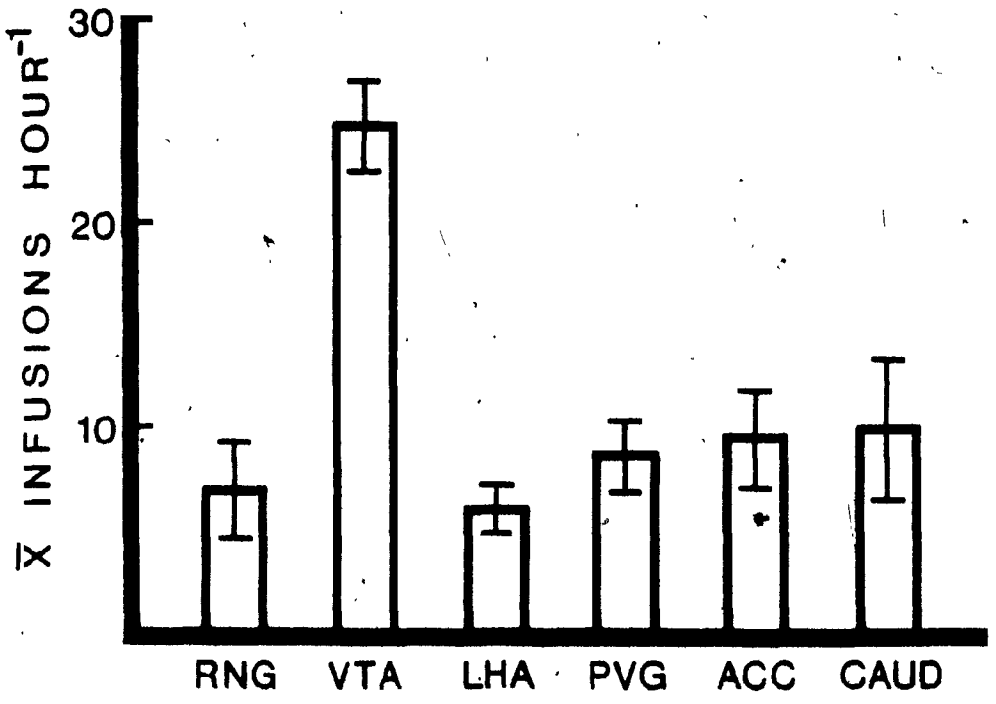

Fig. 3.3. Mean (±SEM) number of infusions per hour averaged across the three sessions of testing. RNG=Ringer's control group; the data are from the first experiment. (See Table 3.1 for abbreviations.)

significant effect for the factor of Groups {$F(5,6)=10.79$, $p<.01$}. Planned comparisons using a Tukey's (a) test (Winer, 1971) demonstrated that the ventral tegmental group was reliably different from all other groups ($p's<.01$). Cannula placements were histologically verified using 40 µm, thionon stained sections (Pellegrino et al., 1979) and representative placements are illustrated in Figure 3.4.

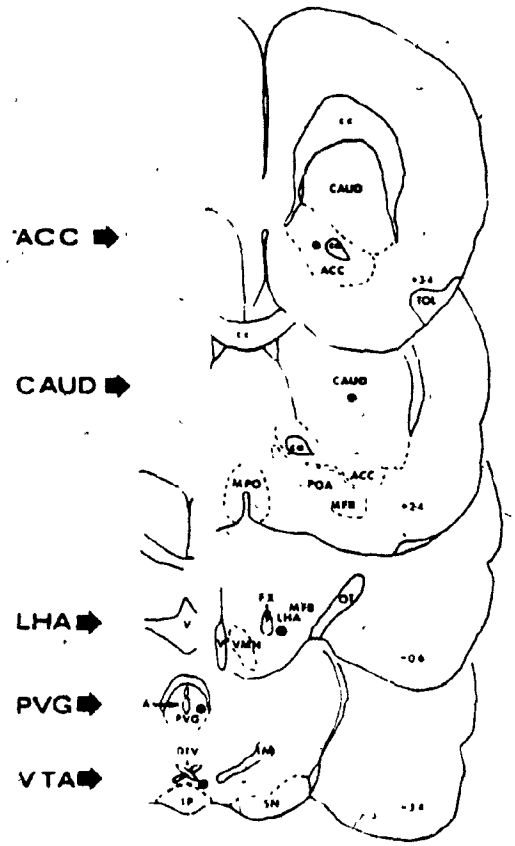

Fig. 3.4. Nominal cannula placements are indicated by the black circles. Brain sections were adapted from Pellegrino et al. (1979). Abbreviations: A, aqueduct; ACC, nucleus accumbens; ca, anterior commissure; CAUD, caudate nucleus; cc, corpus callosum; DTV, decussation of the ventral tegmentum; Fx, fornix; IP, interpeduncular nucleus; LHA, lateral hypothalamic area; MFB, medial forebrain bundle; OT, optic tract; POA, lateral preoptic area; PVG, periventricular gray substance; SN, substantia nigra; TOL, lateral olfactory tract; V, ventricle; VMH, ventromedial nucleus of the hypothalamus.

Some of the rats that received infusions into the nucleus accumbens, caudate nucleus, or periventricular gray regions showed pronounced locomotor excitement during the first 30 minutes of each session. This resulted in a burst of lever contacts, but this behavioral activation was not maintained throughout the four hours of testing. The stimulatory effect of morphine injected into the nucleus accumbens has been previously reported (Pert & Sivit, 1977), and it seems likely that the locomotor excitement produced in some animals by infusions into the caudate nucleus was the result of morphine diffusion to the accumbens. The hyperactivity seen after periventricular gray infusions of morphine has also been reported (Amir, Blair, Shizgal, & Amit, 1979; Blair, Cytrynaik, Shizgal, & Amit, 1980; Jacquet, 1978) although that seen in the present study was far less pronounced than the explosive hyperactivity produced by the higher doses previously studied.[7]

Microinjections of morphine into the ventral tegmental area also produced an increase in locomotor activity. The behavior of these animals, however, was much different than that of rats injected into the nucleus accumbens, caudate nucleus, or periventricular gray area. The locomotor excitement of the latter groups tended to be expressed as erratic bursts of activity lasting less than one hour

---

[7]Explosive motor behavior (i.e., very intense hyperactivity) is produced by the injection of 10 to 250 µg of drug into this region (Blair, Liran, Cytryniak, Shizgal, & Amit, 1978; Jacquet & Lajtha, 1973, 1974; Sharpe, Garnett, & Cicero, 1974).

after the beginning of a session. The ventral tegmental rats showed a sustained increase in activity throughout the four hours of testing. This increase in activity was less pronounced than that of the other groups, and previous tests have shown that it leads to few accidental lever contacts (see Section 3.3). Furthermore, since the microinjections into the ventral tegmental area were unilateral, the motor activity was asymmetrical resulting in circling. As previously reported, the circling was contralateral to the side of injections[8] suggesting that these morphine infusions produced an increase in dopamine release (Iwamoto & Way, 1977; Pert, DeWald, Liao, & Sivit, 1979).

3.4.3 Discussion

The failure to find morphine self-administration into the lateral hypothalamic area is in direct contrast to previous reports (M. Olds, 1979; E. Stein & J. Olds, 1977). These discrepant finds are probably the result of methodological differences. First, the reports showing self administration of morphine into the lateral hypothalamic area used guide cannulae that were considerably larger than those used in this study.[9] Routtenberg (1972) has reviewed

---

[8]The rate of rotation was measured in several animals and found to be around 4 to 5 rotations per minute when measured approximately 1 hour into the test session. Rotational behavior was also observed during the first experiment, but no attempt was made to determine the proportion of animals showing this behavior.

[9]The outside diameter of various gauges of tubing varies somewhat depending on the system of measuring that

evidence suggesting that the use of large diameter cannulae facilitates the diffusion of drug into the cerebral ventricles. The possibility exists that the reported self administration of morphine into the lateral hypothalamus is the result of such ventricular diffusion.[10] A second procedural difference is that the studies showing lateral hypothalamic self-administration used rats that were previously trained to lever-press for rewarding brain stimulation. Since M. Olds (1970) has shown that some drugs can prolong lever pressing for brain stimulation reward even when the electrical stimulation has been discontinued, it is possible that the reported self-administration represents a type of extinction responding. A similar effect has been reported for animals trained to intravenously self-administer various drugs including morphine (de Wit & Stewart, 1981; Gerber & Stretch, 1975; Stretch & Gerber, 1973). Another possibility is that lateral hypothalamic morphine is rewarding but less so than reward from ventral tegmental microinjections. Rats may maintain a previously learned lever-pressing response but fail to acquire a new response because this reinforcement

---

is used (see Hodgman, Weast, Shankland, & Selby, 1962). The specifications for commercially available hypodermic tubing show that 18 gauge tubing is around 0.7 mm in diameter while 22 gauge tubing is approximately 1.2 mm in diameter. Hence, the outside diameter of the 18 gauge cannulae is almost twice that of the 22 gauge cannulae used in the present study.

[10] The fact that the periventricular gray rats do not self-administer morphine eliminates this possibility in the present experiment since the periventricular gray placement is just 2 mm dorsal to the ventral tegmental area.

has a poor efficacy. In any case, it is obvious that reward from ventral tegmental morphine infusions is far more potent than reward from microinjections of morphine into the other brain regions tested in this study.

The fact that morphine was self-administered into ventral tegmental area but not the periventricular gray substance suggests an anatomical separation of the rewarding, analgesic, and sedative properties of opiates. The periventricular gray region has been implicated by other microinjection studies in opiate-induced analgesia (Pert & Yaksh, 1974; Sharpe et al., 1974) and sedation (Broekkamp, Van Den Bogaard, Heynen, Rops, Cools, & Van Rossum, 1976; Pert et al., 1979) while the rewarding properties of morphine appear to be mediated by receptors in the ventral tegmentum and not in the periventricular gray area. The region mediating opiate-induced physical dependence is less clearly defined, but it too seems to involve opiate receptors in the periventricular gray region and perhaps some thalamic regions as well (Wei, 1981; Wei, Loh & Way, 1973). While direct tests of the physical dependence-producing properties of ventral tegmental morphine injections have not yet been made, the present data suggest the possibility that the rewarding properties of opiates may be dissociable from their dependence-producing properties as well as the analgesic and sedative effects of these drugs. Such a dissociation of the rewarding and physical dependence-producing properties of opiates would

have a major significance for theories of addiction and direct tests will be reported in Chapter 7.

3.5 Further Investigation of Intracranial Self-Administration of Morphine into the Lateral Hypothalamic Area

The discordant findings of the present study and those of M. Olds (1979) and E. Stein and J. Olds (1977) are particularly important because they reflect directly conflicting results using the same general paradigm. One of the differences in the procedure used by M. Olds (1979) from that used in the current study concerns the size of the guide cannula implanted in the animals. M. Olds (1979) used 18 gauge cannulae while the experiments reported in Sections 3.3 and 3.4 used 22 gauge cannulae. The diameters of these cannulae are approximately 1.2 and 0.7 mm, respectively. Since the size of the cannulae used in chemical stimulation studies can affect the amount of diffusion up the cannula shaft and into the cerebral ventricles (Routtenberg, 1972), the possibility exists that animals in the M. Olds' (1979) report are self-administering morphine because the rewarding action is dependent on ventricular diffusion to another site of action. To test this hypothesis, the results of the previous study (i.e., Section 3.4) were compared with those obtained in animals implanted with cannulae having a much larger diameter.

3.5.1 Method

Subjects: Male, Long-Evans rats (weighing 350 to

400 g) were unilaterally implanted with chemitrodes[11] stereotaxically aimed at the lateral hypothalamic area using the coordinates listed in Table 3.1. Surgical anesthesia was accomplished using sodium pentobarbital (60 mg/kg, i.p.) and penicillin G (30,000 units, i.m.) was given after surgery. Obturators were fitted 0.5 mm beyond the tip of the cannula portion of the chemitrodes which remained in place except during behavioral testing. The animals had free access to food and water in their home cages and were placed on a 12 hour light-12 hour dark cycle of illumination. All testing occurred during the light cycle.

Apparatus and Procedure: Chemitrodes were made from bipolar electrodes wrapped around 22 gauge guide cannulae. Epoxy cement was used to retain the electrodes to the cannula shafts. The diameters of the chemitrodes ranged from about 1.1 to 1.5 mm and thus were similar in size to the 18 gauge cannulae used by M. Olds (1979). The animals were allowed 6 to 7 days to recover from the surgical procedure. Next, they were tested using the same procedure as described in Section 3.4: all parameters of testing

---

[11] Chemitrodes were originally implanted in these subjects to test another possible explanation of M. Olds' (1979) report of lateral hypothalamic self-administration. The initial protocol entailed training the subjects to press for brain stimulation reward in the lateral hypothalamus and then testing them for intracranial self-administration of morphine. A control procedure consisting of testing chemitrode animals for intracranial self-administration before experience with brain stimulation reward revealed, however, that these experimentally naive rats would self-administer morphine into the lateral hypothalamus. The results of this testing forms the basis of this section.

were identical to those used for the initial anatomical mapping study. The experimentally naive rats were tested for four hours per day every other day for a total of three sessions. Each lever press resulted in the delivery of 100 ng of morphine sulfate dissolved in Ringer's solution. The infusions were delivered over 5 seconds in a volume of 100 nl using the EMIT method described in Section 3.3.1.

3.5.2 Results

Figure 3.5 illustrates the mean number of infusions earned per hour across the three hours of testing. Rats implanted with chemitrodes in the lateral hypothalamic area quickly learned the lever-pressing response. The mean hourly intake of animals self-administering morphine into the ventral tegmental area and animals with cannulae implanted in the lateral hypothalamic area are included for comparison. An ANOVA showed a significant difference among the three groups of animals represented in the figure {$F(2,12)=77.91$, $p<.001$}. A Tukey's (a) test revealed that the rate of lever pressing in chemitrode implanted subjects was significantly higher than that seen when identical placements were tested using cannulae ($p<.01$). Also, the rate of responding was even higher than seen with cannulae implanted into the ventral tegmental area ($p<.01$).

3.5.3 Disucssion

The data presented in this section are preliminary since additional testing is required to draw definitive

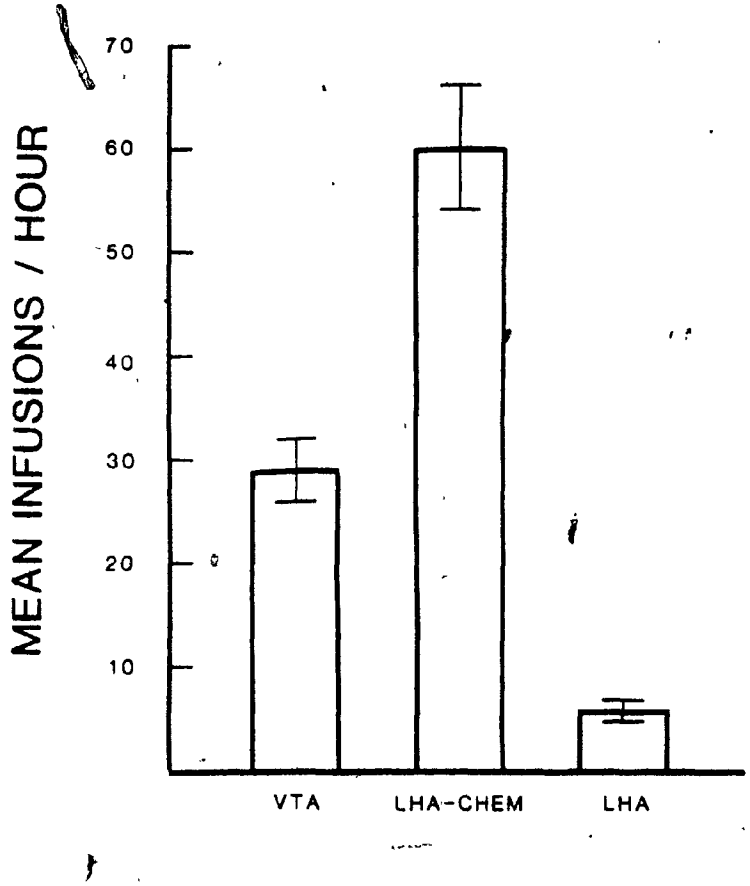

Fig. 3.5. Mean (±SEM) number of infusions per hour averaged across the three sessions of testing. VTA and LHA data are from Figure 3.3 (n=6/group). LHA-chemitrode=lateral hypothalamic placements with chemitrodes, n=3. (See Table 3.1 for other abbreviations.)

conclusions regarding the basis of lateral hypothalamic self-administration. They do suggest, however, that morphine infusions into the lateral hypothalamus are rewarding because ventricular diffusion occurs to a distal site of action. To clearly establish this, one of several procedures must be used. First, the ability of infusions dorsal to this placement to support intracranial self-administration could be evaluated. If the tissue at the tip of the cannulae were responsible for the initiation of the rewarding

effects of morphine infusions into the lateral hypothalamus, then dorsal placements should be ineffective. If ventricular diffusion were responsible for this behavior, then dorsal placements would be expected to be even more effective in establishing and maintaining self-administration. Similarly, self-administration of morphine directly into the cerebral ventricles might be tested to determine if the same pattern of self-administration occurs. Alternatively, kainic acid might be used to selectively destroy the cell bodies in the lateral hypothalamic area (Britt & Wise, 1981bc; Jonsson, 1980; McGeer & McGeer, 1981, 1982; Peterson & Moore, 1980). Since it is likely that the opiate receptors are located on the cell bodies of these neurons and not on the axons of passage (Criado, Aguilar, & De Robertis, 1981; Perry, Mullis, Oie, & Sadee, 1980; Pert, Snowman, & Snyder, 1974; see also Snyder & Matthysse, 1975), this procedure should eliminate intracranial self-administration into the lateral hypothalamic area if this behavior were dependent on the cells in this region. Self-administration which survives kainic acid lesions is likely to be dependent on drug diffusion to a distal site of action outside the lateral hypothalamic area.[12]

The fact that animals self-administer morphine into

---

[12] Another approach would be to use agents that irreversibly inactivate opiate receptors such as the compounds shown to specifically alkalate opiate receptors (Caruso, Larson, Portoghese, & Takemori, 1980; Craviso & Musacchio, 1976; Hazum, Chang, Cuatrecasas, & Pasternak, 1981; Pasternak, Childers, & Snyder, 1980). The application of this approach, however, is likely to be limited by the availability of these agents and their suitability for use in vivo.

the lateral hypothalamus at a higher rate than they self administer it into the ventral tegmentum may be indicative of a diminished potency of lateral hypothalamic morphine infusions. If these infusions of morphine were diffusing to a distal site of action such as the ventral tegmentum, the drug concentration would be appreciably diminished by the time it reached this site. Thus, it would require a larger quantity of morphine injected into the lateral hypothalamus to support self-administration behavior. On the other hand, the rapid acquisition of the lever-pressing response seen in animals with chemitrodes implanted in the lateral hypothalamus suggests that the rewarding effects of these infusions occur soon after injections. It might still be viable that this is the result of ventricular diffusion since ventricular morphine quickly penetrates into many brain regions. The rapid learning of the intracranial self administration response does, however, make the conclusions drawn from this study more tenuous. Plainly, additional testing is required to clearly establish the ability of lateral hypothalamic morphine to support intracranial self administration. The fact that this behavior is seen (in the present study) only in subjects implanted with chemitrodes is suggestive of a ventricular diffusion basis of this behavior.

3.6 General Discussion

The demonstration of behavioral and pharmacological

specificity of intracranial morphine self-administration substantiates this approach as a viable alternative for studying the neural mechanisms of opiate reward. Tests of behavioral specificity (i.e., yoked and Ringer's controls) clearly establish that the animals are working for the rewarding properties of the drug infusions. Since naloxone blocked the self-administration response, it is also apparent that lever-pressing is dependent on a drug action at opiate receptors since narcotic antagonists would not be expected to alter the physico-chemical properties of the morphine solution. Further tests showing that morphine is not self administered at most opiate receptor fields suggest that opiate reward is not the result of an opiate action common to all receptor fields. This observation implies specialization of the various opiate receptor fields for different functions; thus, opiate reward is not a ubiquitous property of these receptor fields, but rather it is associated with the activation of a specific opiate receptor field.

The fact that morphine was self-administered into the ventral tegmental area, but not other brain regions associated with other opiate effects, suggests an anatomical separation of opiate reward and other opiate-induced behaviors. This opens the possibility that use of the intracranial self-administration paradigm might minimize the side-effects caused by whole-brain drug delivery (e.g., intravenous self-administration). Thus, the sedative, analgesic, and physical dependence-producing properties of

opiates may be eliminated as potential sources of confounding during tests of opiate reward.

There are several qualifications which must be considered when generalizing the results of these studies to opiate reward produced by systemic drug injections. First, the anatomical mapping study was based on a protocol proven effective in establishing intracranial self administration into the ventral tegmental area. It is possible that increasing the dose of morphine delivered per infusion, the volume infused, or the number of trials of testing would result in acquisition of the intracranial self administration response for morphine infused into the other brain regions tested in Section 3.4. The present study provided a test only of the relative potency of morphine infused into these brain regions and not a definitive assessment of their ability to support intracranial self administration. Second, although several major opiate receptor fields were tested in this study, the possibility remains that other brain sites not yet studied will support self-administration. Third, the experiments in this chapter revolve around acquisition and different mechanisms may be responsible for the acquisition and maintenance of opiate intake. If this were the case, it might be that morphine infused into one of the other brain regions is actually more rewarding than when delivered into the ventral tegmentum. Fourth, the demonstration of intracranial self administration shows that opiate action at a given brain

region is sufficient for reward, but it does not establish that such opiate action is necessary for reward. This can only be done using tests of the rewarding properties of systemically applied opiate while blocking opiate action at specific receptor fields. It could be that concurrent activation at several opiate receptor fields (e.g., nucleus accumbens, lateral hypothalamus, periventricular gray substance) is equivalent in its rewarding effect to discrete activation of the ventral tegmentum. Thus, opiate action at the ventral tegmental area may not be necessary for this rewarding action to occur when the drug is delivered systemically and thus reaches a number of opiate receptor fields.

With the demonstration of behavioral, pharmacological, and anatomical specificity, the intracranial self administration paradigm is established as a viable method for studying the neural mechanisms of drug reward. To fully evaluate the usefulness of this technique, however, there are four additional aspects of this phenomenon which need to be studied. First, the chronicity of the preparation needs to be evaluated. The studies reported in this chapter reveal minimal tissue damage from these infusions, but there is no indication about how long the intracranial self administration response can be maintained. Second, the effects of changes in unit-dose need to be evaluated. Increasing the dose of drug delivered with each infusion might cause a decrease in response rate, but it is also

possible response rates will increase.[13] It is necessary to document the effects of unit-dose manipulations on response rates before conclusions can be drawn regarding the meaning of increased or decreased rates of responding. Third, the identification of the opiate receptor population mediating the intracranial self-administration response might suggest specific ligands lacking the addictive but retaining the analgesic properties of opiates. Special attention should be focused on evaluating the potency of mu, sigma, kappa, and delta opiate-receptor ligands. Fourth, the degree that intracranial morphine infusions control behavior in a fashion similar to intravenous drug and conventional rewards needs to be investigated. This would reveal whether this method of studying opiate reward is governed by the same principles as other rewards and would suggest the degree of generality of these data to intravenous drug reward and conventional rewards.

---

[13] With continuous reinforcement schedules, increases in unit dose generally lead to decreases in the rate of intravenous opiate self-administration; testing with low doses or partial reinforcement schedules has suggested that there may also be an ascending aspect to the dose-response curve (see Jones & Prada, 1981; Weeks & Collins, 1968, 1979; Young, Swain, & Woods, 1981; Young & Woods, 1980). Thus it is difficult to predict the influence of manipulations of unit dose on intracranial self-administration.

CHAPTER 4

THE NEUROANATOMICAL BOUNDARIES OF THE REWARD-RELEVANT OPIATE RECEPTOR FIELD[1]

4.1 Conditioned Place Preference Paradigm

The conditioned place preference paradigm can make several important contributions to the study of drug reward. First, it offers an independent method of assessing a drug's rewarding properties with a rate-free measure. This is especially useful when assessing the effects of brain lesions that can severely disrupt normal sensory-motor integration. Second, conditioning variables have been implicated in the maintenance of drug-seeking behavior (Crowder, Smith, Davis, Noel, & Cossens, 1972; Schuster & Woods, 1968). The study of conditioned place preference allows a direct comparison of such conditioning effects across different drugs and parameters of testing. Third, this paradigm is extremely quick and easy to use. It avoids the problems associated with intravenous self-administration, and it is a more direct demonstration of a drug's rewarding proper-

[1] Preliminary versions of this report have appeared in M. A. Bozarth and R. A. Wise, Society for Neuroscience Abstracts, 1981, 7, 50, and M. A. Bozarth and R. A. Wise, Localization of the reward-relevant opiate receptors. In L. S. Harris (Ed.), Problems of Drug Dependence, 1981. Washington, D. C.: NIDA Research Monograph Series, 1982.

ties than are paradigms employing brain stimulation reward. Finally, the conditioned place preference paradigm can be combined with microinjection technology to demarcate the anatomical boundaries of the reward-relevant opiate receptor population within a given brain region.

4.2 Conditioned Place Preference from Central Morphine Infusions

Intracranial self-administration studies are perhaps the most direct demonstration of the rewarding properties of central morphine injections. This technique can be used to directly compare the rewarding effects of morphine injected across various brain regions such as in Chapter 3 where the reward-relevant population of opiate receptors was identified in the ventral tegmental area. There is, however, a serious limitation to the use of this paradigm to determine the neuroanatomical boundaries of the population of opiate receptors that are responsible for opiate reward within a given brain region.

In intracranial self-administration studies, the animal controls the number of infusions and hence the total volume of drug injected into its brain. Animals with a high rate of self-administration have a larger field of effective drug spread than animals with low response rates; even microinjections through cannulae that are distal to the site of drug action may be rewarding when significant concentrations of drug have diffused to that region (see Figure 4.1). Therefore, it is difficult to estimate the

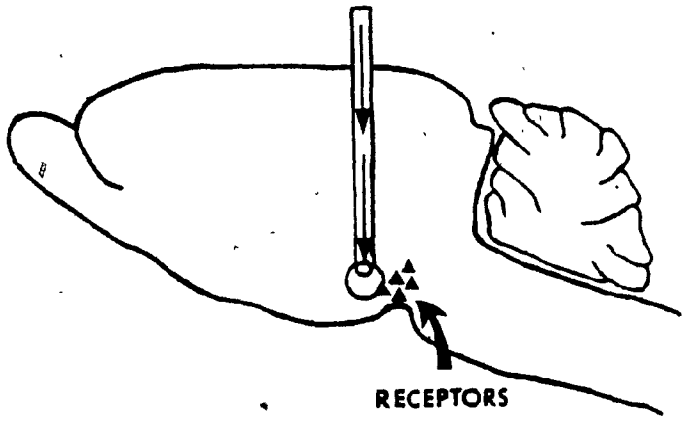

Fig. 4.1. Since changes in the infusion volume produce a concomitant change in the field of effective drug action, attempts to extrapolate the anatomical boundaries of the receptor population mediating reward from cannula placements must use a fixed infusion volume.

distance of the cannula placements to the reward-relevant receptors using intracranial self-administration experiments.

This problem can be overcome by injecting each animal with the same volume of drug and assessing reward using the conditioned place preference paradigm. Cannula placements can then be meaningfully compared to determine the location of the reward-relevant receptors. With this technique, the limits of the brain regions mediating reward from a given drug can be more precisely defined in relation to various microinjection sites.

4.2.1 Method

Subjects: Experimentally naive, male, Long-Evans

rats (weighing 350 to 400 g) were unilaterally implanted with 22 gauge guide cannulae aimed at the ventral tegmental area. With the upper incisor bar 5 mm above the intra-aural line, the coordinates ranged from 2.0 to 4.4. mm posterior to bregma, 0.6 mm lateral to the midsagittal suture, and 7.8 to 8.2 mm ventral from dura. Sodium pentobarbital (60 mg/kg, i.p.) was used as the anesthetic with atropine sulfate (.04 mg/kg, s.c.) and penicillin G (30,000 units, i.m.) given prophylactically. Obturators were fitted approximately 0.25 mm beyond the tips of the guide cannulae and remained in place except during infusions. All testing occurred during the light phase of a 12 hour light-12 hour dark cycle of illumination. Rats were individually housed and had food and water available ad libitum in their home cages.

Apparatus and Procedure: Place preference was measured in a shuttle box (25 x 36 x 35 cm) with a plywood floor on one side and a plywood floor covered with wire mesh on the other. The amount of time spent on each side of the box was automatically recorded. Rats were allowed access to the entire shuttle box for 15 minutes per day on five consecutive days; the last day served as an indication of the animals' initial place preference. After these preconditioning trials, they received four daily injections of morphine while being forced to remain on their nonpreferred sides for 30 minutes. Following the four days of conditioning, the rats were injected with vehicle and tested

again for their place preference (15 minutes). An electrolytic microinfusion transducer (EMIT) was used to unilaterally inject morphine sulfate into the ventral tegmental area immediately before each of the four conditioning trials. A 150 μA infusion current delivered 250 ng of morphine sulfate dissolved in 500 nl of Ringer's solution. Infusions were delivered over 28 seconds through a 28 gauge injection cannula that extended 0.5 to 1.0 mm beyond the guide cannula. An additional 30 seconds was allowed for drug diffusion before the injection cannula was removed from the guide cannula. Ringer's solution was injected prior to the test trial.

Histological Analysis: Following completion of the behavioral testing, the rats were deeply anesthetized with sodium pentobarbital (circa 90 mg/kg, i.p.) and perfused intracardially with isotonic saline followed by formalin. After at least three days of fixation in formalin, the brains were sectioned at 40 micron intervals and then stained using formol-thionin. Brain sections were viewed at approximately 10 times magnification and the cannula placements were identified according to the brain atlas of Pellegrino, Pellegrino, and Cushman (1979). Changes in place preference were then plotted as a function of the number of millimeters that the cannulae were posterior to bregma on de Groot's (1959) plane of sectioning.

Since the determination of cannula placements was of central importance to this study, special attention was

focused on the method used to classify placements. The initial groupings were done with knowledge of the place preference scores for some of the subjects. Next, two additional judges blindly rated the placements for 72% of the animals. The reliability coefficient (Kerlinger, 1973) of these ratings was found to be 0.979 indicating a high degree of interjudge reliability.[2]

4.2.2 Results

Figure 4.2 shows the changes in place preference following the conditioning trials. The scores were derived

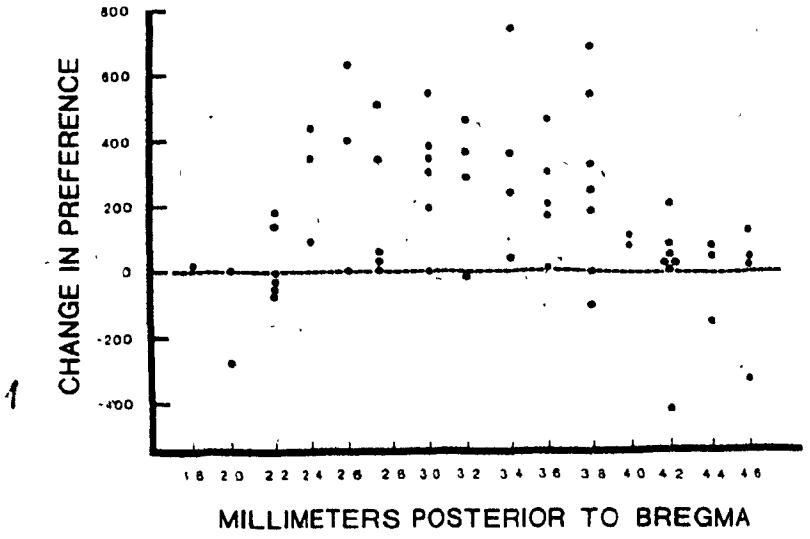

Fig. 4.2. Change in place preference as a function of cannula placement plotted for individual subjects.

[2]The main effect associated with the factor of judges was not significant {$F(2,86)=1.29$, $p>.05$} while the effect associated with differences among rats was reliable {$F(43,86)=50.97$, $p<.001$}. This type of analysis is also discussed in Winer (1971), but the computational formula of Kerlinger (1973) was used.

by subtracting the amount of time spent on the conditioning side during the last preconditioning trial from the time spent on the conditioning side after the conditioning trials. Positive scores indicate an increase in preference for the conditioning side while negative scores show a decrease in the amount of time spent on the conditioning side. The scattergram was used to determine the anatomical intervals for grouping the data for subsequent analysis in Section 4.3.

The nominal cannula placement is shown in Figure 4.3. The amount of tissue damage resulting from these infusions was minimal and probably less than that usually observed after infusions using the microsyringe method of microinjection. Most cannulae were on the lateral border of the interpeduncular nucleus just medial to the substantia nigra. Several subjects with cannulae more dorsal than those illustrated in the figure were eliminated from this study. Also, 10 animals with injection cannulae that were ventral to this region were also tested. These cannulae probably terminated in the ventral cerebral vasculature or cistern as evidenced by the frequent appearance of cerebral spinal fluid flowing up the guide cannulae (placements ranged from 2.6 to 4.0 mm posterior to bregma). The mean change in place preference for this group was 45.7 (SEM=55.8) indicating that infusions into this region were not effective in producing a conditioned place preference. This finding is important, albeit fortuitous, since it eliminates the possibility that morphine

infusions into the ventral tegmental area were rewarding because they had entered the cerebral vasculature or ventral cistern and were transported to a distal site of action.

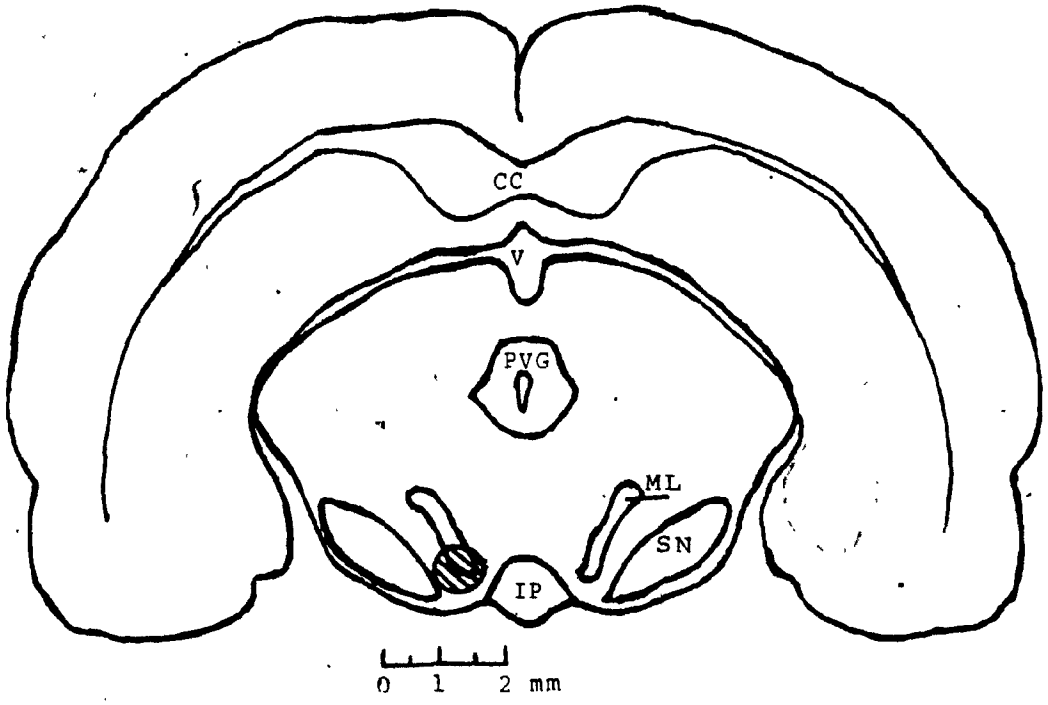

Fig. 4.3. The extent of tissue damage seen following four infusions of 500 nl delivered over 28 seconds is the striped zone. Abbreviations: CC, corpus callosum; IP, interpeduncular nucleus; ML, medial lemniscus; PVG, periventricular gray substance; SN, substantia nigra; V, ventricle.

4.3 Determination of Anatomical Boundaries

To facilitate statistical comparisons of the effects of cannula placement on place preference, the scores illustrated in Figure 4.2 were grouped into anatomical zones at approximately 0.6 mm intervals. This was guided by visual inspection of the scatterplot which suggested that placements rostral to 2.4 mm posterior to bregma and those caudal to 3.8 mm posterior to bregma were ineffective in producing a change in place preference. To decrease the differences in the number of subjects in each zone, the effective range from 2.4 to 3.8 mm posterior to bregma was also divided into two groupings. The results of this analysis is shown in Figure 4.4. An anlysis of variance (Winer, 1971) demonstrated a significant difference among rats implanted with cannulae in the various zones throughout the ventral tegmental area {$F(3,58)=10.267$, $p<.001$}. A Newman-Keuls' test was performed for specific comparisons among the various groups (Winer, 1971). Both the 2.4 to 3.0 mm and the 3.2 to 3.8 mm zones were reliably different from the rostral and caudal placements defined in this procedure ($p's<.01$).

Since the number of subjects tested in each group ranged from 9 to 20, a more conservative approach to data analysis might be to analyze each group separately for

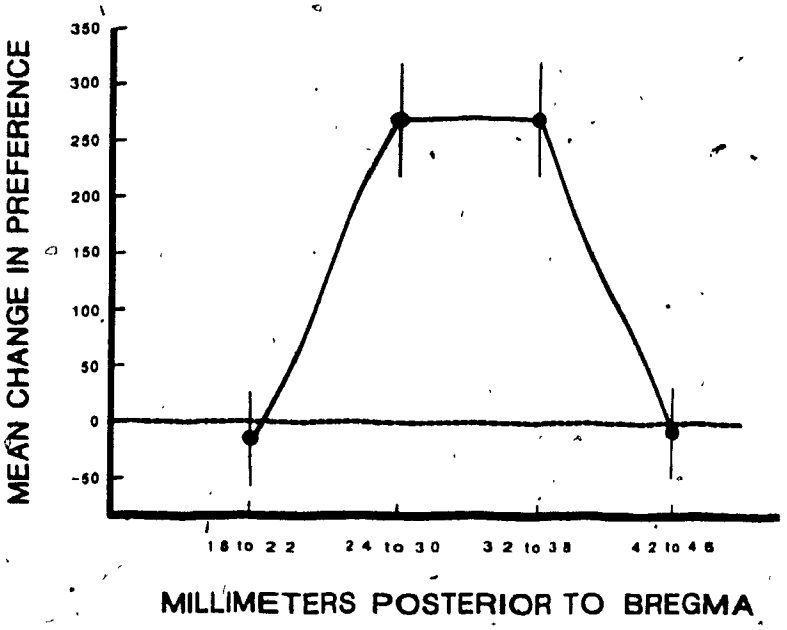

Fig. 4.4. Mean (±SEM) change in place preference for the various anatomical zones.

changes in place preference.[3] This was done by a series of t-tests and by using Fisher's method for specific comparisons (Lindman, 1974) to provide protection against Type I errors. T-tests for correlated measures revealed

[3]The data derived from the analysis by zones are normally distributed and the variances of these groups are not significantly different. Therefore, the effect of unequal sample size is probably negligible (see Lindman, 1974; Winer, 1971). Nonetheless, treating each group as an independent sample and analyzing them separately to assess changes in place preference following morphine infusions would appear to be a somewhat more conservative approach, assuming, of course, that a satisfactory method of holding the alpha level constant is used. The real strength in the conclusions suggested by the analysis of place preference as a function of anatomical zone, however, comes from the fact that both approaches to statistical analysis yield similar results.

TABLE 4.1

STATISTICAL EVALUATION OF THE DATA ILLUSTRATED IN FIGURE 4.3

Zone	N[a]	Mean	SEM	t[b]	p[c]	η^{2}[d]
1.8 to 2.2	9	-15.0	42.7	-0.351	p>.70	1.3%
2.4 to 3.0	17	271.4	51.0	5.322	p<.001	62.5%
3.2 to 3.8	20	272.1	51.3	5.304	p<.001	58.4%
4.0 to 4.6	16	-6.8	41.0	-0.166	p>.70	0.2%

NOTE: Statistical calculations were based on Linton and Gallo (1975). To hold the alpha level constant at p<.05 across the four tests, the alpha level of each test needs to be adjusted to p<.0127. Similarly, to hold the alpha level constant at p<.01, the alpha level for each of the four t-tests needs to be p<.0025. The probability of obtaining one or more type I errors across these four tests using the nominal value of p<.001 is p<.004. See Lindman (1974) for details of Fisher's (1935) method of specific comparisons.

[a]number of subjects tested

[b]t-value for correlated measures

[c]probability level, two tailed test

[d]proportion of variance accounted for by treatment

that rats with cannulae in the 2.4 to 3.0 mm and 3.2 to 3.8 mm groups showed a significant change in place preference while rats with placements rostral or caudal to this region did not change their preferences as a result of the drug injections (see Table 4.1).

Figure 4.5 is a histological reconstruction of the range of cannula placements tested in this experiment. The

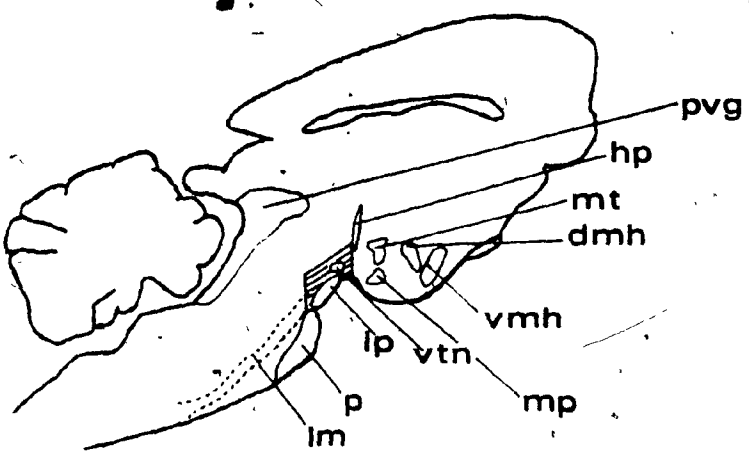

Fig. 4.5. Histological reconstruction of the approximate anatomical boundaries of the reward relevant opiate receptor field. The striped area indicates the zone where morphine infusions produced a conditioned place preference. The dorsal limits of the mid portion of the zone have been extrapolated from Phillips and LePiane (1980). Abbreviations: dmh, dorsomedial nucleus of the hypothalamus; hp, habenulo-interpenduncular tract; ip, interpenduncular nucleus; lm, medial lemniscus; mp, posterior mamillary nucleus; mt, mamillothalamic tract; p, pons; pvg, periventricular gray substance; vmh, ventromedial nucleus of the hypothalamus; vtn, ventral tegmental nucleus of Tsai (adapted from Pellegrino et al., 1979).

zone in which microinjections of morphine produced a conditioned place preference extended throughout the ventral tegmental area covering approximately a 1.4 mm range. This region has previously been shown to contain a moderate level of opiate receptor binding (Duka, Schubert, Wüster, Stoiber, & Herz, 1981; Pert, Taylor, Pert, Herkenham, & Kent, 1980; Snyder & Matthysse, 1975) as well as enkephalin cell bodies and/or terminals (Herkenham & Pert, 1980; Sar, Stumpf, Miller, Chang, & Cuatrecasas, 1978; Uhl, Goodman, Kuhar, & Snyder, 1978; Watson & Barchas, 1979; Yang, Hong, Fratta, & Costa, 1978). In independent fluorescent histochemical mapping of this region for dopamine neurons (Spindler, 1975), this area has also been shown to contain A9 and A10 dopamine cell bodies.[4] Furthermore, recent evidence has suggested that the mesolimbic and nigrostriatal dopamine systems are modulated by enkephalinergic activity in this region (Johnson, Sar, & Stumpf, 1980; Llorens-Cortes,

---

[4]The published papers indicating the location of dopamine-containing cell bodies and projections (Jacobowitz & Palkovits, 1974; Lindvall & Björklund, 1974; Palkovits & Jacobowitz, 1974; Ungerstedt, 1971a) are all based on the plane of sectioning used by König and Klippel (1963). Since this plane is different from that used in the present study (i.e., de Groot, 1959; Pellegrino et al., 1979), assessment of the proximity of the A9 and A10 dopamine cell groups was based on Spindler's (1975) map which used de Groot's (1959) plane of sectioning. Using ventral brain structures as reference points (e.g., interpeduncular nucleus, medial lemniscus, substantia nigra), there appears to be a close correspondence between Spindler's (1975) map and the published maps based on König and Klippel's (1963) plane of sectioning. Specifically, Spindler's (1975) data suggest that the A9 and A10 cell group ranges from about 2.4 to 4.0 mm posterior to bregma.

Pollard, & Schwartz, 1979).

4.4 General Discussion

The results of the present experiment confirm the finding that injections of morphine into the ventral tegmental area are rewarding. In an earlier report, Phillips and LePiane (1980) found that bilateral injections of morphine produced a conditioned place preference that was blocked by naloxone. They also found that if the injections were made 2.5 mm dorsal to the ventral tegmental area, no place preference was shown. The present study extended these findings by showing that unilateral injections were also effective in producing a conditioned place preference and by establishing the rostral-caudal boundaries of the ventral tegmental opiate-receptor field that is capable of producing a place preference. This latter finding was the primary reason for selecting this technique of assessing opiate reward since it overcomes the problem associated with variable infusion volumes seen with the intracranial self-administration paradigm.

There was a great deal of variability in place preference produced by morphine injections into sites that appeared very similar. This is possibly due, in part, to differences in drug diffusion even with seemingly similar placements. Another factor that may account for this variability in responsiveness to morphine infusions is the fact that only about 80% of the animals receiving systemic

heroin injections show a place preference (M. Bozarth, unpublished observations). Thus, even effective sites of chemical stimulation might be expected to produce a place preference in only some fraction of the total subjects tested. It is interesting to note that the magnitude of the place preference was about the same as that produced by bilateral infusions of morphine into the ventral tegmentum (see Phillips & LePiane, 1980).

The anatomical boundaries derived in the present study are not, of course, finely demarcated zones indicating the actual boundaries of the reward-relevant opiate-receptor field. Several factors make these zones only approximations to the actual location of the receptor field mediating opiate reward. First, the extent of drug dispersion following these microinjections has not been determined although it is probably on the order of 0.25 to 0.5 mm from the injection site.[5] The data analysis, which is based on histological zones grouped at 0.6 mm intervals, contains placements that are separated by only a few millimeters and would obviously have partially overlapping fields of effective drug spread. Second, even if the

[5]This estimate is based on the reports of Lomax (1966) and Myers and Hoch (1978) who test functional and physical drug spread, respectively. Even the report by Schubert, Teschemacher, Kreutzberg and Herz (1970), which suggests a much greater radius of drug spread, indicates that a 2.5 µl volume of morphine only spreads about 1.5 mm from the injection site during the first 30 minutes after injection. These estimates of drug spread are for times after injections when the behaviorally relevant drug effects are likely to occur (e.g., drug dispersion 30 minutes post infusion corresponds to the length of the conditioning trial in this experiment).

degree of drug spread were known, the portion of the reward-relevant receptor population that must be chemically activated to result in a place preference remains unknown. If a large percentage of the target receptors must be activated, then the zone identified in the present study may represent the midpoint of a broad homogeneous field. If, on the other hand, only a small proportion of the total receptor field capable of mediating this response needs to be stimulated by the morphine infusions, then the zone demarcated by this technique comes closer to actually mapping the extent of the reward-relevant receptors in the ventral tegmental area. Third, the histological analysis did not provide a high degree of anatomical resolution. The injection cannulae used in this study had a diameter of approximately 0.36 mm, and determination of the brain section that represented the deepest penetration of these cannulae was difficult. The high degree of interjudge reliability, however, suggests consistency in the histological groupings although such determinations of cannula placements are only an anchoring point for the data analysis.

The results of the present study, combined with those of Phillips and LePiane (1980), offer corroborative support to the notion that the ventral tegmental area contains the opiate-receptor field responsible for morphine's rewarding action. The fact that naloxone blocks the development of a conditioned place preference from central morphine

injections (Phillips & LePiane, 1980) demonstrates that the activation of opiate receptors is necessary for this rewarding action (see Section 3.2.2). The failure of injections dorsal to the ventral tegmentum to produce a change in place preference (Phillips & LePiane, 1980) eliminates the possibility that drug was diffusing up the cannula shaft and was spreading to a distal site of action. The present study showed that infusions rostral or caudal to the ventral tegmental area were also ineffective thus eliminating axonal streaming (i.e., drug diffusions along axonal projections; see Routtenberg, 1972) as a possible explanation of this effect. Furthermore, since injections ventral to this region did not result in a place preference, drug dispersion mediated by the ventral cistern or cerebral vasculature could not account for the rewarding property of these infusions. In conclusion, morphine infusions into the ventral tegmental area produce a conditioned place preference that is both pharmacologically and anatomically specific (see Sections 3.2.2 & 3.3.3). The zone of reward relevant opiate receptors has tentatively been defined as extending about 1.4 mm from 2.4 to 3.8 mm posterior to bregma. The fact that this zone corresponds to the approximate location of the mesolimbic and nigrostriatal dopamine cell bodies suggests that opiate reward may be dependent on the modulation of one or both of these dopaminergic systems.

CHAPTER 5

THE EFFECT OF MORPHINE ON BRAIN DOPAMINE FUNCTION

5.1 Introduction

Opiates have seemingly ubiquitous actions affecting both the central and peripheral nervous systems. Some of the effects of opiates on biological activity are independent of opiate-receptor mechanisms and are due to influences on electrolyte balance and membrane stabilization; these effects include some opiate actions on neural impulse conduction, enzyme induction, and cellular metabolism which are not blocked by narcotic antagonists nor demonstrable only with "active" stereoisomers (e.g., Contreras, Contreras, Gonzalez, & Concha, 1980; Durham & Frank, 1981; Frank, 1975; Frank & Marwaha, 1978; Gero, 1979; Goldstein, Lowney, & Pal, 1971; Jacquet, 1980; Seeman, Chau-Wong, & Moyyen, 1972). These opiate actions appear to be independent of classically defined opiate-receptor mechanisms (see Goldstein, 1974; Simon & Hiller, 1978; Snyder & Matthysse, 1975) and are probably relatively unimportant at pharmacologically relevant concentrations of opiates. On the other hand, various neurotransmitter systems, including the catecholamines, show characteristic responses to opiates through a drug

action that is mediated by stereospecific opiate-receptor mechanisms. These responses generally occur at pharmacologically relevant concentrations and are likely to be involved in the central nervous system effects of therapeutically and recreationally administered opiates.

Brain dopamine is involved in a variety of behaviorally relevant processes. Over the past decade an important role for dopamine has been well established for locomotor activity, regulation of feeding and drinking, brain stimulation reward, stereotypy, the behavioral stimulating action of amphetamine and cocaine, and psychopathological disturbances in thought and affect. (For reviews of the involvement of dopamine in these behaviors, see Carlsson, 1978; Crow & Deakin, 1978; Fibiger, 1978; Fibiger & Phillips, 1979; Hall, Bloom, & J. Olds, 1977; Hornykiewicz, 1972; Post, Cutler, Jimerson, & Bunney, Jr., 1981; Snyder, Banerjee, Yamamura, & Greenberg, 1974; Ungerstedt, 1971bd, 1978; Wise, 1978, 1982). Because of the profound influence of opiates on behavior including the development of narcotic addiction, interactions of brain dopamine and opiates have become an area of considerable interest and the subject of numerous reviews (see Clouet, 1975; Eidelberg, 1976; Iwamoto & Way, 1979; Iwatsubo, 1982; Klemm, 1981; Kuschinsky, 1981; Lal, 1975; Martin & Sloan, 1977; Sparber, Gellert, & Fossom, 1979).

5.1.1 Distribution of Opiate Receptors at Dopamine Systems

Opiate-receptor fields have been identified at both dopamine terminal areas and cell bodies.[1] In addition, immunocytochemical procedures have demonstrated the existence of endorphins in these same regions (Haber & Elde, 1982; Hokfelt, Elde, Johansson, Terenius, & Stein, 1977; Sar, Stumpf, Miller, Chang, & Cuatrecasas, 1978; Uhl, Goodman, Kuhar, Childers, Snyder, 1979; Yang, Hong, & Costa, 1977). The distribution of opiate receptors parallels the concentration of dopamine found at various rostral-caudal sections in the striatum (Schwartz, Pollard, Llorens, Malfroy, Gros, Pradelles, & Dray, 1978). A morphological relationship has been established between these opiate receptors and dopamine neurons, largely through the use of selective lesioning procedures. The injection of 6-hydroxydopamine into the striatum results in a selective destruction of the presynaptic dopamine neurons in this region (Breese & Traylor, 1970, 1971; Jonsson, 1980). This is accompanied by a 30 to 55% reduction in striatal opiate binding (Carenzi, Frigeni, & Bella, 1978; Gardner, Zukin, & Makman, 1980; Llorens-Cortes, Pollard, & Schwartz, 1979;

---

[1]Compare the distribution of dopamine neurons (Jacobowitz & Palkovits, 1974; Lindvall & Björklund, 1974; Moore & Bloom, 1978; Palkovits & Jacobowitz, 1974; Ungerstedt, 1971a) with opiate receptors (Atweh & Kuhar, 1977ab; Duka, Schubert, Wuster, Stoiber, & Herz, 1981; Pert, Kuhar, & Snyder, 1975, 1976; Snyder & Matthysse, 1975). Some of the highest opiate-receptor binding is found in the terminal fields of the mesolimbic and nigro-striatal dopamine systems (e.g., nucleus accumbens, amygdala, caudate nucleus). Moderate levels of opiate-receptor binding are also found proximal to the dopamine cell bodies of these systems (i.e., substantia nigra, ventral tegmental area).

Murrin, Coyle, & Kuhar, 1980; Schwartz et al., 1978) suggesting a presynaptic localization of these opiate receptors.[2] Lesions in the cell body region that projects to the striatum (i.e., substantia nigra) also produce a similar decline in striatal opiate-receptor binding (Gardner et al., 1980; Llorens et al., 1979; Schwartz et al., 1978). Similarly, injections of 6-hydroxydopamine into the cell body region of the mesolimbic dopamine system (i.e., ventral tegmental area) or the terminal field in the nucleus accumbens lead to a reduction of opiate receptor binding of the terminal region in the nucleus accumbens (Pollard, Llorens, Bonnet, Costentin, & Schwartz, 1977).

The injection of kainic acid into the striatum, which selectively destroys the cell bodies (Britt & Wise, 1982; Jonsson, 1980; McGeer & McGeer, 1981, 1982; Peterson & Moore, 1980) sparing the presynaptic dopaminergic neurons, produces a 35 to 83% reduction in striatal opiate-receptor binding (Carenzi et al., 1978; Gardner et al., 1980; Llorens et al., 1979; Murrin et al., 1980; Schwartz et al., 1978). This reduction in opiate binding is believed to reflect the loss of postsynaptic opiate receptors. The effects of

---

[2]Several investigators have shown that transsynaptic degeneration can occur after lesioning (Ghetti, Horoupian, & Wisniewsky, 1972; Hattori & Fibiger, 1982; Pinching & Powell, 1971: cells that make synaptic contact with lesioned cells can undergo degeneration even though they were not directly lesioned. It is unlikely that such transsynaptic degeneration could account for all of the loss in opiate receptor binding seen in these lesion studies, but it could influence the magnitude of the reduction in binding.

kainic acid and 6-hydroxydopamine lesions are additive in the striatum (Schwartz, 1979) further supporting the notion that these neurochemical lesions are affecting different opiate receptors (i.e., pre- vs. post-synaptic opiate receptor sites). It is interesting to note that some opiate-receptor binding remains in the striatum after this combined lesioning technique (Schwartz, 1979). This suggests a presynaptic opiate receptor in the striatum associated with a system that originates outside the substantia nigra.

The overlapping distribution of opiate-receptor fields and dopamine systems makes endorphins a likely neuromodulator of dopaminergic activity. Johnson, Sar, and Stumpf (1980) have recently used combined histofluorescence immunocytochemistry to demonstrate the presence of enkephalin fibers juxtapositional to substantia nigra and ventral tegmental dopamine neurons. Their study suggests that enkephalinergic fibers make axo-dendritic or axo-axonic contact with dopamine neurons in this region.

5.1.2 Dopamine Synthesis and Metabolism

The catecholamine synthesis chain begins with the transport of tyrosine into the nervous system and its subsequent conversion by tyrosine hydroxylase to L-3,4-dihydroxyphenylalanine (L-dopa). Next, L-dopa is decarboxylated to dopamine by aromatic amino acid decarboxylase. In nerve terminals containing the catecholamine norepinephrine, dopamine is further acted upon by dopamine

beta hydroxylase to form norepinephrine. The conversion of L-dopa to dopamine is very rapid while the oxidation of tyrosine to L-dopa is much slower. Thus tyrosine hydroxylation is the rate-limiting step in the biosynthesis of dopamine. Dopamine is probably inactivated by several mechanisms: (1) passive diffusion from the synaptic cleft, (2) reuptake which returns dopamine to the presynaptic terminal, and (3) enzymatic degradation. Although there are several metabolic pathways that can eliminate dopamine, the two enzymes primarily responsible for its inactivation are monoamine oxidase which converts dopamine to 3,4-dihydroxyphenylacetic acid (DOPAC) and catechol-O-methyltransferase which metabolizes dopamine to 3-methoxytyramine.[3] Both compounds can be further metabolized to homovanillic acid although appreciable quantities of DOPAC can leave the central nervous system. (For reviews of dopamine biosynthesis and metabolism, see Cooper, Bloom, & Roth, 1978; Hornykiewicz, 1972; Moore & Kelly, 1978; Moore & Wuerthele, 1979; Westerink, 1979.)

5.1.3 Distinction between Neural Activity and Function

It is important to distinguish between the concepts of neurotransmitter activity and function. "Activity" is

---

[3] Westerink and Spaan (1982a) have shown that only about 20% of the homovanillic acid is formed from 3-methoxytyramine. This suggests that this enzymatic pathway is relatively unimportant for the degradation of dopamine.

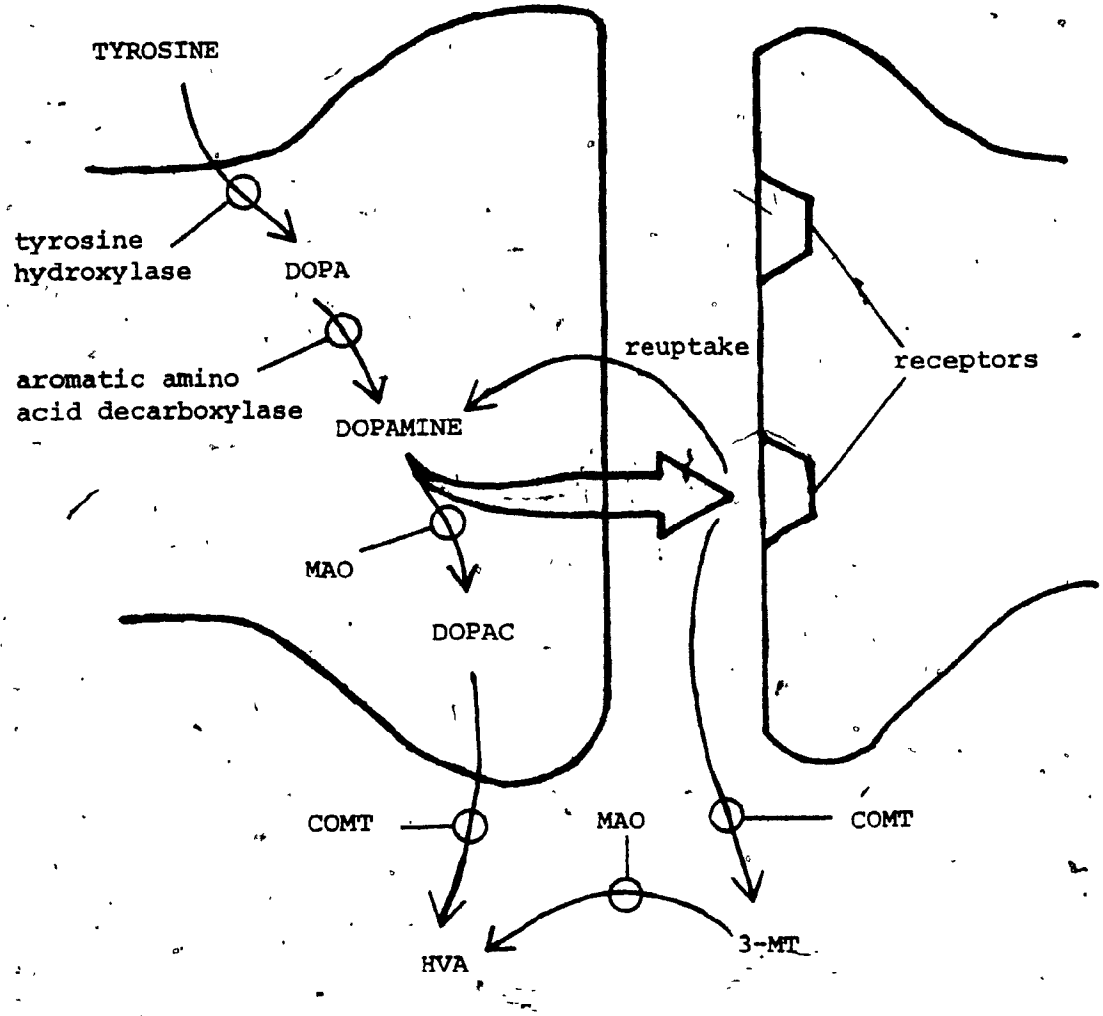

Fig. 5.1. Dopamine is intracellularly metabolized to DOPAC which leaves the brain in appreciable quantities. Much of the DOPAC is further metabolized to HVA in an extraneuronal compartment. Abbreviations: COMT, catechol-O- methyltransferase; DOPA, L-3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; MAO, monoamine oxidase; 3-MT, 3-methoxytyramine.
Note: Intermediate and alternative metabolic pathways have been omitted (e.g., aldehyde dehydrogenase).

used to designate dynamic changes in the parameters of neurotransmitter synthesis, release, and metabolism (i.e., presynaptic and extraneuronal events). An increase in any one (or all) of these parameters can be regarded as an increase in the activity of a particular neurotransmitter. No interpretation should be inferred regarding the efficacy of that increased activity on receptor cells and those postsynaptic events mediated by that neurotransmitter. While it is frequently true that increased activity of a neurotransmitter system can produce an enhancement of the events mediated by that neurotransmitter, it is equally possible that these same parameters of neurotransmitter activity might be the result of an attenuation of neurotransmitter efficacy at the receptor cell.

When the events mediated by the receptor cell are enhanced, there is an increase in the function of that particular neurotransmitter. Thus, "function" can be defined as the events normally mediated by the receptor cell. If the effect of a particular neurotransmitter were to excite a postsynaptic receptor cell, then enhanced neurotransmitter function would result in an increased excitatory action at the receptor cell. Conversely, decreased neurotransmitter function describes an attenuation of those events mediated by that particular neurotransmitter. In this sense function connotes the net result of neurotransmitter stimulation on the receptor cell and directly reflects the efficacy of that action.

The neurochemical determination of neurotransmitter activity generally involves the measurement of turnover (i.e., synthesis rate) or metabolite formation. The activity of a particular cell group which contains a given neurotransmitter can also be assessed by electrophysiological recording of the cell firing rate. Both approaches usually give a clear indication of the rate of neurotransmitter release and, thus, activity. Neurophysiological determination of changes in neurotransmitter function, however, becomes much more difficult. The postsynaptic target cells must be identified and changes in the electrical activity or the biochemical events (e.g., transmitter-gated channels or formation of cyclic adenosine 3',5'-monophosphate) mediated by the action of a particular neurotransmitter on those cells must be studied. Because there is difficulty in determining the neurophysiological consequences of a neurotransmitter on its receptor cells, most neurochemical studies rely on measurement of neurotransmitter activity and assume that the effect of this activity on neurotransmitter function is predictable from known systems.

Electrical stimulation of the substantia nigra or its efferent fibers causes an increase in the release and metabolism of dopamine with a concomitant acceleration of synthesis (Korf, Grasdijk, & Westerink, 1976; Korf, Zieleman, & Westerink, 1976; Murrin & Roth, 1976). The enhanced dopamine activity can be easily measured by elevations in DOPAC and homovanillic acid levels or dopamine turnover rate. The

newly released dopamine is presumed to increase the post-synaptic events mediated by this neurotransmitter system and both neurochemical (see Korf, 1979) and behavioral (e.g., Arbuthnott & Ungerstedt, 1975; Barghon & Costentin, 1980; Roffman, Bernard, Dawson, Sobiski, & Saelens, 1978) indices support this assumption. Amphetamine administration produces an increase in dopamine release and synthesis (see Glowinski, Cheramy, & Giorguieff, 1979; Nicolaou, 1980; Westerink, 1979) without a reliable increase in DOPAC or homovanillic acid formation. These changes in activity are associated with enhanced dopamine function (see Breese, Hollister, & Cooper, 1976; Cole, 1978, Creese & Iversen, 1975; Hornykiewicz, 1972) even though some measures of dopamine activity (i.e., firing rate of dopaminergic neurons, DOPAC formation) are decreased (see Bunney, 1979; Bunney & Aghajanian, 1978; Moore & Kelly, 1978; Nicolaou, 1980; Westerink, 1979). The administration of neuroleptics also increases the release of dopamine, but this effect is accompanied by marked increases in dopamine metabolite formation and the firing rate of these neurons (see Bunney 1979; Bunney & Aghajanian, 1978; Moore & Kelly, 1978; Westerink, 1978, 1979). While neuroleptic treatment produces an increase in dopamine activity, it is the result of a blockade of dopamine receptors on the target cell of this system;[4] hence, there is a decrease in dopamine function.

---

[4]Numerous investigators have shown that a decrease in the postsynaptic consequences of dopamine action produces an increase in the presynaptic activity of these systems (e.g., Bunney & Aghajanian, 1976; Carlsson & Lindqvist, 1963;

(See Seeman, 1980, for a review of the data indicating that neuroleptics block dopamine receptors.)

The enhanced firing rate, elevated neurotransmitter release, and increased metabolite formation are so closely associated with the postsynaptic blockade of dopamine sensitive cells that they characterize neuroleptic action (Bunney, 1979; Westerink, 1978, 1979) and have been used to conclude that the effect of various drugs is neuroleptic-like. This is in direct contrast to the normal neurophysiological situation where increases in neurotransmitter activity would be expected to result in increases in neurotransmitter function.

5.2 Effects of Morphine on Parameters of Dopamine Neurophysiology

Perhaps the most common method of studying the effects of opiates on dopaminergic systems is to determine the actions of these compounds on various aspects of dopamine neurophysiology. There are three primary variables to be considered. First, the electrical activity of cells that release or respond to the application of dopamine can be determined. Potential excitatory and inhibitory effects are reflected by increases and decreases in the spontaneous firing rate of these cells. Multiple unit and single unit activity give indices of the effects of morphine on large or small populations of neurons, respectively. Caution must be

---

Cheramy, Leviel, & Glowinski, 1981; Glowinski, Michelot, & Cheramy, 1980; Tissari & Gessa, 1981), although there is considerable controversy regarding the nature of this feedback mechanism.

exercised in the interpretation of the results of these experiments, however, in regard to whether the changes in electrical activity represent changes in dopamine activity or function. A second approach measures changes in the release and metabolism of dopamine. This neurochemical method, however, would appear to be limited to changes in dopamine activity and has no direct relationship to the functional aspects of that activity. The third approach determines the effect of opiates on various systems believed to reflect the functional result of dopaminergic activity--the biochemical processes that mediate the effects of dopamine on the target cells of these systems. Of these three techniques, the latter approach would be the most direct measure possible of the effects of opiates on dopamine function. It is also, however, the most problematic since the postsynaptic consequences of dopaminergic activation of the target cells are not easily identifiable.

5.2.1 Electrophysiological Indices

Opiates affect the electrical activity of many neural systems. The predominant effect of both systemic and microiontophoretic application of opiates seems to be a depression in the spontaneous firing rate and the evoked activity of cells (Baldino, Beckman, & Adler, 1980; French & Siggins, 1980; Gebhart, 1982; Klemm & Mallari, 1980; North, 1979; Zieglgansberger, Siggins, French, & Bloom, 1978). There are several exceptions to this; the

most notable is the excitatory action of opiates at hippocampal cells (Corrigall & Linseman, 1980; Gahwiler, 1980; Gahwiler & Maurer, 1981; Robinson & Deadwyler, 1980). This action, however, is probably due to disinhibition and not to the result of a direct excitatory action (see Nicoll, Alger, & Jahr, 1980; Zieglgansberger et al., 1978; Zieglgansberger, French, Siggins, & Bloom, 1979). Most studies of the effects of opiates on electrical activity have used unidentified populations of neurons. That is, the neurotransmitter involved in the normal activity of these units has not been determined by the experimenter. Nonetheless, since the predominant effect of opiates on unit activity is a decrease in firing rate, many investigators have apparently concluded that this inhibitory action is also likely to occur in dopaminergic neurons. This conclusion, however, is contrary to the empirical evidence regarding the effect of opiates on cells identified as containing dopamine as their neurotransmitter.

Morphine treatment increases the firing rate of the dopamine-containing cells of the substantia nigra (Gysling & Wang, 1982; Iwatsubo & Clouet, 1977; Ostrowski, Chiodo, Keller, & Caggiula, 1981; Matthew & German, 1982). This effect has also been reported for the ventral tegmental dopamine cells (Gysling & Wang, 1982; Matthew & German, 1982). The microiontophoretic application of morphine into the substantia nigra produces an increase in the firing rate of these dopamine-containing cells; it has been found, how-

ever, that the ventral tegmental dopamine neurons are much more sensitive to morphine treatment than are the substantia nigra dopamine cells (Matthew & German, 1982). Lesions of the efferent fibers of the nigrostriatal system attenuate the response of substantia nigra neurons to morphine treatment while lesions of the efferent connections of the mesolimbic system have no effect on the enhanced firing of ventral tegmental neurons following morphine treatment (Gysling & Wang, 1982).

Both the systemic and the microiontophoretic application of morphine directly onto caudate (Dafny, Brown, Burks, & Rigor, 1979ab; Frederickson & Norris, 1976, 1978) and globus pallidus (Huffman & Felpel, 1981) neurons inhibit the neural activity of these units. This parallels the action of dopamine at these neurons (Bernardi, Marciani, Morocutti, Pavone, & Stanzione, 1978; Herrling & Hull, 1980; Siggins, 1978; but see also York, 1979). Thus the effect of morphine on the terminal projections of the substantia nigra dopamine system seems to mimic the action of dopamine on these cells.

In an elegant experiment by Finnerty and Chan (1981), units from several populations were simultaneously studied in an attempt to determine the effects of systemic morphine on several links in the nigrostriatal dopamine system. Units in the pars compacta of the substantia nigra which contain dopamine cells, units in the caudate nucleus which is one of the major terminal projections of this system, and units in

the zona reticulata were studied in the same preparation. The latter region contains cells that are not dopaminergic and is believed to send axons dorsally to the pars compacta to modulate the activity of these dopamine-containing neurons. The systemic injection of morphine caused a dose dependent decrease in activity in the units recorded from the zona reticulata while units in the dopamine-containing cell bodies of the zona compacta increased their spontaneous firing rates. The caudate nucleus showed a decrease in cell firing indicating that morphine inhibited the postsynaptic target cells of the nigrostriatal projection. This latter finding is especially interesting since the action of iontophoretically applied dopamine on these units is inhibitory. Hence, systemic morphine injections mimic the effects of dopamine release in the caudate nucleus. Finnerty and Chan (1979, 1981) suggested that morphine causes a functional increase in dopamine activity by disinhibiting cells in the zona compacta of the substantia nigra. Regardless of the nature of this action, their study demonstrates that morphine can increase both the activity and function of this dopaminergic system.

Palmer and Hoffer (1980) have reported that the microiontophoretic application of an enkephalin analog decreases the unit activity of cells in the frontal cortex. This region receives input from the ventral tegmental dopamine system and the distribution of these neurons parallels that seen for enkephalins (see Palmer & Hoffer,

1980). The depressant action of enkephalin on these cells was blocked by neuroleptics which block dopamine-receptors but not by their inactive stereoisomers. Furthermore, the destruction of the dopaminergic input to the frontal cortex was shown to attenuate the action of enkephalin on these cells.

In summary, while morphine decreases the activity of most units studied in the central nervous system, dopamine containing cells in both the substantia nigra and ventral tegmental area show an increase in unit activity in response to morphine treatment. Similarly, the target cells of these projections show changes in activity that parallel the effect of dopamine application.

5.2.2 Neurochemical Measures

Morphine increases the synthesis and release of dopamine as well as the formation of two of its metabolites, DOPAC and homovanillic acid (Chesselet, Cheramy, Reisine, & Glowinski, 1981; Clouet, Johnson, Ratner, Williams, & Gold, 1973; Clouet & Ratner, 1970; Costa, Cheney, Racagni, & Zsilla, 1975; Gauchy, Agiel, Glowinski, & Cheramy, 1973; Kuschinsky & Hornykiewicz, 1972; Moleman, 1977; Westerink & Korf, 1976). This indicates that there is an increase in the activity of dopaminergic systems in response to morphine treatment. The nature of this enhanced activity, however, is not clear. The application of amphetamine, which is known to increase dopaminergic function, produces a decrease

in the metabolism of dopamine. Neuroleptic treatment, which has been shown to block dopamine receptors, produces an increase in the synthesis and metabolism of dopamine. This has led several investigators to suggest that morphine blocks dopamine receptors and the increase in dopaminergic activity is the result of a compensatory increase in the firing of dopamine-containing cells similar to that proposed for neuroleptic drugs. The electrical activation of dopamine-containing cells in the substantia nigra also produces an increase in the synthesis and metabolism of dopamine and this effect is assumed to be related to an increase in the functional activity of this system. Thus, the increase in dopaminergic activity (i.e., synthesis and metabolism) may be associated with either a diminished or enhanced function of these dopaminergic neurons. Therefore, measures of dopamine activity do not establish whether morphine increases or decreases the function of these dopamine systems.

5.2.3 Putative Models of Dopamine Function

Another approach to assessing the effect of morphine on dopamine function is to directly measure the influence of morphine on the postsynaptic consequences of dopamine. That is, if the effects of dopamine on its target cells were identified, then the action of morphine on these postsynaptic events could yield a direct measure of morphine's effect on dopamine function. There are three models of dopamine func-

tion that will be considered in this regard: (1) the formation of 3-methoxytyramine which is believed to reflect the extraneuronal release of dopamine, (2) the formation of cyclic adenosine 3',5'-monophosphate which has been proposed as a second messenger in cells receiving dopaminergic innervation, and (3) the release of prolactin which has been shown to be inhibited by dopamine.

It has been proposed that the metabolism of dopamine to 3-methoxytyramine reflects the functional, extraneuronal release of dopamine while the metabolism of dopamine to DOPAC and homovanillic acid is the result of nonfunctional, intraneuronal dopamine release (Carlsson & Lindqvist, 1963; Di Giulio, Groppetti, Cattabeni, Galli, Maggi, Algeri, & Ponzio, 1978; Kehr, 1976; Westerink, 1979; Wood, Stotland, Richard, & Rackham, 1980). The administration of drugs believed to increase dopaminergic function through an action on endogenous dopamine (i.e., amphetamine, cocaine, methylphenidate) causes an increase in 3-methoxytyramine while the direct acting dopaminergic agonist apomorphine does not (Di Giulio et al., 1978; Waldmeir, Lauber, Blum, & Richter, 1981; Westerink, 1979; Westerink & Spaan, 1982ab; Wood et al., 1980). Similarly, electrical stimulation of the dopamine cells in the substantia nigra causes a frequency-dependent increase in striatal 3-methoxytyramine formation (Wood, Nair, & Bozarth, 1982) further supporting the notion that functional increases in dopamine neurotransmission are accompanied by

an increase in this metabolite. Waldmeier et al. (1981) have challenged this proposal suggesting that 3-methoxytyramine is rapidly converted to homovanillic acid and thus maintains a steady-state concentration despite increased metabolism of dopamine to 3-methoxytyramine (see also Westerink & Spann, 1982ab). This argument attributes much of the increase in 3-methoxytyramine following amphetamine treatment to the monamine oxidase inhibiting properties of amphetamine. This would explain why some drug treatments (e.g., neuroleptics) thought to increase the extraneuronal release of dopamine fail to produce increased levels of 3-methoxytyramine (cf. Westerink, 1978). The increased formation of 3-methoxytyramine following electrical stimulation, however, clearly demonstrates that the rate of deamination of 3-methoxytyramine to homvanillic acid is not sufficient to maintain a steady-state concentration of this potentional index of functional dopamine-release (Wood et al, 1982).

Wood et al. (1980) have examined the effect of morphine on striatal dopamine metabolism and found an increase in DOPAC and homovanillic acid formation accompanied by no change in 3-methoxytyramine in the rat. This is in contrast to their finding of an increase in 3-methoxytyramine levels in mouse brain. They relate these findings to the behavioral depressant effect of morphine in rats compared with the behavioral activation found in mice. Thus the effect of morphine

on striatal 3-methoxytyramine levels depends on the species tested as does the behavioral effects of morphine treatment.

Recently, the same laboratory group has examined the effect of morphine on 3-methoxytyramine levels in several projections of the ventral tegmental dopamine system. Wood (personal communication) has found an increased formation of 3-methoxytyramine in the nucleus accumbens[5] but not in the frontal cortex[6] or septal terminal fields of this system. Therefore, the effect of morphine on this putative model of dopamine function depends on the dopamine terminal field studied. Further testing is required before the validity of this model can be fully evaluated.

Considerable evidence suggests that cyclic adenosine 3',5'-monophosphate (cAMP) may mediate the postsynaptic consequences of some neurochemical transmission including that of dopamine. Treatments known to enhance dopaminergic neuro-

[5]This finding substantiates an earlier report by Westerink (1978) which showed an increase in 3-methoxytyramine in the nucleus accumbens and olfactory tubercle of rats following morphine treatment. Westerink (1978), however, used a monoamine oxidase inhibitor to retard the formation of homovanillic acid and thus slow the metabolism of 3-methoxytyramine. Inhibition of this enzyme has been suggested by Roth, Salzman, and Nowycky (1978) to alter catecholamine synthesis.

[6]Bannon, Bunney, and Roth (1981) have recently shown that the turnover of dopamine in the frontal cortex is approximately four times faster than that in the striatum. This rapid turnover might make it difficult to detect significant drug-induced changes in 3-methoxytyramine levels since the basal level of this metabolite is probably proportionally higher in the frontal cortex than in the striatum.

transmission (e.g., electrical activation of either pre- or post-synaptic elements, amphetamine administration) increase the formation of cAMP in striatal tissue while treatments that decrease dopaminergic function (e.g., dopaminergic receptor blockade) lower the levels of cAMP in dopamine-rich brain areas. Manipulations that alter cAMP levels have also been shown to influence events believed to be dependent on dopaminergic neurotransmission. (For reviews of the involvement of cAMP in neurotransmission, see Bloom, 1975; Briggs & McAfee, 1982; Cooper et al., 1978; Greengard, 1976, 1981; Kebabian & Greengard, 1971; Kebabian, Zata, & O'Dea, 1977; Libet, 1979).

The effect of opiates on cAMP formation has been proposed as a molecular model of opiate action (see Brandt, Traber, Glaser, & Hamprecht, 1978; Collier, 1979, 1980; Hamprecht, 1978; Klee, 1979; Wollemann, 1981). This model has been specifically applied to the action of opiates on dopaminergic systems by studying the effects of opiates in preparations where the effects of dopamine are known (e.g., Carenzi, 1978; Costa, Carenzi, Guidotti, & Revuelta, 1973; Neff, Parenti, Gentleman, & Olianas, 1981; Stefano, Catapane, & Kream, 1981).

There are two problems with using cAMP as a model of opiate action on dopaminergic function. First, the relevance of cAMP to dopamine function has been challenged. The dopamine-receptor population that mediates the effect of dopamine on cAMP formation is different from the receptor population responsible for the mediation of the other effects

of dopamine-receptor activation (Hartley, Spuhler, Prasad, & Seeman, 1980; Marchais, & Bockaert, 1980; Seeman, 1980; Takimoto & Weiner, 1981). Libet (1979) has suggested that cAMP mediates the modulatory effect of dopamine on acetylcholine neurotransmission but not the hyperpolarization response seen in many dopaminergic target cells. Although there is strong evidence that dopamine can influence the formation of cAMP, the relevance of this action to the behavioral or neurophysiological effects of dopamine is unclear (see Seeman, 1980). Another problem with the use of cAMP as a model of the effect of opiate action on dopaminergic function concerns the nature of the interaction of opiates with this system. Costa et al. (1973) have reported that cAMP formation is enhanced by amphetamine in a dose-dependent manner and that a similar effect was observed with morphine. The threshold dose of morphine necessary to stimulate dopamine turnover, however, was lower than that required to alter the formation of cAMP. Other investigators have reported conflicting results with opiates inhibiting, stimulating, or not affecting the formation of cAMP (see Simon & Hiller, 1978; Wollemann, 1981). Fantozzi, Mullikin-Kilpatrick, and Blume (1981) have shown that in neuroblastoma x glioma cells[7] a reduction in the number of

---

[7]Neuroblastoma x glioma cells (NG108-15) are a hybrid cell culture frequently used to study the effects of opiates on cAMP. These cells show stereospecific opiate binding that is blocked by naloxone and have even been used to demonstrate tolerance to opiates (see Brandt et al., 1978; Collier, 1980; Hamprecht, 1978).

opiate receptors does not affect the ability of an enkephalin analog to inhibit the formation of cAMP. In addition, both intracellular and extracellular recording techniques have been used to show that the in vitro inhibitory effect of opiates on single neurons is not affected by manipulations of cAMP formation (Karras & North, 1979; North & Vitek, 1980). Therefore, the proposed relationship of cAMP and opiate actions may be a promising approach to studying the molecular effects of opiates, but the existing data do not support its use as a model of dopaminergic function.

Prolactin secretion from the pituitary is inhibited by dopamine, and drug-induced increases in the release of prolactin have been suggested to indicate a blockade of dopamine receptors. (For reviews of the control of prolactin secretion, see Cronin, 1982; Moore & Johnston, 1982; Moore & Wuerthele, 1979.) In fact, the antipsychotic potency of neuroleptics, which is believed to result from their ability to block dopamine receptors (see Seeman, 1980; Snyder et al., 1974), is highly correlated with their ability to stimulate prolactin secretion (Langer, Sachar, Gruen, & Halpern, 1977). Furthermore, the stimulatory action of neuroleptics on prolactin secretion appears to be independent of cAMP formation (Lal, Niar, Iskandar, Etienne, Wood, Schwartz, & Guyda, 1982) as is the antipsychotic action of these drugs (Seeman, 1980).

Opiates and endorphins stimulate prolactin secretion (Collu, Tache, & Chaepenet, 1980; Gold, Redmond, Donabedian,

Goodwin, & Extein, 1978; Meites, Bruni, Van Vugt, & Smith, 1979) and this effect has been attributed to an inhibition of dopamine function (Collu et al., 1980; Labrie, Cusan, Dupont, Ferland, & Lemay, 1978; Van Loon, De Souza, Ho, & Shin, 1980). The effect of opiates on the dopaminergic inhibition of prolactin release in vitro is unclear with some investigators reporting an attenuation of dopamine's effect (Enjalbert, Ruberg, Arancibia, Priam, & Kordon, 1979) while others report no change (Login & MacLeod, 1979).

Although blockade of dopamine receptors clearly stimulates prolactin secretion, two lines of evidence challenge the use of prolactin secretion as a general model of brain dopamine function. First, opiates increase the activity of the nigrostriatal and mesolimbic dopamine systems (see Section 5.2.1 and 5.2.2) but decrease the activity of the tuberoinfundibular dopamine system which is believed to control prolactin release (Alper, Demarest, & Moore, 1980; Demarest & Moore, 1981). The reason for this difference has not been established, but it may be related to functional differences in the regulation of dopamine activity in these systems (see Moore & Wuerthele, 1979). Second, there is reason to suspect that morphine's effect on prolactin secretion is not mediated by changes in dopamine function but by alterations in serotonergic (Demarest & Moore, 1981; Koenig, Mayfield, Coppings, McCann, & Krulich, 1980; Muller, Locatelli, Cocchi, Spampinato, Apud, Ferri, & Racagni, 1980) or cholinergic (Muraki, Tokunaga, Nakadate,

& Kato, 1979) systems. Recently, Johnson (1982) has shown that the microinjection of morphine directly into the serotonergic cell bodies of the dorsal raphe nucleus stimulates prolactin secretion. Thus the action of opiates on the tuberoinfundibular dopamine system may even be irrelevant to the prolactin-releasing effects of these drugs. In any case, the effect of opiates on prolactin release is not a suitable model to study the action of these drugs on the function of other dopaminergic systems.

5.3 Effects of Dopamine Manipulations on Morphine-Induced Behaviors

Since the effect of morphine on dopamine function cannot be determined by observing changes in the parameters of dopamine neurophysiology produced by opiate administration, alternative methods must be used to assess the possible interactions and interdependences of morphine and dopaminergic mechanisms. One approach is to study the effect of dopamine manipulations on morphine-induced effects such as analgesia and physical dependence. If manipulations of dopamine function (e.g., synthesis blockade, receptor blockade, potentiation by precursor loading or agonist administration) can be shown to govern the expression of some morphine induced effects, then an action of morphine on brain dopamine function might be inferred from this relationship. It is important to note that the effect of manipulations of dopamine function on morphine-induced behaviors could be the result of the independent actions of these compounds on the

behavior under study. Morphine may initiate a sequence of events that produces analgesia, but an independent action of a drug which enhances dopamine function may uncouple this response. That is, hyperactivity induced by a dopamine agonist may mask the expression of analgesia which is the primary effect of morphine. These data should not be construed to indicate that dopamine agonists block morphine induced analgesia.

Some of the most prominent effects of morphine on behavior are analgesia, the development of physical dependence, and reward or addiction to opiates. It is difficult to evaluate the effects of drugs which alter dopamine activity on these morphine-induced behaviors. Profound changes in motor activity can occur from alterations of dopamine function. Generally, treatments that enhance dopamine function lead to increased responsiveness to both internal and external stimuli while attenuation of dopamine function produces marked lethargy and sedation (see Dominic & Moore, 1969; Hornykiewicz, 1972; Lloyd & Hornykiewicz, 1975; Randrup, Munkvad, Fog, & Ayhan, 1975). It is not surprising to find many studies that report increased responsiveness to morphine-induced analgesia (e.g., Eidelberg & Erspamer, 1975; McGilliard & Takemori, 1979) and catalepsy from dopamine antagonists or increased withdrawal behaviors (Huang, Yano, & Takemori, 1978; Laschka, Gramsch, Blasig, & Herz, 1978) associated with drugs that potentiate dopamine function. (For reviews of the effects

of catecholamine manipulations on morphine-induced behaviors, see Akil, Watson, Holman, & Barchas, 1978; Clouet, 1975; Eidelberg, 1976; Lal, 1975; Martin & Sloan, 1977.) It would be predicted that an animal sedated by catecholamine depletion or dopamine-receptor blockade would show behavioral signs of enhanced analgesia. The pharmacological specificity of these effects, however, is speculative.

In contrast, studies reporting enhanced analgesia from dopamine agonists and attenuated analgesia from dopamine depletion or receptor blockade (e.g., Chan, 1979; Jensen & Smith, 1982; Nakamura, Kuntzman, Maggio & Conney, 1973; Price & Fibiger, 1975; Robertson, Weston, Lewis, & Barasi, 1981) cannot be discounted as the product of nonspecific arousal or sedation since the effects are opposite those predicted from the individual actions of these manipulations. Therefore, studies that demonstrate changes in morphine induced effects where the individual actions of these treatments directly summate cannot be construed as evidence supporting an inhibitory action of morphine on dopamine function, while the results of studies where the effect of alterations in dopamine function are opposite those produced by the individual actions of these agents add some support to a model depicting dopaminergic mediation of morphine induced effects. Furthermore, the finding that dopamine did not play a crucial role in a given morphine-induced effect would not indicate that morphine fails to modify brain dopamine function.

The presentation of data supporting a dopaminergic mediation of morphine's effects and the discounting of data to the contrary creates a seemingly one-sided argument. Perhaps the best approach is to examine systems where this potential bias is absent.

5.4 Effects of Morphine on Dopamine-Mediated Behaviors

An alternative approach for studying the effects of morphine on dopamine function is to use behavioral "end-points" where the relevant pharmacology has already been determined. There are a variety of behaviors which appear to be mediated or regulated by dopaminergic mechanisms and a number of experiments have examined the effects of morphine on these behaviors. It is not suggested that dopamine is exclusively involved in these behaviors, but rather that dopamine has an executive function whereby drugs modifying dopaminergic function produce predictable changes in behavior. In this sense, dopaminergic neural transmission may be tentatively viewed as a crucial synaptic element whereby morphine may express some of its effects.

5.4.1 Drug Discrimination Studies

The subjective similarities of amphetamine and morphine in humans are perhaps best exemplified by the development of psychometric scales designed to identify euphoria producing and mood-elevating properties of drugs. Using factor analytic techniques, Haertzen (1966) developed the

Morphine-Benzedrine Group (MBG) scale in the Addiction Research Center Inventory. This questionnaire was constructed to determine the subjective effects of various abused substances and to assess the abuse liability of new compounds. Although many drugs of abuse appear to produce an elevation in mood (Martin, 1973), the MBG scale specifically identifies drugs which produce morphine- and amphetamine-like euphoria. Humans can readily distinguish between morphine and amphetamine injections, but the pronounced similarities in the type of euphoria common to these drugs are inherent in the development of the MBG scale (see Haertzen, 1966; Hill, Haertzen, Wolbach, & Miner, 1963). It is not surprising that these two classes of drugs produce distinct stimulus properties since ex-addicts can even discriminate between injections of morphine and heroin showing a marked preference for the latter (Martin & Fraser, 1961; see also Eddy, Halbach, & Braenden, 1957). This is probably due to differences in the pharmacokinetics of these opiates since heroin is quickly deacetylated in the brain to morphine (Jaffe & Martin, 1975). Hence, differences in the subjective effects of these compounds would appear to result from the more rapid penetration of heroin into the central nervous system (Oldendorf, Hyman, Braun, & Oldendorf, 1972). Similarly, the subjective differences between morphine and amphetamine might result from the differences in their secondary actions: morphine's stimulus properties probably include effects related to its analgesic, sedative, and

emetic effects (see Jaffe & Martin, 1975; Martin & Sloan, 1977) while the predominant effects of amphetamine include psychomotor stimulation and autonomic actions associated with general arousal (see Gunne, 1977; Innes & Nickerson, 1975). The fact that these secondary effects may overshadow the marked similarities in mood-elevation is not surprising, and interviews with several persons reporting experience with both of these drugs support this assertion (M. Bozarth, unpublished observations).

Animals can easily be trained to discriminate the stimulus properties of various drugs (for reviews, see Colpaert, 1978; Ho, Richards, & Chute, 1978; Lal, 1977; Seiden & Dykstra, 1977). The drug discrimination paradigm has been proposed as an animal model of the subjective effects of various drugs including the opioids and amphetamines (Colpaert, 1978; Colpaert, Lal, Niemegeers, & Janssen, 1975; Shannon & Holtzman, 1979). The procedure typically involves training the subjects, in a two-lever choice situation, to press one lever during the drug state and another lever during saline treatment. Lever-pressing is usually followed by a food reward or by the postponement of some aversive event such as electric shock. After some criterion of correct choices (i.e., discriminations between the training drug and some other condition) has been reached, the subjects are injected with the test drug. The proportion of responding on each of the two levers is then tabulated to determine whether test drug is more like the training drug

or saline. This type of drug discrimination procedure has been extended to include not only differences in the stimulus properties of a drug and saline but also differences between various drugs and even different doses of the same training drug. One of the most important advantages of this technique is that drug treatments that disrupt motor behavior should not affect the animal's choice of levers. If the subject were severely sedated by the drug treatment, longer latencies to respond or even fewer completed trials might be produced, but the outcome of the choice between the two lever associations should not be altered.

The stimulus properties of amphetamine appear to be mediated by dopaminegic mechanisms (Ho & McKenna, 1978; Schechter & Cook, 1975). Similarly, the stimulus properties of morphine are attenuated by dopamine-receptor blockade (McCarten & Lal, 1979; see also Colpaert, Niemegeers, & Janssen, 1977) but not by inhibition of serotonin synthesis (Miksic, Shearman, & Lal, 1978). This suggests that the subjective effects of morphine in rats are also dependent on a dopaminergic mechanism.

Animals trained to discriminate amphetamine from saline do not generalize to a variety of agents including nicotine, mescaline, LSD, caffeine, and phenobarbital (see Seiden & Dykstra, 1977) indicating that the interoceptive cue produced by amphetamine is relatively specific. Moderate doses of morphine (5.6 mg/kg and greater) do not generalize to amphetamine while a lower dose (1.75 mg/kg) does (Shannon

& Holtzman, 1979). In rats trained to discriminate apomorphine from amphetamine, low doses of morphine consistently result in apomorphine-like "responding" (Hernandez, Holohean, & Appel, 1978). Drugs that block dopamine receptors do not generalize to morphine in the discrimination paradigm (Miksic et al., 1978; Shannon & Holtzman, 1976).

The apparent similarities in the subjective effects of morphine and amphetamine in both humans and animals point to common mechanisms of action for these mood-elevating drugs. Dependence on dopaminergic mechanisms for both the narcotic cue and amphetamine discrimination appears to suggest that morphine can facilitate dopamine function. It is important to note that generalization only occurs with low doses of morphine even though the interoceptive cue produced by larger doses is also attenuated by dopamine-receptor blockade.

5.4.2 Brain Stimulation Reward

Increasing dopamine function (e.g., precursor loading, blocking reuptake, direct-agonist stimulation) has been shown to increase rates of lever pressing for brain stimulation reward, while decreasing dopamine function (e.g., synthesis blockade, neurotransmitter depletion, receptor blockade) generally attenuates brain stimulation reward (see Broekkamp, 1976; Fibiger, 1978; J. Olds, 1977; Wise, 1978a). Similar effects have been shown with thresholds for rewarding brain stimulation, and various techniques have

been developed to distinguish between drugs that impair response capacity from those that blunt the rewarding impact of the stimulation (Atalay, Bozarth, & Wise, 1982; Fouriezos & Wise, 1976; Zarevics & Setler, 1979). Although several neurotransmitters may influence brain stimulation reward, dopamine appears to have a special role in its regulation and expression. Manipulations of dopamine neurotransmission produce predictable changes in responding for brain stimulation reward (for reviews, see Broekkamp, 1976; Fibiger, 1978; Fibiger & Phillips, 1979; Hall et al., 1977; J. Olds, 1977; Wauquier & Rolls, 1976; Wise, 1978ab,1982); many drugs which modify brain stimulation reward seem to do so by inducing alterations in dopamine function although other neurotransmitter systems cannot be ruled out at this time (see Crow & Deakin, 1978; L. Stein, 1980). Since treatments that impair dopamine function produce a clear attenuation of brain stimulation reward while pharmacological manipulations that enhance dopamine function lead to facilitated responding for brain stimulation reward, this paradigm provides another technique to examine the effects of morphine on a behavior presumed to be dependent on brain dopamine function.

Morphine and other opioids produce a facilitation of brain stimulation reward which is reflected as both an increased rate of responding for a fixed intensity of stimulation (Adams, Lorens, & Mitchell, 1972; Bush, Bush, Miller, & Reid, 1976; Lorens & Mitchell, 1973; Reid & Bozarth, 1978)

and a lowering of threshold for brain stimulation reward (Bozarth, Gerber, & Wise, 1980; Esposito & Kornetsky, 1977; Kelley & Reid, 1977; Marcus & Kornetsky, 1974); this facilitory action seems to be specific to the rewarding properties of the brain stimulation (for reviews, see Bozarth, 1978; Esposito & Kornetsky, 1978). Initial injections of moderate doses of morphine (circa 10 mg/kg) cause a depression in responding followed hours later by a facilitation of brain stimulation reward. This apparently biphasic action of morphine on brain stimulation reward might be interpreted as indicating that the enhanced responding for brain stimulation reward is the result of rebound hyperexcitability.[8] This hypothesis is similar to one proposed by Smee and Overstreet (1976) and asserts that the primary action of morphine is to block dopamine receptors and that this action results in compensatory increases in the release and synthesis of dopamine. As the receptor blockade attenuates, rebound hyperactivity prevails because the turnover of dopamine continues at an enhanced rate.

Several lines of evidence converge to challenge this hypothesis. First, naloxone blocks the facilitation effect in animals injected chronically with morphine (Bozarth & Reid, 1977). If facilitation were the result of rebound

---

[8] This model was first proposed by Himmelsbach (1943) to explain cellular tolerance and dependence on opiates. See Martin and Sloan (1977) for a discussion of the contemporary derivations of this model.

hyperexcitability, the rapid displacement of morphine from the opiate receptor should induce enhanced responding for brain stimulation reward. Second, small doses of morphine (Reid, Lind, Bozarth, Merriman, & Stapleton, 1978) or heroin (Gerber, Bozarth, & Wise, 1981) produce facilitation of brain stimulation reward which is not preceded by a depression in responding. Third, it is possible to anatomically separate the brain loci involved in the inhibitory- and facilitatory-actions of morphine (Broekkamp, 1976). Microinjection studies have revealed that suppression of responding not followed by facilitation is produced by injections of morphine directly into the periventricular gray region while "pure" facilitation results from injections into the ventral tegmental nucleus (origin of the mesolimbic dopamine system). As suggested in other behavioral tests, the periventricular gray region appears to be responsible for an initial inhibitory action which may be related to general decreases in motility (Pert, 1978; Pert, DeWald, Liao, & Sivit, 1979; Wilcox & Levitt, 1978) and not specific to brain stimulation reward.

The only study which specifically tested the hypothesis of dopaminergic mediation of morphine-induced facilitation of brain stimulation reward showed that catecholamine synthesis inhibition abolished morphine's effects on brain stimulation reward (Pert, 1975). The effect of synthesis inhibition was specific to morphine-augmented brain stimulation reward since no change in pre-morphine rates of

responding was noted. Also, the inhibition of serotonin synthesis was without effect. Unfortunately, this important study has never been replicated and no attempt was made to distinguish between the effects of dopamine and norepinephrine synthesis blockade. The reported lack of effect for catecholamine synthesis inhibition on pre-morphine rates of responding is surprising since this treatment has previously been shown to attenuate responding for brain stimulation reward. It is theoretically viable, however, that the inhibition of catecholamine synthesis was not sufficient to affect baseline rates of responding but did effectively block the accelerated synthesis and release of dopamine induced by morphine treatment.

A dependence of morphine-induced facilitation of brain stimulation reward on dopamine is probable, but no direct evidence exists to support or to refute this hypothesis. Modification of dopamine function seems to be the most sensitive, and perhaps crucial, aspect of brain stimulation reward. Drugs that enhance dopamine function clearly facilitate responding for brain stimulation reward and morphine also facilitates this behavior. Although a dopaminergic basis of this effect has not been established, the proposition that morphine treatment causes a decrease in brain dopamine function is clearly untenable on the basis of these data.

5.4.3 Catalepsy

Drugs that deplete dopamine or block postsynaptic dopamine-receptors produce a profound state of immobility termed catalepsy (see Costall & Naylor, 1973; Hornykiewicz, 1972; Lal, Gianutsos, & Puri, 1975). In fact, this behavioral response has been so closely associated with diminished dopaminergic function (e.g., dopamine-receptor blockade) that catalepsy has been used to behaviorally predict neuroleptic activity (e.g., Costall & Naylor, 1974a; Janssen, Niemegeers, & Schellekens, 1965; Niemegeers & Janssen, 1979). Moderate to high doses of morphine (10 mg/kg and greater) also produce cataleptic-like immobility but with the added feature of extreme rigidity of the skeletal muscles (see Costall & Naylor, 1973, 1974b; Havemann & Kuschinsky, 1982; Lal et al., 1975; Wand, Kuschinsky, & Sontag, 1973). Because catalepsy has been identified with decreased functioning of striatal dopamine neurons, many researchers have hastily concluded that morphine's effects on motility must also result from impaired dopaminergic function in the striatum (e.g., Kuschinsky & Hornykiewicz, 1972; Lal, 1975; Lat et al., 1975; Sasame, Perez-Cruet, Di Chiara, Tagliamonte, Tagliamonte, & Gessa, 1972).

Despite the superficial similarities between neuroleptic-induced catalepsy and catatonia produced by opioids, there are important differences seemingly ignored by many reviewers. For this reason, the term catalepsy shall be restricted to immobility produced by neuroleptic drugs and catatonia used to note the added feature of

rigidity induced by moderate doses of opioids.

The most obvious differences in catalepsy and catatonia can be realized with behavioral observations. Catalepsy lacks the muscular rigidity characteristic of catatonia (Costall & Naylor, 1974a; Wand et al, 1973). Other marked differences have been noted between the state of immobility produced by opiates and neuroleptics including differences in their effects on the postural support system, righting reflex, and clinging response (De Ryck, Schallert, & Teitelbaum, 1980; Rossier & Bloom, 1979). Furthermore, tolerance rapidly develops to the catatonic effect of morphine while repeated testing is often necessary to demonstrate maximum catalepsy (Moleman, Versluis, & Bruinvels, 1978; Stanley & Glick, 1976).

Catalepsy and catatonia can also be distinguished pharmacologically. Anticholinergic drugs readily antagonize neuroleptic-induced catalepsy but have no effect on catatonia (Ezrin-Walters, Muller, & Seeman, 1976). Low doses of apomorphine decrease catatonia without affecting neuroleptic induced catalepsy (Ezrin-Walters et al., 1976). The immobility produced by the maximal cataleptic-inducing dose of a neuroleptic is further increased by morphine (Kuschinsky & Hornykiewicz, 1974) suggesting that these effects are mediated by separate pharmacological actions. In addition, other pharmacological manipulations have produced differential effects on catalepsy and catatonia (Ezrin-Walters et al., 1976; Kuschinsky & Hornykiewicz, 1974; Mason, Roberts,

& Fibiger, 1978; Moleman et al., 1978).

The neuroanatomical substrates of catalepsy and catatonia also differ. Lesions of the striatum effectively abolish or attenuate the cataleptic action of neuroleptics while a potentiation of catatonia is observed (Costall & Naylor, 1973, 1974b; Havemann, Winkler, Genç, & Kuschinsky, 1981; Koffer, Berney, & Hornykiewicz, 1978; Turski, Czuczwar, Turski, & Kleinrok, 1982).[9] The periventricular gray region and amygdala have been implicated in the induction of morphine-induced catatonia, while the striatum is firmly established as the locus of neuroleptic-induced catalepsy (Costall, Fortune, & Naylor, 1978; Costall & Naylor, 1973, 1974b; Koffer et al., 1978; Pert et al., 1979; Pert & Sivit, 1977; Sanberg, 1980; Sanberg, Pisa, & Fibiger, 1981; Wilcox & Levitt, 1978).

It is interesting to note that brain lesions attenuating catatonia reveal a pronounced behavioral excitement induced by acute morphine injections (Costall & Naylor, 1973,

---

[9]Kuschinsky's laboratory group has collected considerable evidence that muscular rigidity is mediated by an opiate action at the caudate nucleus while the akinetic response to opiates is the result of a drug action at another brain region (e.g., Havemann & Kuschinsky, 1981; Havemann, Winkler, Genç, & Kuschinsky, 1980; Havemann, Winkler, & Kuschinsky, 1980; Havemann et al., 1981; see Havemann & Kuschinsky, 1982, for a review). Rigidity, in these experiments is measured electromyographically and the relevance of this measure to the extreme rigidity seen with systemic opiate injections has not been established. It seems perplexing that an animal could display catatonic-like muscular rigidity without being akinetic. In fact, most behavioral observations usually fail to show any effect of intracaudate opiate infusions on motor behavior (M. Bozarth, unpublished observations; Dunstan, Broekkamp, & Lloyd, 1981; Pert, 1978).

1974b). This, along with other data which anatomically and pharmacologically characterize morphine's effects on motor behavior, has led to the suggestion that morphine simultaneously activates neural substrates responsible for catatonia and behavioral arousal (e.g., locomotor excitement and stereotypy) with sedation being the dominant effect (Broekkamp, 1976; Costall & Naylor, 1973, 1974b; Moleman, 1977; Moleman et al., 1978; Pert et al., 1979; Roberts, Mason, & Fibiger, 1978). In fact, the finding that amphetamine treatment enhances morphine-induced catatonia has led to the suggestion that morphine's cataleptic actions may be the result of the activation of extrastriatal (e.g., mesolimbic or mesocortical) dopamine systems (Moleman et al., 1978; see also Moleman, 1977).[10]

5.4.4 Locomotor Activity

The catecholamines have been implicated in the induction and regulation of locomotor activity (for reviews, see Hornykiewicz, 1972; Krauthamer, 1975; Lloyd & Hornykiewicz, 1975; Ungerstedt, 1978). Drugs that augment dopamine function have been shown to increase locomotor activity while

---

[10] Moleman's (1977; Moleman et al., 1978) suggestion that morphine may produce catalepsy by an activation of dopaminergic neurons seems contrary to what is generally known about the role of dopamine in motor behavior. Nonetheless, a high dose of amphetamine injected into the nucleus accumbens has been observed to produce a sedative effect (M. Bozarth, unpublished observation). No tests were performed to determine the pharmacological specificity of this effect, however, and it is very likely that the behavioral sedation was the result of a nonspecific, local anesthetic action of the extremely high concentration of amphetamine sulfate.

impairment of dopamine function attenuates these drug-induced increases in motor activity. Similarly, direct stimulation of postsynaptic dopamine-receptors with apomorphine increases behavioral excitement and this effect is antagonized by drugs that block dopamine receptors (Di Chiara & Gessa, 1978). Lesions which deplete striatal or mesolimbic dopamine abolished or attenuated both spontaneous (Fink & Smith, 1979a; 1980a) and amphetamine-induced (Fink & Smith, 1980b; Kehne, Sant, & Sorenson, 1981; Kelly, Seviour, & Iversen, 1975; Roberts, Zis, & Fibiger, 1975) locomotor activity. The stimulatory action of apomorphine is unaffected by these treatments, but the blockade of postsynaptic receptors in lesioned animals abolishes apomorphine's effect (Fink & Smith, 1980ab). The evidence strongly supports the notion that stimulant-induced as well as spontaneous motor activity is dependent on dopaminergic mechanisms probably associated with the nigrostriatal and/or mesolimbic pathways (Breese et al., 1976; Cole, 1978; Di Chiara & Gessa, 1978; Fink & Smith, 1979ab, 1980ab; Kelly et al., 1975; Roberts et al., 1975; Thornburg & Moore, 1973).

Morphine produces locomotor excitement in mice which appears to be mediated by catecholamines: pharmacological manipulations that deplete catecholamines or block their synthesis attenuate morphine's stimulatory effect while monoamine oxidase inhibition, which potentiates the effect of released catecholamines, enhances the locomotor stimulation caused by morphine (Carroll & Sharp, 1972; Fuchs &

Coper, 1980; Villarreal, Guzman, & Smith, 1973). The locomotor stimulation produced by amphetamine is also decreased by catecholamine synthesis inhibition, but not by the depletion of stored catecholamines, suggesting that the mechanisms of morphine- and amphetamine-induced locomotor excitement differ in some respects (Villarreal et al., 1973).

In rats the effects of morphine on locomotor activity are more complex. Moderate doses of morphine (circa 10 mg/kg) produce an initial depression in motor activity which is followed hours later by enhanced activity (Babbini & Davis, 1972; Domino, Vasko, & Wilson, 1976; Smee & Overstreet, 1976; Vasko & Domino, 1978). With repeated injections tolerance rapidly develops to the depressive effect of morphine, and behavioral excitement prevails (Babbini & Davis, 1972; Domino et al., 1976). This biphasic action of morphine has been reported for a number of physiological responses and behaviors (see Domino et al., 1976).

Low doses of morphine (1 to 5 mg/kg) produce behavioral activation which is not preceded by a depression in locomotor activity (Brady & Holtzman, 1981; Roberts et al., 1978; Vasko & Domino, 1978). This effect is blocked by inhibition of catecholamine synthesis (Davis, Babbini, & Khalsa, 1972; Eidelberg & Schwartz, 1970) but not by lesions that selectively deplete forebrain norepinephrine (Roberts et al., 1978).

The effect of morphine on locomotor activity is dependent upon the species, dose, and time after the injec-

tion that the behavior is measured. The effect of low doses parallels that observed with drugs stimulating dopamine function. The initial effect of moderate to high doses of morphine closely resembles that seen with drugs attenuating dopamine function while behavioral activation is seen some time after the initial depressive phase. It has been suggested that morphine blocks dopamine receptors and activates compensatory mechanisms that increase the release and synthesis of dopamine (Kuschinsky & Hornykiewicz, 1974; Puri & Lal, 1973). According to this hypothesis, the biphasic nature of moderate doses of morphine on locomotor activity can be attributed to an initial blockade of dopamine receptors followed by a compensatory increase in dopamine activity (Smee & Overstreet, 1976). As the receptor blockade attenuates, the increase in dopamine synthesis and release leads to heightened locomotor activity. It is interesting to note that tolerance has not been demonstrated to the behavioral-exciting effect of morphine (Babbini & Davis, 1972; Brady & Holtzman, 1981; Vasko & Domino, 1978).

While the hypothesis of dopamine-receptor blockade followed by compensatory increases in dopamine release and synthesis could account for the biphasic action of morphine on locomotor activity and other behaviors, the pure facilitatory action of low doses of morphine (e.g., Domino et al., 1976) cannot be explained by this model. It is possible that high doses of morphine produce a functional decrease in dopamine neural transmission (cf. Lal et al., 1975)

which is not essential for the accelerated release and synthesis of dopamine induced by small doses of morphine. Furthermore, naloxone antagonizes the motor depressant effect of morphine without precipitating behavioral excitement. This would be predicted from a model asserting a direct effect of morphine on dopamine release and synthesis and not from a hypothesis that attributes an enhanced function induced by dopamine-receptor blockade. Also, acute dopamine-receptor blockade induced by neuroleptics does not produce a latent locomotor excitability.

The microinjection of morphine or endorphins onto the dopamine-containing cell bodies of the ventral tegmental area produces an increase in locomotor activity (Arnt & Scheel-Kruger, 1979; Broekkamp & Phillips, 1979; Joyce & Iversen, 1979). This enhanced motor activity is blocked by neuroleptics (Joyce & Iversen, 1979; Joyce, Koob, Strecker, Iversen, & Bloom, 1981; Sanchez-Blazquez, Garzon, & Del Rio, 1980) and by lesions of the mesolimbic dopamine system (Kelley, Stinus, & Iversen, 1980; Stinus, Koob, Ling, Bloom, & Le Moal, 1980). Furthermore, the hyperactivity produced by systemic morphine injections in cats is blocked by injections of a narcotic antagonist into the ventral tegmental area (Van Dongen, Broekkamp, & Cools, 1979).

The data suggest that the apparent biphasic effect of opiates on locomotor activity is due to drug actions at two distinct neural systems. One system is responsible for the sedative action; opiate receptors in the periventricular

gray region probably initiate this effect and there is no conclusive evidence for a mediation by dopaminergic systems. Another system is responsible for the excitatory action; opiate receptors in the ventral tegmental area (or substantia nigra) probably initiate this effect and this action is dependent on an enhanced dopaminergic function of the mesolimbic dopamine system. The acute effect of moderate to high doses of an opiate may result in an action on both motor inhibitory and excitatory opiate-receptor fields, but the depressant effects of the drug treatment predominate. As tolerance develops to the sedative action of opiate treatment, the excitatory action, which does not show appreciable tolerance, emerges and becomes progressively more pronounced. This notion of multiple and antagonistic opiate actions had been suggested by Tatum, Seevers, and Collins (1929) but did not gain appreciable support until recent years when the necessary procedures became available to demonstrate the anatomical basis of this effect.

5.4.5 Stereotypy

While low doses of amphetamine and other drugs that potentiate dopaminergic function produce locomotor excitement, higher doses result in a series of compulsive, repetitious behaviors collectively termed stereotypy. Like amphetamine-induced locomotor activity, stereotypy appears to be mediated by a dopaminergic mechanism associated with the nigrostriatal or mesolimbic dopamine systems (for

reviews, see Cole, 1978; Creese & Iversen, 1975; Moore & Wuerthele, 1979; Randrup & Munkvad, 1970; Randrup et al., 1975).

It is not surprising that doses of morphine producing catatonia inhibit stimulant-induced stereotypy (Blumberg & Ikeda, 1978; Feigenbaum, Moon, & Klawans, 1978). Chronic injections of morphine alone produce stereotypy which emerges increasingly sooner after injections (Ayhan & Randrup, 1972; Charness, Amit, & Taylor, 1975). Small doses of morphine produce stereotypy and this behavior seems to be dependent on a dopaminergic mechanism in both the tolerant and nontolerant subjects (Ayhan & Randrup, 1972, 1973; Randrup et al., 1975). Charness et al., (1975) have suggested, however, that the reduction in stereotypy seen with neuroleptic treatment is the result of general sedation. In a comparison of the percentage of time stereotypic activity was present when the subjects were active, they found that haloperidol did not decrease the proportion of stereotypic activity. This sort of analysis illustrates the care which must be exercised when evaluating experiments involving drugs with sedative side-effects.

Apomorphine-induced stereotypy is enhanced by moderate doses of morphine (up to 30 mg/kg) while higher doses abolish this response (Feigenbaum et al., 1978; Vedernikov, 1970). Rats made physically dependent on morphine by pellet implantation show enhanced responsiveness to the

stereotypic action of apomorphine (Cox, Ary, & Lomax, 1976). This increased sensitivity to apomorphine is blocked by naloxone (Cox et al., 1976).

The facts that small doses of morphine induce stereotypy and that larger doses potentiate the stereotypic effect of dopamine agonists suggest that morphine may induce a functional increase in dopamine mediated behaviors involved in the induction or expression of stereotypy. Higher doses of morphine stimulate brain mechanisms related to catatonia and this depressant effect could result in an attenuation of stimulant-induced stereotypy. In this way the attenuation of stimulant-induced stereotypy could be a nonspecific response to morphine which does not reflect changes in dopamine mediated behavior, but, rather, it represents a masking of the direct action of morphine on this response. There is no evidence supporting the notion that morphine inhibits the neural mechanisms directly involved in stereotypy, while it is evident that morphine can both directly activate and potentiate these mechanisms.

Chronic treatment with drugs blocking dopamine receptors has been reported to enhance the locomotor and stereotypic responses to dopamine agonists (see Akiyama, Sato, & Otsuki, 1982; Christensen & Nielson, 1979; Muller & Seeman, 1978; Seeger, Thal, & Gardner, 1982; Thornburg & Moore, 1975). Supersensitivity to dopamine agonists has also been reported following chronic morphine treatment, and this effect has been interpreted as evidence supporting

a dopamine-receptor blockade hypothesis of opiate action (Carlson & Almasi, 1978; Puri & Lal, 1973; Tye, Horsman, Wright, & Pullar, 1979). Some researchers, however, have reported a failure to demonstrate enhanced responsiveness to dopamine agonists following chronic morphine treatment (Babbini, Gaiarda, & Bartoletti, 1978; Kuschinsky, 1975), so this effect is, at best, controversial. Furthermore, Carlson and Seeger (1982) have reported that chronic opiate treatment does not alter the dopamine receptor density in striatal or mesolimbic regions as does chronic treatment with neuroleptics (Akiyama et al., 1982; Goldstein, Lew, Asano, & Ueta, 1980; Seeger et al., 1982). This finding suggests that the neurochemical basis of enhanced responsiveness to apomorphine following chronic opiate or neuroleptic treatment is different.

A number of investigators have demonstrated that repeated administration of drugs enhancing dopamine function can produce reverse tolerance and supersensitivity to dopamine agonists (Bailey & Jackson, 1978; Conway & Uretsky, 1982; Kilbey & Ellinwood, 1977; Kilbey, Ellinwood, & Easler, 1979; Klawans & Margolin, 1975; Kuczenski & Leith, 1981; Segal & Mandell, 1974; Segal, Weinberger, Cahill, & McCunney, 1980). This has been termed "agonist-induced" supersensitivity as opposed to denervation supersensitivity which occurs after prolonged postsynaptic receptor blockade or after lesions of presynaptic dopaminergic elements.

It is not possible to differentiate the behavioral

or physiological mechanisms involved in these manifestations of supersensitivity; therefore, since both dopamine agonists and antagonists can induce an enhanced responsiveness to dopamine agonists, the behavioral data cannot be interpreted in terms of increased or decreased dopamine function through chronic morphine treatment. Furthermore, stimuli that have been associated with either morphine's or amphetamine's actions can elicit increases in general activity and stereotypy (Hayashi, Ohashi, & Tadokoro, 1980; Mucha, Volkovskis, & Kalant, 1981). It would appear feasible that similar conditioning factors may contribute to the enhanced responsiveness to dopamine agonists following chronic opiate treatment (see also Hinson & Poulos, 1981; Post, Lockfeld, Squillace, & Contel, 1981; Schiff, 1982).

5.4.6 Rotational Model of Dopamine Function

Normally, an animal exhibits no preference with respect to turning and circling behaviors.[11] Anden, Dahlstrom, Fuxe, and Larsson (1966) demonstrated that if a rat were lesioned unilaterally in the nigrostriatal pathway, it would display asymmetrical behavior (e.g., turning in circles) ipsilateral to the lesioned side; if the subject

---

[11] It has recently been shown that striatal asymmetries exist in untreated animals and that amphetamine injections enhance this asymmetry as indexed by rotational behavior (Glick & Cox, 1978; Jerussi & Glick, 1976; Glick, Weaver, & Meibach, 1981; but see Myslobodsky & Braun, 1980). This slight bias in the direction of turning, however, is usually unnoticeable in the untreated animal unless rotational behavior is measured over a long period of time.

were administered amphetamine, this effect was exaggerated. If a direct dopamine agonist (e.g., apomorphine) were administered to the lesioned animal, it would display contralateral asymmetry. Supersensitivity of the intact postsynaptic receptors was hypothesized to account for this differential effect. L-dopa, like apomorphine, produces contralateral turning.[12] Direct electrical stimulation of the fibers (Arbuthnott & Ungerstedt, 1975) or cell bodies (Roffman et al., 1978) of the nigrostriatal dopamine system also produces circling contralateral to the stimulation site, and this rotation is attenuated by drugs that block dopamine receptors (Roffman et al., 1978; Saranak & Goldfarb, 1981). The microinjection of neuroleptics into the terminal fields of this system (i.e., caudate nucleus) also results in circling, but in this case the direction is ipsilateral to the injection site (Costall, Naylor, & Olley, 1972; Ungerstedt, Butcher, Butcher, Anden, & Fuxe, 1969). Anden and Grabowska Anden (1978) have used KCL-induced, subcortical spreading depression to reversibly inactivate one side of the striatum. With this technique a dopamine-receptor antagonist produced contralateral asymmetry while agonists caused ipsilateral rotation. Iwamoto, Loh, and Way (1976) have also demonstrated contralateral rotation in lesioned animals treated with a dopamine-receptor blocking drug.

---

[12] Dopa decarboxylase is apparently present extraneuronally (Kaplan, Harman, & Creveling, 1979) and can convert L-dopa to dopamine outside the neurons.

This method, developed by Ungerstedt (e.g., 1971b) has been used to test the effects of a variety of agents on dopaminergic function in the nigrostriatal system. Agents that directly mimic dopamine produce contralateral asymmetry; agents that potentiate the activity or release of endogenous dopamine display exaggerated ipsilateral asymmetry; numerous agents that have no effect on dopamine neurons do not modify asymmetry (for reviews, see Glick, Jerussi, & Fleisher, 1976; Pycock, 1980; Ungerstedt, 1971b).

Iwamoto et al. (1976) have shown that rats with unilateral lesions in the substantia nigra display ipsilateral asymmetry in response to systemic morphine injections. This was interpreted as an indication that morphine potentiates dopamine function through mechanisms which are similar to those activated by amphetamine. Pert et al. (1979) reported that morphine injections initially failed to produce rotation in unilaterally lesioned animals, but that, with repeated injections, ipsilateral asymmetry emerged by the second day of testing. The morphine-induced circling was blocked by naloxone and by haloperidol (Pert et al., 1979) suggesting that rotation was a specific pharmacological action dependent upon a dopaminergic mechanism. Blundell, Crossman, and Slater (1976) also reported that systemic morphine did not produce rotation and, in fact, decreased circling induced by amphetamine. Blundell et al. (1976), however, failed to test their subjects during continued morphine injections.

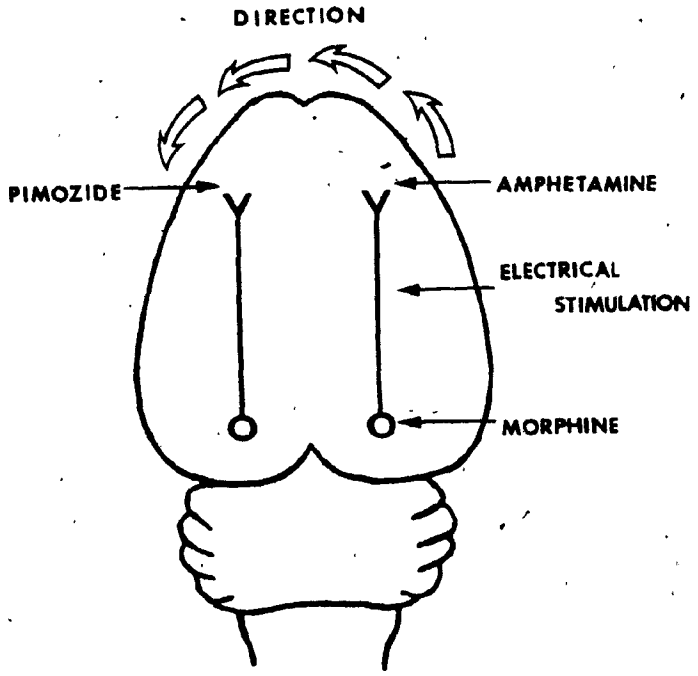

Fig. 5.2. The rotational model was developed to assess functional asymmetries in the activity of the nigrostriatal and/or mesolimbic pathways (Anden et al., 1966; Glick et al., 1976; Pycock, 1980; Ungerstedt, 1971b). Essentially the animal circles away from the most active side (i.e., contralateral rotation) of these dopaminergic systems. The asymmetrical activity can be produced by unilateral lesions or by direct activation caused by electrical or chemical stimulation. With microinjections directly into the brain, the direction of circling indicates whether the unilateral treatment enhances (contralateral rotation) or attenuates (ipsilateral rotation) dopaminergic function. In lesioned animals receiving systemic drug injections, the direction of rotation is reversed with enhanced activity indicated by ipsilateral rotation and attenuated activity indicated by contralateral rotation. It is interesting to note that animals trained to circle show increased dopamine release in the contralateral caudate nucleus (Yamamoto & Freed, 1982; Yamamoto, Lane, & Freed, 1982).

Unilateral microinjections of morphine directly into the substantia nigra produce contralateral rotation which is blocked by pretreatment with naloxone (Pert et al., 1979; see also Welzl, Flack, & Huston, 1982). Since the motor suppressive effects of morphine appear to be mediated by a substrate outside the nigrostriatal system (Pert, 1978; Pert et al., 1979; Wilcox & Levitt, 1978), the failure to find circling during initial injections of systemic morphine (i.e., Blundell et al., 1976; Pert et al., 1979) is probably due to the activation of extrastriatal neurons. This is further supported by the observation that microinjections into the caudate nucleus do not produce a depression in activity (Broekkamp, 1976; Pert, 1978; Pert et al., 1979). Systemic morphine injections would appear to simultaneously activate neurons responsible for both the motor suppressive and rotation-effects. As tolerance develops to the depressive effect of morphine on locomotor activity (Babbini & Davis, 1972; Vasko & Domino, 1978), rotation caused by stimulation of dopaminergic neurons in the substantia nigra is unmasked.

Although the effect of morphine on rotation may sometimes appear to be biphasic, the observed behavior seems to be produced by the simultaneous activation of two distinct systems. The acute effect of morphine on striatal neurons leads to a functional increase in dopaminergic activity which may be masked by concurrent stimulation of inhibitory processes outside this system. Chronic injections produce

tolerance to the catatonic effect of morphine revealing ipsilateral circling in unilaterally lesioned animals. In fact, repeated injections of morphine may lead to enhanced responding in this rotational model of dopamine function (Pert et al., 1979). This effect parallels the continued enhancement of dopamine turnover reported by Clouet and Ratner (1970).

5.5 Conclusions

The data concerning the effects of morphine on parameters of dopaminergic activity cannot be construed as demonstrating an increased or decreased dopaminergic function. Manipulations which lead to enhanced or diminished dopaminergic function produce identical changes in dopamine cell firing, synthesis, and metabolism. Studies employing morphine-induced behaviors are also impossible to interpret in terms of pharmacological specificity since profound changes in behavior occur in subjects with altered dopaminergic function not augmented by morphine. In this way, the behavioral "end-points" are confounded by a direct action of dopaminergic manipulations on the responses measured. Only in cases where the individual actions of morphine and dopaminergic manipulations are opposite may the data be inferred to support a specific pharmacological action. In these studies dopaminergic agonists have been shown to enhance the behavioral effects of morphine. Reports of increased morphine-like effects from inhibition of dopaminer-

gic function are confounded by the possibility of summation resulting from the activation of separate systems regulating arousal level.

The neuroanatomy and neurochemistry of the mesolimbic and nigrostriatal pathways are well known and manipulations of these systems produce predictable changes in behavioral "end-points" (Bunney & Aghajanian, 1978; Friedhoff, 1975; Moore & Bloom, 1978; Moore & Kelly, 1978). This offers a unique opportunity to study the effects of morphine on dopamine-mediated behaviors and to draw tentative conclusions regarding the role of dopamine in morphine-induced effects.

Low doses of morphine increase locomotor activity, produce stereotypy, and cause rotation which are indicative of enhanced dopaminergic function in the mesolimbic and nigrostriatal systems. Higher doses of morphine attenuate a variety of behaviors, and this action appears to be related to a catatonia-like mechanism. The extant data suggest that catatonia only superficially resembles catalepsy induced by the blockade of dopamine receptors. The behavioral-depressant effect of morphine seems to be dependent on extrastriatal, non-dopaminergic mechanisms. Furthermore, as tolerance develops to the catatonic properties of morphine, behaviors indicative of enhanced dopaminergic functioning emerge. It appears that the excitatory effects are initially masked by catatonia-producing doses of morphine; therefore, the acute and chronic effects of both low and high doses of morphine may lead to enhanced dopaminergic function.

CHAPTER 6

INVOLVEMENT OF DOPAMINE IN OPIATE REWARD[1]

6.1 Introduction

Several lines of evidence have implicated central catecholamines in the mediation of reward (Fibiger, 1978; Fibiger & Phillips, 1979; Wise, 1978a, 1982). In particular, a dopaminergic substrate appears to be involved in food (Ungerstedt, 1971c; Wise, Spindler, deWit, & Gerber, 1978; Wise, Spindler, & Legault, 1978; Zis & Fibiger, 1975) and water (Gerber, Sing, & Wise, 1981; Ungerstedt, 1971d) reward as well as reward from electrical brain stimulation (Fouriezos, Hansson, & Wise, 1978; Fouriezos & Wise, 1976; Franklin, 1978; Franklin & McCoy, 1979; Mora, Sanguinetti, Rolls, & Shaw, 1975; Phillips & Fibiger, 1978). The rewarding properties of psychomotor stimulants (e.g., amphetamine, cocaine) are also dependent on a dopaminergic mechanism as demonstrated by the changes in stimulant self-administration following dopamine-receptor blockade (deWit, & Wise, 1977; Yokel & Wise, 1975, 1976) or by lesions of dopaminergic terminal fields in the nucleus accumbens (Lyness, Friedle, & Moore, 1979; Roberts,

---

[1]Portions of this chapter have appeared in M. A. Bozarth and R. A. Wise, Heroin reward is dependent on a dopaminergic substrate. Life Sciences, 1981, 29, 1881-1886.

Corcoran, & Fibiger, 1977; Roberts, Koob, Klonoff, & Fibiger, 1980). The unique role of dopaminergic mechanisms in these different sources of reward suggests that a common reward system may mediate these effects, although different sources of reward might activate different elements in this reward system. Because many investigators have interpreted neuropharmacological and behavioral data as indicating that morphine blocks dopamine function (e.g., Eidelberg & Erspamer, 1975; Koffer, Berney, & Hornykiewicz, 1978; Lal, 1975; Puri, Reddy, & Lal, 1973), it is of special interest to examine the involvement of dopamine in the mediation of opiate reward. It would be surprising if opiate reward were produced by impaired dopaminergic neurotransmission since these other rewards seem to enhance brain dopamine function.

Previous reports have shown that opiate self administration is attenuated by neuroleptics that block dopamine receptors (Ettenberg, Koob, Pettit, & Bloom, 1981; Glick & Cox, 1975; Hanson & Cimini-Venema, 1972; Pozuelo & Kerr, 1972), but the pattern of responding seen after neuroleptic treatment makes the data difficult to interpret. Unlike the case of intravenous self-administration of amphetamine (Yokel & Wise, 1975, 1976), neuroleptic challenge of opiate self-administration does not usually produce the compensatory increase in drug intake that is associated with competitive antagonists.[2] Since an apparent side-effect

[2]In intravenous amphetamine self-administration, treatment with a neuroleptic produces a dose-dependent

of either neuroleptic treatment (Costall & Naylor, 1973) or opiate injections (Domino, Vasko, & Wilson, 1976) is sedation, a simple decrease or cessation in responding does not necessarily indicate an attenuation of reward.

6.2 Opiate-Induced Conditioned Reinforcement

To evaluate the possibility that neuroleptics decrease opiate self-administration by a sedative action produced by these two drugs, Smith and Davis (1973) have tested the effect of a neuroleptic on morphine reward using a conditioning paradigm. The animals received a series of intravenous morphine injections that were associated with a buzzer. They were later tested in a lever-pressing procedure for the self-administration of drug vehicle (i.e., isotonic saline) which was accompanied by the presentation of the buzzer. Animals that were not pretreated with a narcotic antagonist showed sustained self-administration of saline while animals who underwent conditioning with prior injections of naloxone showed little responding. The effect of neuroleptic treatment during the conditioning trials has also been tested and was shown to have no effect on subsequent responding for saline in the presence of the buzzer stimulus. From this Smith and Davis (1973) concluded that

---

increase in amphetamine intake (Yokel & Wise, 1975, 1976). This relationship would be expected if amphetamine reward were dependent on an action at dopamine receptors, since competitive antagonists can be displaced by increasing the concentration of agonist available at the receptor site (see Fingl & Woodbury, 1975; Tallardia, Cowan, & Adler, 1979; Tallardia & Jacob, 1979; Waud, 1975).

neuroleptic treatment fails to block the rewarding properties of morphine injections and low doses of a neuroleptic may even enhance the rewarding effects of morphine. Thus, the observation that neuroleptic treatment attenuates intravenous opiate self-administration was asserted to be the result of a nonspecific sedative action of these drugs.

This conditioned reinforcement paradigm (Davis & Smith, 1976; Smith and Davis, 1973, 1975) provides a novel and important approach to assessing the rewarding properties of drugs. It would appear that this method allows the determination of the impact of various manipulations on drug reward in a paradigm that is insensitive to the motoric side-effects produced by some drug treatments. There are, however, two apparent problems with this technique. First, there have been no published reports of other laboratories successfully using this technique. In fact, there has been difficulty in replicating the initial demonstration of conditioned reinforcement (H. deWit, personal communication). This may be due to the fact that Smith and Davis (1973) use an extremely rapid infusion procedure and other investigators have been reluctant to use this method. Second, Smith and Davis (1973) failed to control for nonspecific increases in general activity. This potential confound is particularly problematic since they use a relatively small test chamber and a very large lever.[3] This combination may be necessary

[3]Smith and Davis' test chamber has a 25 cm diameter (e.g., Davis & Smith, 1976; Smith & Davis, 1973). The only

to assure adequate lever contacts during the tests for conditioned reinforcement since they use subjects that have not been lever trained. Without an appropriate control procedure, the effect of nonspecific motor activity on the measure of conditioned reinforcement cannot be evaluated; the animals could be responding to the presentation of the buzzer stimulus, following an accidental lever contact, and reacting to some aversive consequence associated with the rapid infusion regimen used for conditioning. This possibility, as well as the failure of other laboratories to replicate the basic conditioning effect, needs to be investigated before this work can be meaningfully interpreted.

6.2.1 Method

Subjects: Under sodium pentobarbital anesthesia (60 mg/kg, i.p.), male, Long-Evans rats (weighing 350 to 450 g) were catheterized with Silastic medical grade tubing (Dow Corning). The catheters (1.19 mm O.D.) were placed in the right external jugular and extended approximately 3 cm into the vein. A single injection of penicillin G (30,000 units, i.m.) was given prophylactically following surgery. Heparin (circa 0.5 U.S.P. units) dissolved in isotonic saline was used to flush the catheters periodi-

---

indication of the lever size used in these experiments is illustrated in Smith and Davis (1975). It would appear that an adult rat would have a great deal of difficulty moving in this test chamber without activating the lever. Thus any appreciable change in motor activity would result in a greatly inflated lever-press score.

cally and thus confirm their patency. The animals were individually housed in a 12 hour light-dark cycle of illumination with food and water available ad libitum in the home cages. All behavioral testing occurred during the light cycle of illumination.

Apparatus: The rats were tested in a 26 x 26 x 28 cm test chamber with a small exhaust fan providing ventilation and masking of peripheral noise. On one end of the test chamber, two 4.5 x 5 cm levers were located 10 cm above the floor. The catheter of each subject was attached to a fluid line allowing intravenous infusions without disturbing the animal. Solid-state programming equipment controlled a syringe pump which was used to deliver the infusions.

Procedure: After at least one week to recover from the surgical procedure, subjects were placed in the test chamber and intravenously infused with 100 µg/kg of heroin hydrochloride every 15 minutes for three hours. Each infusion was delivered over 28 seconds in a volume of 0.25 ml and accompanied by the presentation of a cue light above one of the two levers. These conditioning trials lasted for five consecutive days resulting in a total of 60 stimulus-infusion pairings. Three hours before each conditioning trial, each animal was injected with either pimozide (0.5 mg/kg, i.p.; n=9) or drug vehicle (tartaric acid; 1 ml/kg, i.p.; n=10). The subjects remained in their home cages for two days following the last conditioning

trial without further treatment.

Pimozide was used because it is a selective neuroleptic blocking primarily dopamine receptors with little effect on other catecholaminergic systems (Janssen, Niemegeers, Schellekens, Dresse, Lenaerts, Pinchard, Schaper, Van Nueten, & Verbruggen, 1968). Furthermore, pimozide has been shown to attenuate the impact of a variety of rewards at doses that do not cause appreciable motor impairment (Fouriezos et al., 1978; Fouriezos & Wise, 1976; Wise et al., 1978ab; Yokel & Wise, 1976).

Testing for conditioned reinforcement consisted of allowing the subjects to self-administer isotonic saline by pressing one of two levers. The second lever was inactive and served as a control for nonspecific lever-presses (see Section 3.2.1). Each press on the active lever resulted in a 0.25 ml infusion of saline with the concurrent presentation of the cue light for 28 seconds. Presses on the other lever had no scheduled consequence. Testing lasted for three hours per day and was continued for three days.

6.2.2 Results

The number of presses on the active lever during each of the three sessions of testing was averaged for each group (see Figure 6.1). The mean number of presses on the inactive lever was also computed for each group. The group pretreated with tartaric acid during their conditioning

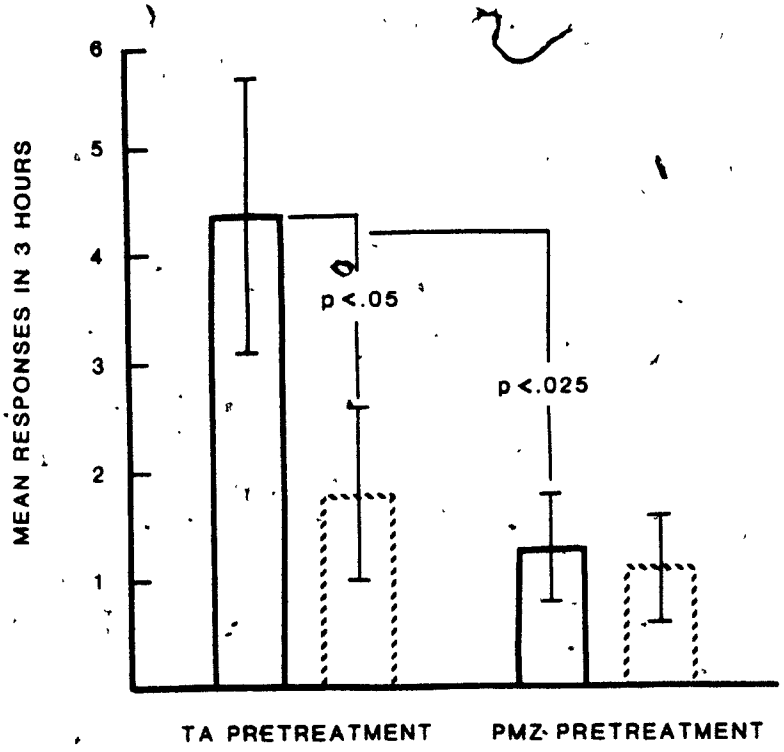

Fig. 6.1. The mean (±SEM) number of responses made during the three hours of testing. Animals pretreated with tartaric acid (TA pretreatment) during their conditioning trials made significantly more lever presses than did subjects pretreated with pimozide (PMZ pretreatment) during their conditioning trials {$t(17)=2.36$, $p<.025$}. The tartaric acid pretreated animals also pressed the lever associated with the cue light (solid bar) reliably more than the inactive lever (striped bar) {$t(8)=2.25$, $p<.05$}. Note: Since these differences were predicted a priori, one-tailed probability levels were used with no adjustment for an increased probability of obtaining a type I error with repeated tests (see Lindman, 1974).

trials pressed the lever associated with the cue light significantly more often than the inactive lever; the group pretreated with pimozide pressed each lever with equal frequency and their lever pressing on the lever associated

with the cue light was significantly lower than that of the tartaric acid pretreated group.

6.2.3 Discussion

The development of conditioned reinforcement produced by the association of a cue light with heroin infusions was blocked by pimozide pretreatment. Since the animals are drug free during the tests for conditioned reinforcement, neither the sedative effects of pimozide nor heroin can account for these data. The fact that the vehicle-pretreated group pressed the active lever significantly more than the inactive lever suggests that this measure of conditioned reinforcement was not the result of accidental lever contacts. Whether using the rapid infusion technique of Smith and Davis (1973) would have resulted in appreciable contacts with the inactive lever remains to be established.

Smith and Davis (1973) report that their animals make around 100 lever presses in a 6 hour session, but this is only twice the rate of the lever pressing seen prior to conditioning. In the present study, only about 4½ responses were made in three hours of testing, but the vehicle pretreated animals pressed about three times as much as those pretreated with pimozide (see Figure 6.1). The strength of the conditioned response in the present study was very weak; the amount of variance accounted for by the treatment, as measured by η^2, was 24.85% (Linton & Gallo, 1975). Smith

and Davis (1973) do not report any strength of association measure for their data.

6.3 Conditioned Place Preference

Another approach to assessing the effect of neuroleptic treatment on opiate reward is to use the conditioned place preference paradigm. As discussed in Chapter 4, rats that had experienced rewarding drug effects associated with a specific environment will return, when given the opportunity, to the place where they experienced these effects (Rossi & Reid, 1976; Schwartz & Marchok, 1974; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980a; Sherman, Roberts, Roskam, & Holman, 1980b; Stapleton, Lind, Merriman, Bozarth, & Reid, 1979). Since the animals are tested in the drug-free state, this technique, like conditioned reinforcement, is not subject to the limitations inherent in paradigms sensitive to the response depressant effects of drugs.

6.3.1 Method

The apparatus for measuring place preference consisted of a shuttle box (25 x 36 x 35 cm) with a plywood floor on one side and a plywood floor covered with wire mesh on the other. The amount of time spent on each side of the box and the number of crosses were automatically recorded. Rats were allowed access to the entire shuttle box for 15 minutes on five consecutive days. After these preconditioning trials, they received four daily injections

of drug while being forced to remain on the plywood side for 30 minutes. Following the four days of conditioning trials, the rats were injected with vehicle and tested again for their place preference during a 15 minute trial. Increases in the time spent on the side of putative conditioning were interpreted as an indication of drug-induced reward.

Fifty-five male, Long-Evans rats (weighing 350-450 g) were divided into 5 groups and received different pretreatments during their conditioning trials. Three groups of rats received heroin (0.5 mg/kg, s.c.) preceded by either saline (1 ml/kg, i.p.), naloxone (3 mg/kg, i.p.), or pimozide (0.5 mg/kg, i.p.). The remaining two groups of rats were injected with either naloxone (3 mg/kg, i.p.) or pimozide (0.5 mg/kg, i.p.) followed by saline (1 mg/kg, i.p.). All drugs were injected immediately before the conditioning trials except pimozide which was injected four hours prior to conditioning.

6.3.2 Results

The rats showed a marked preference for the wire mesh side of the box during their preconditioning trials. The 99% confidence interval for the last preconditioning trial is depicted in Figure 6.2. Heroin produced a shift in place preference similar to that found in Chapter 4 and that previously reported for morphine (Rossi & Reid, 1976; Schwartz, & Marchok, 1974; Sherman et al., 1980a),

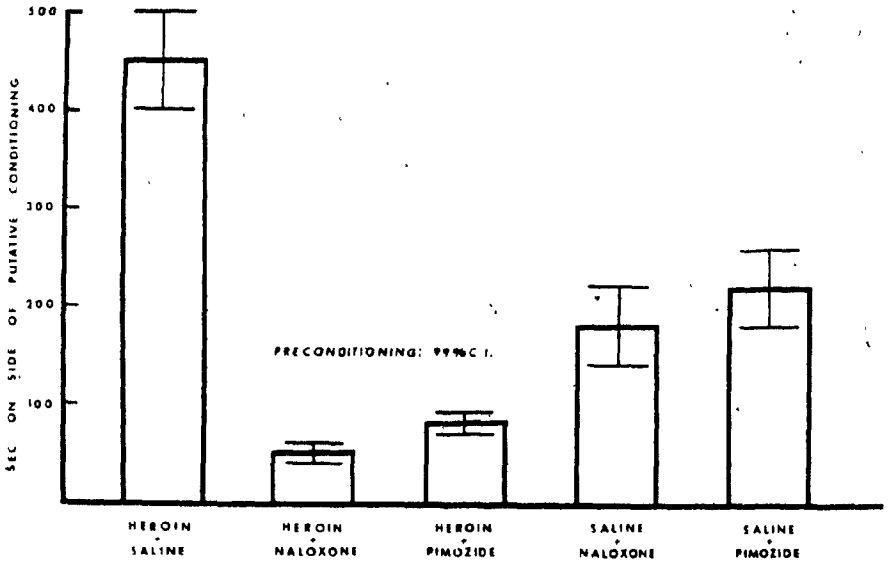

Fig. 6.2. The mean (±SEM) time spent on the side of putative conditioning for each group (n=11/group). An analysis of variance (ANOVA) showed no significant differences (Winer, 1971) among the groups during preconditioning {$F(4,50)=1.43$, $p>.20$}; the 99% confidence interval (99% C.I.) of these scores is shown on the figure. After conditioning, an ANOVA revealed a significant treatment effect {$F(4,50)=19.73$, $p<.001$}. A Newman-Keuls' test (Winer, 1971) demonstrated significant differences between heroin plus saline and all other treatments ($p<.01$); also, both heroin plus naloxone and heroin plus pimozide decreased the amount of time spent on the side of putative conditioning when compared with saline plus naloxone and saline plus pimozide, respectively ($p's<.05$).

d-ala^2-methionine enkephalin (Stapleton et al., 1979), or amphetamine (Sherman et al., 1980b). Pretreatment with either naloxone or pimozide resulted in a decrease in the amount of time spent on the conditioning side for rats also receiving heroin injections. Neither naloxone nor pimozide produced a reliable shift in place preference in rats not

injected with heroin.

A comparison of the effects of these various treatments on locomotor activity is shown in Table 6.1. Although all shuttle scores were increased on the test trial, the only significant increase was seen in the saline plus pimozide group. The fact that an increase in shuttle activity was not seen in the heroin plus saline group suggests that the place preference demonstrated by this group was not the result of nonspecific increases in activity or exploration. This observation is important since the heroin plus saline group only spent about half of the total test-time on the

TABLE 6.1

CHANGES IN LOCOMOTOR ACTIVITY

Condition	Mean Difference Score
Heroin plus saline	↑1.9±1.5
Heroin plus naloxone	↑0.9±1.5
Heroin plus pimozide	↑0.8±1.3
Saline plus naloxone	↑1.1±2.6
Saline plus pimozide	↑8.5±1.8

Note: Mean difference (±SEM) in activity (number of crossings) between the last preconditioning trial and the post-conditioning trial. An ANOVA revealed no significant differences in the locomotor scores between groups on the last preconditioning day {$F(4,50)=1.04$, $p>.20$}. Following the conditioning trials, there were no reliable changes in locomotor activity (p's$>.20$ to $.50$) except for the group given pimozide plus saline during the conditioning trials {$t(10)=4.72$, $p<.002$}.

conditioning side, and increases in locomotor activity could potentially inflate the measure of place preference by simply distributing the time spent on each side more equally.[4]

6.3.3 Discussion

These results are concordant with those of the first experiment and with other studies showing an attenuation of opiate reward following neuroleptic treatment (e.g., Hanson & Cimini-Venema, 1972; Pozuelo & Kerr, 1972). Since place preference is tested in drug-free animals, it is obvious that pimozide's blockade of heroin reward cannot be attributed to a general sedative effect. Unlike the first experiment, however, the effect demonstrated in this study was relatively strong; the amount of variance accounted for by treatment, as indexed by ω^2, was 61.2% (cf. Table 4.1).

The fact that the same dose of an opiate can produce both reward and aversion is well established (Sherman et al., 1980a; White, Sklar, & Amit, 1977), and it has been suggested that opiates produce mixed affective consequences (Gorman, De Obaldia, Scott, & Reid, 1978). The apparent aversion to the side of putative conditioning seen in rats treated with heroin plus naloxone or heroin plus pimozide may point to a

---

[4]This hypothesis has been directly tested (M. Bozarth, unpublished observations). Rats injected with amphetamine (1 or 3 mg/kg, i.p.) show marked increases in locomotor activity as measured by the number of crossings in 15 minutes. This increased locomotor activity did not produce a shift in the amount of time the animals spent on their nonpreferred side of the test chamber even though activity was increased dramatically.

separation of the rewarding and aversive properties of opiates. This learned aversion to stimuli associated with heroin plus naloxone or heroin plus pimozide, however, needs to be confirmed using other techniques, such as the conditioned taste aversion paradigm, before any firm conclusions can be reached regarding this effect.

6.4 General Discussion

Since neuroleptic treatment produces a simple response attenuation in intravenous opiate self administration,[5] this technique cannot be used to determine the effect of dopamine-receptor blockade on opiate reward. In fact, the decrease in intravenous self-administration is dose dependent, so it might be concluded that this effect is the result of a general sedative effect of neuroleptic treatment. Therefore, the effect of neuroleptic

[5]The assertion that neuroleptics cause a simple decrease in intravenous opiate self-administration is not true. In fact, low doses of a neuroleptic have been shown to increase opiate self-administration (Hanson & Cimini Venema, 1972; Smith & Davis, 1973; M. Bozarth, unpublished observations). This effect has not been well documented and the only full report of increased responding during neuroleptic treatment attributes this effect to an enhancement of opiate reward (i.e., Smith & Davis, 1973). The series of studies reported by Yokel and Wise (1975, 1976) emphasized the importance of accelerated drug intake in eliminating the possibility that general sedation is responsible for altered intravenous self-administration. It should be noted, however, that a simple cessation in responding for intravenous drug does not prove that the effect is due to motor sedation. The observation that low doses of a neuroleptic increase intravenous opiate self administration could be interpreted as evidence against a motor sedation hypothesis, and thus supporting the notion that neuroleptics attenuate opiate reward.

treatment on opiate reward needs to be assessed with animals tested in the drug-free condition. Two such procedures have been used: (1) Smith and Davis-type conditioned reinforcement, and (2) conditioned place preference. In the present study, both indices of opiate reward were blocked by pretreatment with pimozide. The strength of the conditioning seen in subjects not pretreated with pimozide, however, was relatively weak in the Smith and Davis-type measure (cf. strength of association measures were 24.8% and 61.2% for Smith and Davis-type conditioned reinforcement and conditioned place preference, respectively). The reason for the discrepant findings of the present study with Smith and Davis (1973) is unclear, but it is apparent that additional testing and replication by other laboratories are required before the results of either study can be meaningfully interpreted.

Schwartz and Marchok (1974) have reported that the place preference produced by morphine was blocked by neuroleptic treatment, but their paper has drawn little attention. The present study confirmed this assertion by showing that the heroin-induced conditioned place preference is blocked by pimozide pretreatment and this effect cannot be attributed to an aversive side-effect of pimozide treatment.[6] Phillips, Spyraki, and Fibiger (1982) have

[6]Stewart and Grupp (1981) have shown a place aversion can also develop with some drug treatments. If pimozide treatment alone were to decrease the amount of time spent on the side of conditioning, then pimozide's attenua-

also replicated this finding making the results of these three laboratories in agreement.

The demonstration that opiate reward appears to be dependent on a dopaminergic mechanism suggests that it may share a common neural substrate with other sources of reward. This proposition is strengthened by the demonstration in Chapter 3 that rats will learn a lever-pressing response to inject morphine directly into the ventral tegmental area. Independent corroboration of the rewarding properties of morphine injected into this region of dopamine-containing cells has been obtained in studies showing a conditioned place preference from these injections (see Chapter 4; Phillips & LePiane, 1980).

---

tion of heroin-induced conditioned place preference might be due to an additive effect and not the result of a direct action on heroin reward.

CHAPTER 7

DISSOCIATION OF THE REWARDING AND PHYSICAL DEPENDENCE-PRODUCING PROPERTIES OF OPIATES[1]

7.1 Introduction

According to operant psychologists, there are three cardinal principles governing behavior: (1) punishment, where an aversive event decreases the frequency of the behavior it follows, (2) negative reinforcement, where the frequency of a response is increased by the avoidance or termination of an aversive event, and (3) positive reinforcement, where the frequency of a response is increased by the presentation of some rewarding (i.e., usually emotionally positive) event (see Bindra, 1976; Skinner, 1953; Young, 1959). Punishment decreases the occurrence of behaviors it follows while both negative and positive reinforcement increase the occurrence of behaviors they affect. Theories concerning the nature of drug addiction generally involve either negative or positive reinforcement mechanisms although there is no a priori reason to suspect that both

---

[1]Portions of this chapter were presented to the 44th Annual Meeting of the Committee on Problems of Drug Dependence, Toronto, June, 1982, and will appear in M. A. Bozarth and R. A. Wise, Dissociation of the rewarding and physical dependence-producing properties of morphine. In L. S. Harris (Ed.), Problems of Drug Dependence, 1982. Washington, D.C.: NIDA Research Monograph Series, 1983 (in press).

could not be concurrently operating.

Addiction to opiates is usually characterized by the repetitive intake of large quantities of drug and the development of marked physical dependence. Upon discontinuation of opiate intake (or the injection of a narcotic antagonist), withdrawal symptoms appear which include anorexia, restlessness, irritability, tremor, and diarrhea accompanied by subjective experiences of weakness and depression (Jaffe, 1975; Martin, 1966, 1977; Martin & Jasinski, 1977). Although recent definitions of drug addiction emphasize the behavioral aspects of compulsive drug intake (Eddy, 1973; Jaffe, 1975), several investigators have postulated that the development of physical dependence is essential for establishing compulsive drug use (Dole, 1980; Lindesmith, 1938, 1970; Nichols, 1965; Seevers, 1967; Spragg, 1940; Wikler & Pescor, 1967). Specifically, the withdrawal discomfort produced by the discontinuance of opiate intake in physically dependent persons has been postualted to provide the motivation to ingest opiates to relieve the distress produced by abstinence. This type of negative reinforcement model of addiction seems to fit with many of the patterns of opiate intake, but it fails to account for the initial acquisition of drug-taking habits. Nonetheless, the notion that opiate addicts self-administer drugs to alleviate withdrawal distress remains a very popular view.

The self-administration of drugs by laboratory animals has suggested that many of the substances abused by

humans can serve as positive reinforcers (Deneau, Yanagita, & Seevers, 1969; Griffiths & Balster, 1979; Pickens, Meisch, & Thompson, 1978; Seiden & Dykstra, 1977; Spealman & Goldberg, 1978; Woods & Schuster, 1968, 1971). Although some early studies of animal self-administration used subjects that were made physically dependent on an opiate before testing for self-administration (e.g., Thompson & Schuster, 1964; Weeks, 1962), later studies have shown that naive subjects will learn and sustain self-administration of opiates at dose levels that do not produce obvious signs of physical dependence (Deneau et al., 1969; Woods & Schuster, 1971). On the other hand, it has been shown that physically dependent animals will "work" to avoid injections of narcotic antagonists that would precipitate withdrawal (Downs & Woods, 1973; Goldberg, Hoffmeister, & Schlichting, 1972; Goldberg, Hoffmeister, Schlichting, & Wuttke, 1971). Furthermore, Young, Swain, and Woods (1981) have shown that there is a high positive correlation between the ability of an opiate to suppress withdrawal signs and its pharmacological potency in maintaining intravenous self-administration. Thus it seems that negative reinforcement (i.e., the avoidance of withdrawal distress) is also capable of maintaining behavior. This has led to controversy regarding the relative importance of positive and negative reinforcement mechanisms in maintaining opiate intake in humans. Even studies reporting no clear signs of physical dependence in animals self-administering opiate cannot eliminate the possibility that

the animals are experiencing some degree of withdrawal discomfort during periods of abstinence.

One approach to studying the relative contribution of the positive and negative reinforcing properties of opiates to the maintenance of drug intake is to identify the underlying neural substrate of each and differentially manipulate them to assess their impact on behavior. Usually, however, it is not possible to produce physical dependence without the concomitant positive reinforcing properties of opiate administration nor is it possible to test for positive reinforcement without the possibility that some degree of physical dependence has developed as a consequence of drug intake. This chapter reports the results of two lines of investigation designed to delineate the relationship between opiate reward and the development of physical dependence. The first experiment was designed to determine if the opiate receptor field responsible for positive reinforcement is also involved in the production of physical dependence on morphine, and a second experiment was designed to assess the rewarding properties of a single injection of heroin. These studies should help to clarify the importance of physical dependence in drug taking behavior by examining the degree to which positive reinforcement and physical dependence mechanisms can be dissociated.

7.2 Physical Dependence from Central Morphine Injections

In early studies of the brain site responsible for

opiate physical dependence, rats were made physically dependent by systemic injections of morphine and withdrawal was precipitated by central narcotic antagonist administration. In an experiment by Wei, Loh, and Way (1973), rats were made dependent by morphine pellet implantations and crystalline naloxone was applied to various brain sites to assess their contributions to opiate physical dependence. Sites in the thalamic and periventricular gray regions were shown to be involved in physical dependence while the application of naloxone to the caudate or other forebrain sites seldom resulted in precipitated abstinence. Aware of the problem of extensive drug spread with lipophilic substances like naloxone (see Section 2.1.2), Laschka, Teschemacher, Mehraein, and Herz (1976b) used intraventricular injections of a narcotic antagonist following the insertion of "plugs" into various parts of the ventricular system. This procedure restricted the entry of the narcotic antagonist to certain parts of the cerebral ventricular system. These studies identified the caudal ventricular system (i.e., fourth ventricle) as being proximal to the opiate receptor field responsible for physical dependence. The failure to find precipitated withdrawal from narcotic antagonist injections restricted to the rostral ventricular system would appear to be in conflict with Wei et al.'s (1973) report of withdrawal from naloxone application in thalamic sites. This may be due to either (i) the spread of naloxone to more distal sites in the periventricular gray region in Wei et al.'s (1973)

study, (ii) the failure of ventricular naloxone infusions to produce effective antagonist concentrations in thalamic sites in Laschka et al.'s (1976b) study, or (iii) the use of narcotic antagonists of the mixed agonist-antagonist type[2] by Laschka et al. (1976b). Whatever the case, both lines of investigation eliminate a forebrain site of action for opiate-induced physical dependence and suggest the importance of caudal midbrain and periventricular opiate receptor fields.

A more direct assessment of what brain sites are responsible for the development of physical dependence on opiates involves the direct intracranial application of morphine into various brain regions. Wei (1981) and Wei and Loh (1976) have reported that rats chronically administered morphine into the periaqueductal gray-fourth ventricle region,

---

[2]Herz's laboratory group (e.g., Albus, Schott, & Herz, 1970; Blasig, Hollt, Herz, & Paschelke, 1976; Herz, Teschemacher, Albus, & Zielglansberger, 1972; Laschka, Herz, & Blasig, 1976a; Laschka et al., 1976b) has used several narcotic antagonists in their studies of various opiate effects (e.g., precipitated withdrawal) including levallorphan (l-3-hydroxy-N-allylmorphinan) and dially-normorphinium bromide which are structural analogs of nalorphine (N-allylnormorphine). Like their parent compound, however, they have mixed agonist-antagonist properties (Houde, 1979; Martin, 1967), and several compounds in this drug class have been reported to produce moderate levels of physical dependence (e.g., Fennessy & Laska, 1979, Koga & Inukai, 1981a; Laska & Fennessy, 1978; Martin, Fraser, Gorodetzky, & Rosenberg, 1965; Martin & Gorodetzky, 1965; McCarthy, Metcalf, & Howe, 1982). Generally, nalorphine precipitates withdrawal in opiate dependent subjects (Wikler, Fraser, & Isbell, 1953), but the possibility remains that the discrepancy between the data of Wei et al., 1973, and Herz's group is due to their use of markedly different compounds (see Jacob, Michaud, & Tremblay, 1979; Jasinski, 1979; Martin, 1967, 1979). Nonetheless, it is much more likely that even crystalline naloxone is diffusing to a distal site of action.

show pronounced withdrawal signs when challenged with systemic naloxone injections. In these studies, morphine had been infused chronically using an osmotic minipump (Alzet Corp.) and various withdrawal signs were observed following a challenge dose of naloxone. Although a variety of withdrawal signs are demonstrable after central morphine injections, the most clear appear to have been escape attempts from a cylindrical enclosure, teeth chattering, and wet-dog shakes.

The first experiment in this chapter involved the procedure described by Wei (1981) to study the ability of morphine injected directly into the ventral tegmental area to produce physical dependence in rats. This brain region has previously been shown to contain the opiate receptor population that is critical for the rewarding properties of morphine (Bozarth & Wise, 1980b, 1981a, 1982; Britt & Wise, 1981a Phillips & LePiane, 1980; see also Chapters 3 and 4). The effects of chronic morphine injections into other brain regions were also assessed to determine the loci of the opiate receptor fields mediating the development of physical dependence.

7.2.1 Method

Subjects: Male, Long-Evans rats (weighing 300 to 375 g) were stereotaxically implanted with 21 gauge cannulae aimed unilaterally at the various brain regions listed in Table 7.1. While the rats were anesthetized with sodium

pentobarbital (60 mg/kg, i.p.), cannulae that had been filled with morphine were connected to osmotic minipumps (model 2001) and the cannula tips were lowered to the brain region under study. The minipumps delivered 1.0 µl/hr of 0.5 µg/µl morphine sulfate[3] dissolved in Ringer's solution. Some of the cannulae were angled at 20 to 30 degrees[4] from the midline to avoid penetration of the periventricular gray region. The minipumps were implanted subcutaneously between the scapulae of the animals, and polyethylene tubing (PE 60) was used to connect the minipumps to the implanted cannulae (see Wei, 1981, or Yates, 1977, for details). Penicillin G (30,000 units, i.m.) was administered prophylactically during the surgery. After recovery from the anesthetic, animals were housed in a 12 hr light-12 hr dark cycle of illumination. All behavioral testing occurred during the light phase of this cycle. Food and water were available in the home cages ad libitum.

Because of a latency of about four hours in reaching their nominal flow rate of 1 µl/hr (Yates, 1979, 1981), the

---

[3]This dose is the equivalent of 1.5 nmole of morphine per µl of solution. The infusion parameters used in the present study were based on Wei's (1981) study which showed that maximum physical dependence was demonstrable after approximately three days of continuous morphine infusions and a challenge dose of 5 mg/kg of naloxone. The dose of morphine selected for the chronic infusions was shown to produce a high level of physical dependence.

[4]A Stoelting stereotaxic apparatus (Stoelting Co., Chicago) was used to perform the surgeries since angled placements can easily be done without adjusting the stereotaxic coordinates.

TABLE 7.1

STEREOTAXIC COORDINATES USED FOR MORPHINE INFUSIONS
(Adapted from Pellegrino, Pellegrino,
& Cushman, 1979)

Placement	anterior-posterior[a]	lateral[b]	ventral[c]	subjects[d]
FCX	+4.4	±2.5	2.0	14
NAS	+3.5	±1.6	6.2	15
CPU	+2.0	±3.0	6.0	12
AMYG	+0.4	±4.8	7.8	16
LHA	-0.6	±1.5	9.2	10
THAL	-1.0	±2.0	6.0	17
D3V	-3.8	±0.0	4.9	13
VTA	-3.8	±0.6	8.2	12
VTA-S	-3.8	±0.6	8.2	14
VTA-30°	-3.8	±0.6	8.8	19
RF	-3.8	±1.7	6.8	14
SC	-3.8	±2.8	5.8	10
PVG-R	-3.8	±0.6	5.8	14
PVG-C	-6.8	±0.0 to ±0.6	4.5	20

NOTE: Two groups of animals implanted with cannulae in the ventral tegmental area had cannulae angled 20 to 30° from the sagittal plane to avoid the periventricular gray region. Superior colliculus and reticular formation placements were also angled 20 to 30° from the sagittal plane so that they followed the same trajectory as the angled ventral tegmental cannulae. The central dorsal third-ventricle placement was angled at 15° from the sagittal plane to avoid the midsagittal sinus. All other cannulae were paralled to the sagittal plane. Abbreviations: FCS, frontal cortex; NAS, nucleus accumbens; CPU, caudate nucleus; AMYG, amygdala; LHA, lateral hypothalamic area; THAL, thalamus; D3V, medial aspect of the dorsal third-ventricle; VTA, ventral tegmental area (naloxone challenged); VTA-S, ventral tegmental area (physiological saline challenge); VTA-30°, ventral tegmental area (cannulae angled 20 to 30°); RF, reticular formation; SC, superior colliculus; PVG-R, periventricular gray substance (caudal aspect including the fourth ventricle and locus coeruleus).

[a] mm from bregma

[b] mm from the midline

[c] mm from dura

[d] number of subjects tested

minipumps were kept in isotonic saline at 37 degrees Celsius for 16 to 24 hours prior to implantation in the rats. Also, since the minipumps deliver 1.0 µl/hr for a minimum of 200 hours, each was reused in a second subject. The minipumps were bathed in isotonic saline at 37 degrees Celsius to maintain their equilibrated flow rate between the first and second group of rats to receive the same pumps. Fluorometric determinations of flow rate performed by Alzet Corporation showed that the nominal infusion rate (for this lot of minipumps) was .99 µl/hr ±0.1 µl (see Yates, 1981).

After completion of the behavioral testing, the rats were given an anesthetic dose of sodium pentobarbital and intracranially perfused with isotonic saline followed by a 10% Formalin solution. Their brains were then removed and fixed in 10% Formalin for at least two days and frozen sections were taken at 40 micron intervals. Following formalthionin staining, the cannula placements were examined at approximately 10 times magnification and the subjects were grouped according to histological placements.

Procedure: After 72 hours of continuous drug infusions, the rats were placed in a Plexiglas cylinder (23 x 25 cm). Following a five minute adaptation period, they were challenged with an intraperitoneal injection of naloxone hydrochloride (5.0 mg/kg) and placed back in the Plexiglas cylinder. The presence of teeth chattering and wet-dog shakes and the number of escapes from the cylinder were scored in five minute segments for the next 20 minutes (see

Table 7.2). Other withdrawal signs were also observed, but no attempt was made to quantify them since they have been shown to be more variable than the measures assessed in this study (see Wei et al., 1973).

TABLE 7.2

VARIOUS WITHDRAWAL SIGNS USED TO SCORE PRECIPITATED WITHDRAWAL

Withdrawal Sign	Criterion
Escape behavior	Three or more paws on the top rim of the cylindrical enclosure.
Teeth-chattering	An audible sound made by rapid mastication-like movements of the jaws.
Wet-dog shakes	Rotational movements of the body which resemble the motion of a dog shaking himself when wet.
Diarrhea	A soft, formless stool without definite shape.
Vocalization	Squealing upon touch or spontaneously.
Aggression	Attack behavior directed toward the experimenter when touching the subject.
Weight-loss	More than 2 g loss of body weight within 20 minutes of the naloxone challenge.

NOTE: Aggression, diarrhea, and weight-loss were observed in only a few cases of the 200 animals tested in the present study. Vocalization scores were dropped when it became apparent that what was rated as spontaneous vocalization was frequently produced by the animal being approached by the experimenter.

7.2.2 Results

Injections into either the rostral or caudal regions of the periventricular gray substance produced marked physical dependence as quantified by escape behavior following naloxone challenge (see Figure 7.1). The animals with

cannulae in the ventral tegmental area also demonstrated significant escape behavior, although at about one third the rate of subjects that received morphine into the rostral periventricular gray region. An analysis of variance (Winer, 1971) revealed a significant effect for the factor of cannula placement {$F(3,61)=12.406$, $p<.001$}. A Newman-Keuls' test (Winer, 1971) further showed that the escape behavior associated with the rostral periventricular gray placement was significantly greater than that found with the other placements shown in this figure (p's<.01). Ten unoperated rats were also tested for withdrawal signs following an injection of naloxone (5.0 mg/kg, i.p.). None

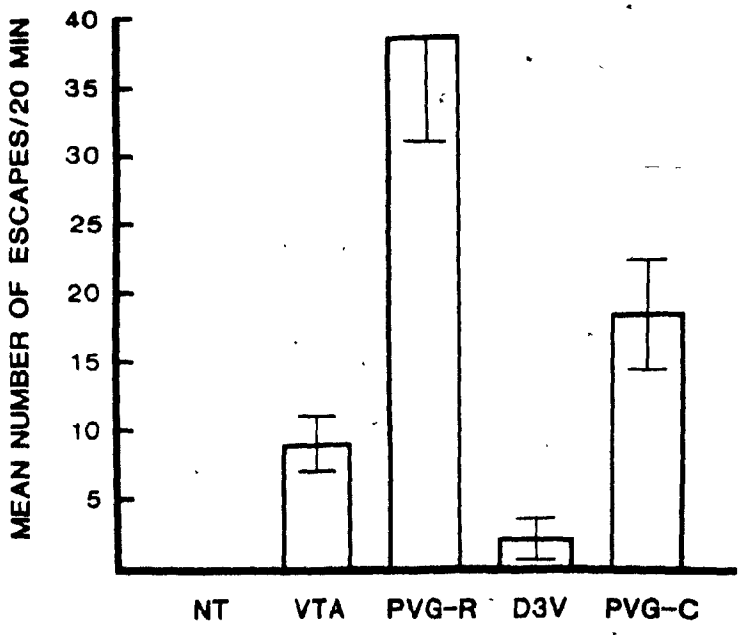

Fig. 7.1. Mean (±SEM) number of escapes in 20 minutes. NT=unoperated control animals challenged with naloxone. (See Table 7.1 for abbreviations.)

of these rats demonstrated any signs of physical dependence including escape behavior although several of the subjects did attempt to leave the cylinder during the five minute adaptation period prior to naloxone injections.

Other withdrawal signs were also seen in the rats injected with morphine into the periventricular gray regions. As shown in Table 7.3, teeth chattering was seen with similar frequency in these two sites, but wet-dog shakes were primarily observed following infusions into the caudal periventricular gray region. In fact, this conclusion is even more strongly supported if a measure that not only represents frequency but is also sensitive to the intensity

TABLE 7.3

PERCENTAGE OF ANIMALS SHOWING VARIOUS WITHDRAWAL SIGNS FOR MORE THAN ONE FIVE MINUTE PERIOD FOLLOWING NALOXONE CHALLENGE

Placement	escape behavior	teeth-chattering	wet-dog shakes
FCX	0	0	0
NAS	33	27	7
CPU	0	0	0
AMYG	12	0	6
LHA	40	0	0
THAL	41	0	0
D3V	21	14	0
VTA	76	0	6
VTA-S	25	0	0
VTA-30^{c}	32	10	0
RF	100	14	0
SC	0	0	0
PVG-R	100	43	14
PVG-C	70	25	45

NOTE: See Table 7.1 for abbreviations.

of the withdrawal sign is used. Figure 7.2 was derived by computing the mean number of periods each sign was present during testing. A subject that displayed a given withdrawal sign during only one of the four five-minute periods was assigned a score of one while a subject displaying the withdrawal sign for the entire 20 minutes of observation (i.e., all four five-minute periods) received a score of four. These values were then converted to the percentage of time the withdrawal sign was present during the observation period (4 periods=100%). A t-test showed that the incidence of wet-dog shakes was significantly higher for animals with cannulae in the caudal periventricular gray region {$t(32) = 2.09$, $p<.05$}. This supports the notion that different brain

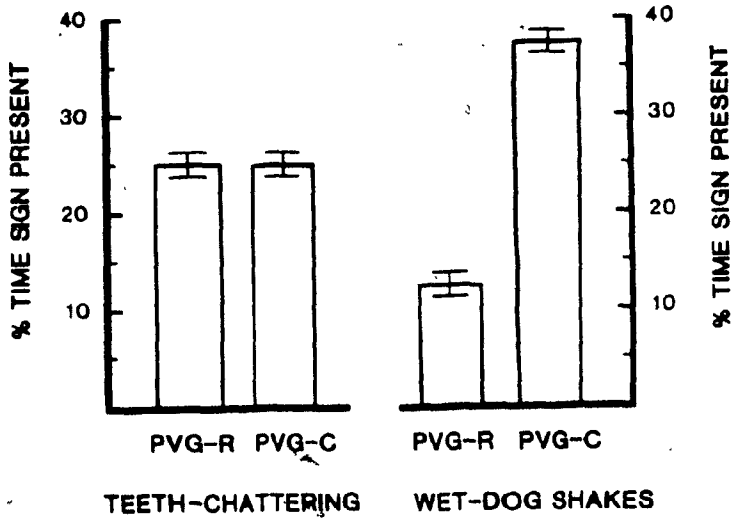

Fig. 7.2. Mean (±SEM) percentage of the 20 minute observation period that teeth chattering or wet-dog shakes were present. (See Table 7.1 for other abbreviations.)

regions may be responsible for the production of different signs of physical dependence (Wei et al., 1973) and cautions against consideration of the withdrawal syndrome as a unitary phenomenon generated by a single brain mechanism. Furthermore, a comparison of Table 7.2 with Figure 7.2 demonstrates the importance of using a measure that takes into account the influence of intensity or duration of the withdrawal sign. Tables that only illustrate the percentage of animals that show a given response can be misleading, and rather arbitrary changes in response criterion can radically alter the conclusions derived from such tables.[5]

The physical dependence produced by morphine infusions into the mesencephalic region was further investigated by implantation of cannulae in the ventral tegmental area at a 20 to 30 degree angle to avoid penetration of the periventricular gray region. Additional animals were implanted with cannulae using the same angled penetration but approximately 2.5 and 3.5 mm dorsal to the angled ventral tegmental

---

[5]For example, changing the values in Table 7.2 to represent the percentage of animals that display more than 10 escapes in 20 minutes would drop the percentage of rostral periventricular gray subjects from 100% to 79%, but more dramatically, the percentage of angled ventral tegmental subjects (VTA-30) decreases form 32% to 5%. Some changes in criterion, however, do not produce markedly different results (e.g., percentage of subjects showing teeth chattering from rostral periventricular morphine infusions only drops from 57% to 43% if the criterion is changed from one or more responses to greater than one response). Note also that both the rostral periventricular gray region and the reticular formation infusions of morphine produced physical dependence in 100% of the subjects tested, but the intensity of the withdrawal escape behavior is markedly different (cf. Figures 7.2 and 7.4).

placement terminating in the reticular formation and superior colliculus, respectively. Morphine was also infused into the central aspect of the dorsal third-ventricle (just dorsal to the medial periventricular gray region) to determine what proportion of the withdrawal response might be due to the ventricular diffusion of drug to a distal site of action. Another group of rats with unangled ventral tegmental cannulae were challenged with physiological saline instead of the naloxone injections. This latter test was used to determine if any of the escape behavior observed in animals implanted in the ventral tegmentum was the result of mechanical irritation from the microinfusions.

Figure 7.3 shows the results under each of the various conditions. Rats with morphine infused through ventral tegmental cannulae angled to avoid the periventricular gray region showed a small, but significant, number of escapes when challenged with systemic naloxone. Animals that received infusions into the dorsal third-ventricle demonstrated a similar low level of escape behavior while rats infused in the reticular formation showed escape responding that was intermediate between the rostral periventricular gray and unangled ventral tegmental placements. Rats challenged with physiological saline displayed about the same level of escape behavior as those with angled ventral tegmental cannulae and challenged with naloxone. This suggests that some of the responding that remains with cannulae that avoid penetration of the periventricular gray substance may

be the result of mechanical irritation in the ventral tegmentum. None of the rats with cannulae implanted in the superior colliculus showed any behavioral sign associated with withdrawal.

Other brain sites were also tested to determine their ability to produce physical dependence with chronic morphine injections. Figure 7.4 shows the results of naloxone challenge after morphine infusions into several forebrain and midbrain regions. The only region that was associated with any appreciable escape behavior was the nucleus accumbens. In this group, three subjects had shown a pronounced level of escape responding (mean=20), but a post hoc histological examination failed to discern any difference between these rats and the other subjects with cannula placements in this region. Likewise, several of the

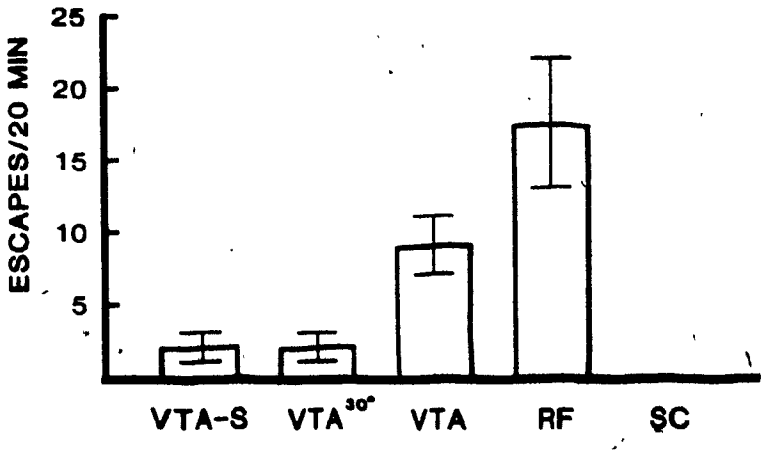

Fig. 7.3 Mean (±SEM) number of escapes in 20 minutes. (See Table 1 for abbreviations.)

animals that received morphine infusions into the amygdala, thalamus, and lateral hypothalamus displayed a low level of escape behavior, but no histological differences were apparent among responders and nonresponders in these groups. In general the level of responding seen after chronic infusions into forebrain and midbrain regions was very low and tests for ventricular diffusion were not undertaken since the primary site of action would appear to be associated with the mesencephalic regions which support a much higher level of escape behavior.

Blasig, Herz, Reinhold, and Zieglgansberger (1973) have suggested that the withdrawal syndrome precipitated after a narcotic antagonist challenge can be divided into dominant and recessive signs. They reported that there was an inverse relationship between the appearance of dominant

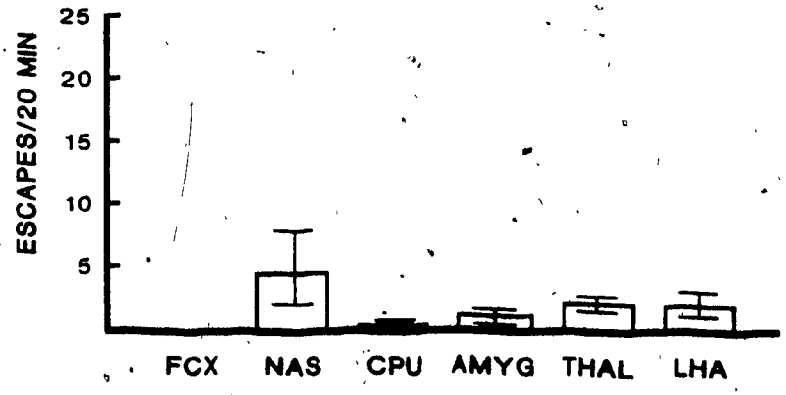

Fig. 7.4: The mean (± SEM) number of escapes in 20 minutes for forebrain and midbrain placements. (See Table 7.1 for abbreviations.)

signs such as escape behavior and teeth chattering and recessive signs such as wet-dog shakes. They suggested that this relationship was a function of both the level of physical dependence and the time since the antagonist challenge. Figure 7.5 illustrates the distribution of escape behavior, teeth chattering, and wet-dog shakes over the 20 minute observation period for the rostral and caudal periventricular gray groups. There was no evidence that the incidence of wet-dog shakes increased toward the end of the observation period (cf. Blasig et al., 1973, Figure 6). In fact, the percentage of animals showing these three signs was highest during the initial five minute period. The discrepant findings of the present study and of Blasig et al. (1973) could be due to the fact that the latter study quantified the intensity of all three measures while the present study quantified escape behavior but simply tabulated the percentage of animals showing teeth chattering and wet-dog shakes. These signs might have increased in frequency within subjects while the number of animals showing a given sign decreased. Nonetheless, the present study does not support the claim of Blasig et al. (1973) and suggests that this issue may be more complicated than previously suggested.

A number of other withdrawal signs have been reported by other investigators. These include abnormal posturing, diarrhea, hyperactivity, mastication, and vocalization (e.g., Blasig et al., 1973; Martin, Wikler, Eades, & Pescor, 1963; Menon, Tseng, Loh, & Clark, 1980; Teiger, 1974; Wei et al.,

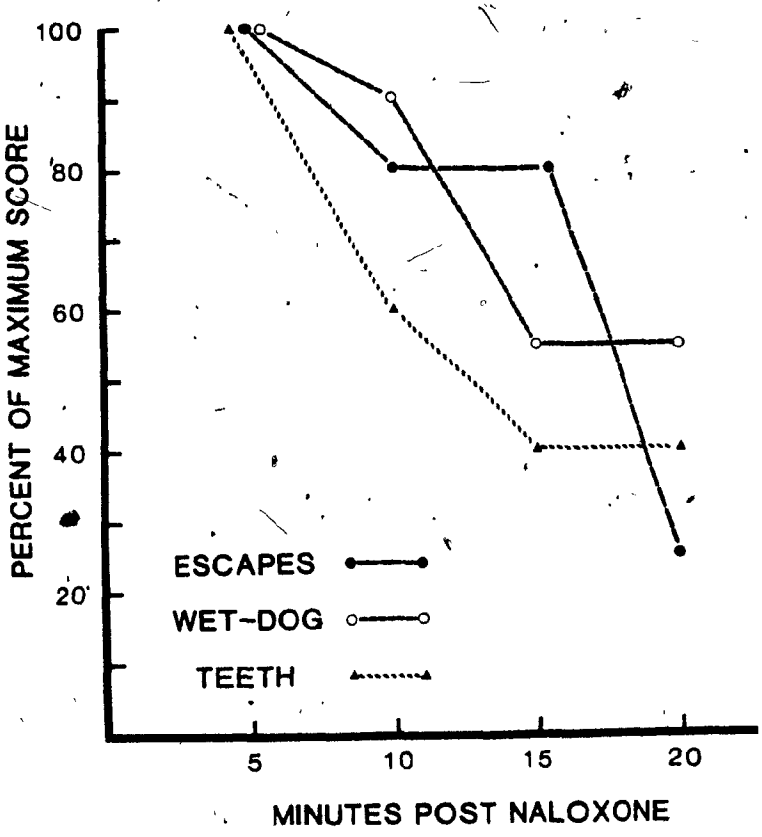

Fig. 7.5. Distribution of the various withdrawal signs over the 20 minute observation period. Only subjects that displayed the corresponding withdrawal sign during at least one of the observation periods were included in the computations. Escape means represent the number of escape responses in a given period while both wet-dog shakes and teeth chattering means represent the percentage of subjects showing these signs in each period.

1973). All of these signs except diarrhea were seen in the present study. Because there is difficulty in the quantification of these signs, however, they were not used to assess the intensity of opiate withdrawal. The failure to observe diarrhea was surprising since (i) opiates have both centrally

and peripherally mediated effects on intestinal motility (Schulz, Wuster, & Herz, 1979; Steward, Weisbrodt, & Burks, 1978), (ii) Schreier and Burks (1981) have reported that intraventricular clonidine inhibits withdrawal diarrhea, and (iii) Herz has reported that the systemic administration of a narcotic antagonist that does not cross the blood-brain barrier fails to precipitate withdrawal diarrhea (Herz, unpublished observation, cited in Laschka et al., 1976a). The fact that morphine chronically infused into the caudal periventricular gray region (and surrounding fourth ventricle) did not result in diarrhea following naloxone challenge does not support the notion that withdrawal diarrhea is mediated by an opiate action at the locus coeruleus (see Gold & Pottash, 1981).

Representative histologies are illustrated in Figure 7.6. Cannulae aimed at the frontal cortex, nucleus accumbens, and caudate nucleus were dispersed within about a 1.0 mm radius of the placements shown in the figure. No signs of withdrawal were seen in any of the rats in this range of placements except in the case of several nucleus accumbens animals previously discussed. Cannula placements in the thalamus were generally in the caudal portion of the ventral nucleus while some lateral hypothalamic cannulae extended to the zona incerta. Sites tested in the amygdala included the central, cortical, and pars lateralis amygdaloid nuclei. Histological analyses were unable to discern any relationship between withdrawal behavior and within site variations in

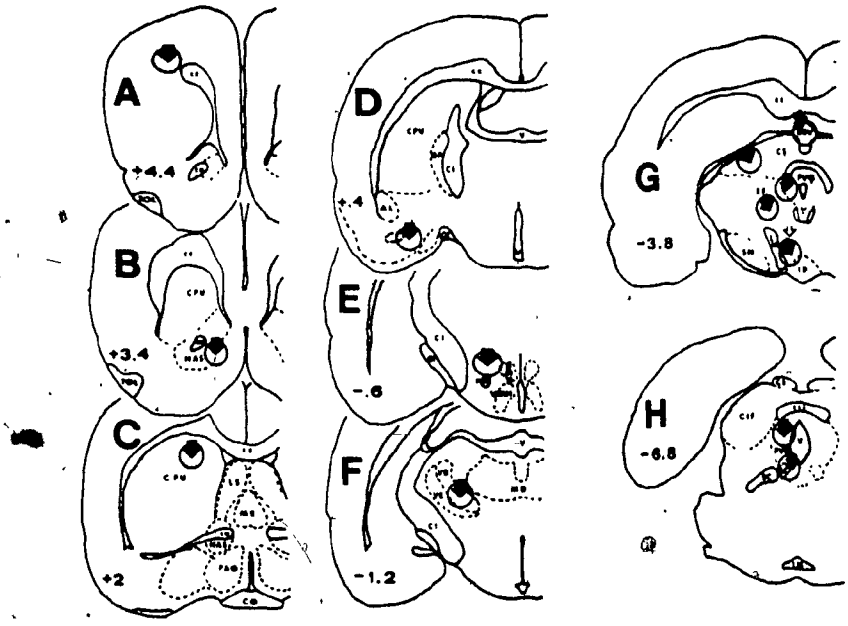

Fig. 7.6. Representative histological reconstructions of the various brain regions tested in this study. Each placement is indicated by a circle and the angle of cannula penetration by an arrow. It should be noted that the placements illustrated in sections A through F are approximate modal values and appreciable dispersion of cannulae occurred around these sites. Abbreviations: AL, lateral amygdaloid nucleus; BC, brachium conjunctivum (superior cerebellar peduncle); ca, anterior commissure; cc, corpus callosum; cci, commissure of the inferior colliculus; CI, internal capusle; CIF, inferior colliculus; CO, optic chiasm; CPU, caudate nucleus-putamen; CS, superior colliculus; D3V, dorsal third-ventricle; DMH, dorsomedial nucleus of the hypothalamus; FX, fornix; GP, globus pallidus; IP, interpeduncular nucleus; LC, locus coeruleus; LM, medial lemniscus; LS, lateral septal nucleus; MD, dorsomedial nucleus of the thalamus; MS, medial septal nucleus; NAS, nucleus accumbens; OT, optic tract; PAO, lateral preoptic area; PVG, periventricular gray substance; RF, reticular formation; SN, substantia nigra; TOL, lateral olfactory tract; V, ventricle; VD, ventral nucleus of the thalamus, dorsal part; VE, ventral nucleus of the thalamus; VMH, ventromedial nucleus of the hypothalamus (adapted from Pellegrino et al., 1979; ventricular nomenclature from Mitro & Palkovits, 1981).

cannula placements. Even if regional variations existed, it would be unlikely that such fine anatomical distinctions could be made because of the relatively high hourly infusion rate of morphine and its probable spread which is likely to exceed a millimeter.

A number of subjects whose data were included in the caudal periventricular gray group had cannulae aimed specifically at the locus coeruleus. Because the size of this structure is about the same as the 21 gauge cannulae used to infuse the morphine, lesions were frequently created by the insertion of the cannulae. Furthermore, since the locus coeruleus is just ventral to the fourth ventricle, infusions delivered to this area are accompanied by appreciable diffusion into the ventricular system. Likewise, morphine infusions into the caudal periventricular gray region readily reach the locus coeruleus by ventricular diffusion. In addition, several subjects tested early in the study had cannulae aimed at the lateral aspect of the caudal periventricular gray region. Even these placements, however, usually resulted in rupturing the fourth ventricle and subsequent cannulae were lowered down the midline. Histological examination did not reveal any anatomical factor that could account for the variability of behavioral responses associated with this brain region. Hence, these subjects were treated as a single group.

Since the primary aim of this study was to determine the involvement of ventral tegmental opiate receptors in the development of physical dependence, the area surrounding

the ventral tegmentum was examined in much greater detail.[6] Cannulae were angled 20 to 30 degrees from the midline to avoid penetration of the lateral aspect of the rostral periventricular gray substance. Other cannulae were placed 2.5 and 3.5 mm dorsal to the ventral tegmentum along the same line of trajectory followed by the angled cannulae. This resulted in placements that included the reticular formation and the superior colliculus. Cannulae in the reticular formation were frequently more medial than the placement illustrated in Figure 7.6. Cannulae in the superior colliculus ranged dorsal to the dorsal third-ventricle and as far anterior as the lateral nucleus of the posterior thalamus and the lateral geniculate body. The ventral tegmental region tested for its ability to produce physical dependence from morphine infusions included the zone shown in Chapter 4 to contain the reward relevant opiate receptors and ranged from about 3.2 to 4.2 mm posterior to bregma. The rostral periventricular gray region included an anterior-posterior dispersion similar to the ventral tegmental placements.

Figure 7.7 shows the range of tissue damage by the infusion of 1 µl/hr for 72 hours. Many of the histologies showed cell loss comparable to that seen in the first brain section but most were intermediate between the low and

---

[6]Ex post facto this has proved to be the most interesting brain region for the study of physical dependence, at least in terms of the production of escape behavior.

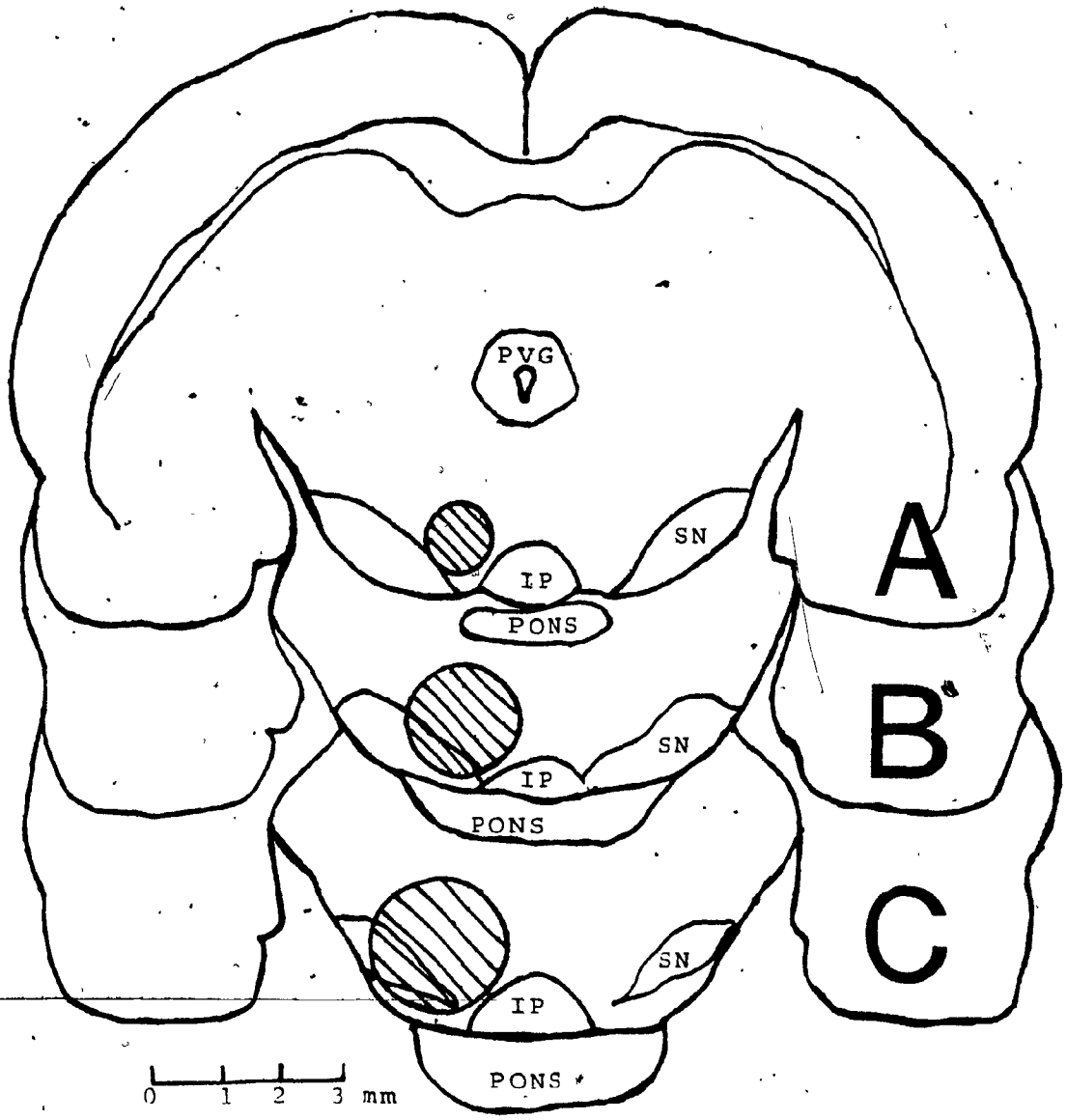

Fig. 7.7. The extent of tissue damage produced by chronic produced by chronic infusions is illustrated by the striped zone. Abbreviations: IP, interpeduncuclar nucleus; PVG, periventricular gray substance; SN, substantia nigra.

moderate levels of damage. Several animals had extensive damage produced by this chronic infusion regimen and these subjects were deleted from the data analysis (i.e., see Figure 7.7-C). The large variability in cell loss is surprising since all subjects received identical osmotic minipumps and since care was exercised to insure that the nominal flow rate of 1 µl/hr had been reached prior to implantation. Nonetheless, there were large variations in the extent of tissue damage following chronic infusions of morphine. Even the low level of tissue damage was higher than that encountered with a higher infusion volume delivered over a shorter time interval (see Section 3.3.2).

7.2.3 Discussion

Figure 7.8 is a sagittal reconstruction of the brain sites tested in this study. Escape behavior was chosen for this comparison because it only occurs after the development of moderate to severe physical dependence and because it is the most suitable measure for quantification (Blasig et al., 1973; Laschka et al., 1976b; Wei et al., 1973).[7] The degree

---

[7]The significance of the various withdrawal signs studied in animals to those observed in humans has not been systematically investigated (see Wei, 1981). It is interesting to note, however, that in an early study by Wikler et al. (1953), narcotic addicts described nalorphine as a drug that "cleans you out of dope and makes you climb the walls" (p. 12). Unlike the mouse (Bhargava & Way, 1972; Way, Loh, and Shen, 1969), rats do not show spontaneous withdrawal jumping when challenged with a narcotic antagonist (M. Bozarth, unpublished observations). It would appear that the escape behavior observed in rats is in fact ESCAPE BEHAVIOR and motivated by withdrawal distress. Thus, this term is probably more descriptive of the phenomenon than the term "jumping."

of physical dependence produced by the same regimen of infusions is indicated by the relative density of the various brain sections in the figure. The highest level of escape behavior was associated with infusions into the rostral periventricular gray region while the infusion of morphine into the reticular formation and caudal periventricular gray region also produced appreciable escape behavior. The withdrawal signs observed after infusions into the ventral tegmentum with unangled cannulae appear to be the result of diffusion up the cannula shaft since angling the cannulae to avoid the lateral aspect of the periventricular gray substance virtually eliminated this behavior. Furthermore, the escape behavior seen after reticular formation infusions was probably the result of drug diffusion to the rostral periventricular gray region which is just 1.5 mm medial to this infusion site (see Figure 7.6-G). Ventricular diffusion of morphine was eliminated as a possible explanation because infusions into the central aspect of the dorsal third-ventricle or the superior colliculus (just ventral to the lateral aspect of the dorsal third-ventricle) failed to produce any significant escape behavior. Teeth chattering was also seen after infusions into the rostral periventricular gray region, although wet-dog shakes were seldom observed. These observations provide empirical support for the suggestion of Calvino, Lagowska, & Ben-Ari (1979) and Wei et al. (1973) that different brain sites are responsible for the production of specific withdrawal signs.

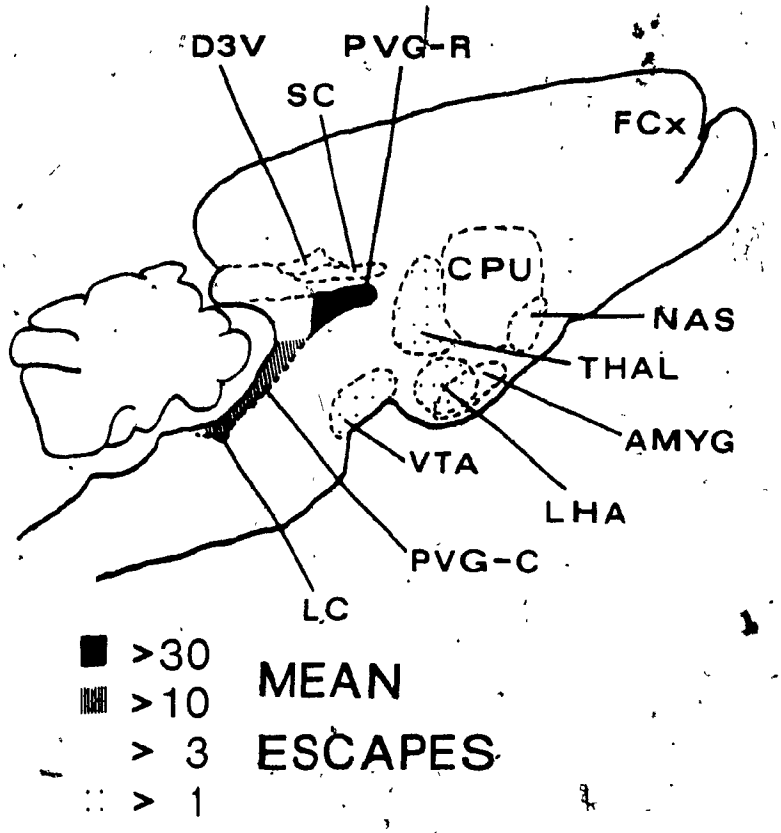

Fig. 7.8. Sagittal reconstruction of the brain regions tested for their ability to produce physical dependence on morphine. The relative density of the various regions indicates the degree of physical dependence produced by chronic morphine infusions as assessed by escape behavior during precipitated abstinence. The reticular formation placement is lateral to the rostral periventricular gray region and is not shown on the figure. The data for the ventral tegmental placement is taken from the animals with cannulae angled to avoid the periventricular gray substance. LC=locus coeruleus. (See Table 7.1 for other abbreviations.)

There are several lines of evidence that suggest that the locus coeruleus is involved in the production of physical dependence on opiates (see Gold & Pottash, 1981). First, morphine inhibits the spontaneous activity of locus coeruleus

neurons (Korf, Bunney, & Aghajanian, 1974) and tolerance develops to this effect following chronic morphine treatment (Aghajanian, 1978). The microiontophoretic application of naloxone onto morphine-tolerant locus coeruleus neurons produces a "withdrawal response" characterized by a dramatic increase in the firing rate of these units (Aghajanian, 1978). Second, electrical activation of the locus coeruleus produces behaviors that are similar, in some respects, to opiate withdrawal symptoms (Redmond & Huang, 1979; Redmond, Huang, & Gold, 1977). Third, the locus coeruleus contains the largest concentration of noradrenergic cell bodies in the brain (Lindvall & Bjorklund, 1974; Moore & Bloom, 1979) and neurochemical evidence has suggested that norepinephrine mediates at least some of the symptoms of physical dependence on opiates: the systemic injection of clonidine, an alpha-2-noradrenergic agonist,[8] attenuates many of the withdrawal signs in both animals (Cervo, Rochat, Romandini, & Samanin, 1981; Fielding, Wilker, Hynes, Szewozak, Novick, & Lal, 1978;

---

[8]Clonidine's action on alpha-2-noradrenergic receptors produces a suppression firing, probably by activating inhibitory autoreceptors on the noradrenergic neurons (see Aghajanian, 1978, 1981; Dixon, Mosimann, & Weiner, 1979; Engberg, Elam, & Svensson, 1982; Medgett, McCulloch, & Rand, 1978). Tolerance develops to this effect and increased unit activity is reported after the cessation of chronic clonidine treatment (Engberg et al., 1982). Furthermore, the action of clonidine on locus coeruleus neurons may be associated with a clonidine withdrawal reaction which is observed in humans and resembles some of the features of opiate withdrawal (Engberg et al., 1982; Parker & Atkinson, 1982). Recently, Franz, Hare, & McCloskey (1982) have suggested that clonidine's suppression of opiate withdrawal may be due in part to its ability to attenuate hyperactivity at spinal sympathetic neurons.

Herz, Blasig, & Papeschi, 1974; Meyer & Sparber, 1976; Samanin, Cervo, Rochat, Poggesi, & Mennini, 1980; Sparber & Meyer, 1978) and humans (Gold & Pottash, 1981; Gold, Pottash, Sweeney, & Kleber, 1980; Gold, Redmond, & Kleber, 1978; Washton & Resnick, 1980, 1981ab).[9] The proposed role of the locus coeruleus in withdrawal distress is particularly enticing because there is independent evidence that this structure may be involved in anxiety and distress (Mason & Fibiger, 1979; Redmond & Huang, 1979; Redmond, Huang, Snyder, & Maas, 1976).

The caudal periventricular gray region tested in the present study is in close proximity to the locus coeruleus (see Pellegrino et al., 1979). In fact, a number of animals were infused directly into this structure although preliminary data analysis showed no difference between these rats and others infused with morphine into the surrounding periventricular gray-fourth ventricle region. Therefore, the data from these subjects and that from subjects infused into the fourth-ventricle and the surrounding periventricular gray substance were treated as a single group. It should be noted that many of these cannula tracks were continuous with

---

[9]It is interesting to note that clonidine has also been reported to attenuate the withdrawal syndrome associated with ethanol dependence in humans (Walinder, Balldin, Bokstrom, Karlsson, & Lundstrom, 1981). Furthermore, lesions of the locus coeruleus are reported to effectively block the development of physical dependence on ethanol in animals (Kostowski & Trzaskowska, 1980).

the fourth ventricle, and physical dependence produced by morphine infusions into this placement is the least likely (of those tested in the present study) to be dependent on an opiate action proximal to the infusion site.[10] Thus, no estimate of the extent of diffusion from these infusions is possible and the evidence suggesting a role for the locus coeruleus in the development of physical dependence remains indirect. The present data regarding teeth chattering and wet-dog shakes are consistent with the proposed role of the locus coeruleus in opiate dependence. Escape behavior, however, does not seem to result from a drug action in this region since a much more pronounced level of escape behavior has been produced by infusions into the rostral periventricular gray region. This latter finding is consistent with the observation by Cervo et al. (1981) and Samanin et al. (1980) that clonidine fails to reduce escape behavior during opiate

[10] Since the locus coeruleus is juxtaposed to the ventral aspect of the fourth ventricle (see Pellegrino et al., 1979), infusions into this region can be assumed to result in the diffusion of appreciable quantities of morphine both from the locus coeruleus to the fourth ventricle and vice versa. Attempts to infuse morphine directly into the locus coeruleus were complicated by the fact that the insertion of the 21 gauge cannulae (used in this study) into the locus coeruleus functionally lesioned this small structure. Thus, infusions directly into the locus coeruleus might be expected to produce less effect than more dorsal infusions into the fourth ventricle. To specifically test the involvement of this structure in opiate-induced physical dependence, it would be more viable to assess the effect of locus coeruleus lesions on the development of physical dependence from systemic opiate injections. Nonetheless, the caudal periventricular gray infusions should result in significant opiate action at the locus coeruleus and these placements provide at least an estimate of the relative importance of this structure in the development of physical dependence.

withdrawal.

Early work in the study of the central mechanisms of opiate dependence relied on the production of physical dependence from systemic drug injections or from pellet implantation and precipitated withdrawal by central narcotic-antagonist application. The amygdaloid complex (Calvino et al., 1979; Lagowska, Calvino, & Ben-Ari, 1978; Tremblay & Charton, 1981), medial thalamus (Tremblay & Charton, 1981; Wei et al., 1973), and parts of the striatum (Tremblay & Charton, 1981; Wei et al., 1973) have all been reported to be sensitive to direct naloxone application. In contrast to these reports, the present study failed to find any appreciable physical dependence after chronic morphine infusions into the amygdala, thalamus, or striatum (see Figure 7.4), although if the criterion were changed to the percentage of animals showing a given withdrawal sign, the present study would confirm Tremblay and Charton's (1981) and Wei et al.'s (1973) observations of withdrawal following naloxone injections into the thalamus.[11] Rather than confirming the role of the thalamus in the development of physical dependence, however, this comparison would appear to emphasize the importance of using a measure which

---

[11] In the present study, 41% of the subjects showed escape behavior (see Table 7.2) while Tremblay and Charton (1981) reported responding in 42% of their animals. Wei et al. (1973) reported that 58% of their animals challenged with naloxone in the medial thalamus showed withdrawal behavior (i.e., at least two escape attempts OR three wet-dog shakes).

reflects not only the proportion of subjects responding but also the intensity of the response.

Because of the problem of excessive drug diffusion following the liquid injection of drugs, both Calvino et al. (1979) and Wei et al. (1973) used crystalline naloxone application to precipitate withdrawal. The study by Calvino et al. (1979) showed anatomical resolution that would suggest that crystalline naloxone spreads only a fraction of a millimeter from the application site (see Calvino et al., 1979, Figures 3 and 4). This is surprising since Routtenberg, Sladek, and Bondareff (1968) have shown that crystalline drug application can result in appreciable diffusion after only 11 to 12 minutes of application. The Routtenberg et al. (1968) study reported three diffusion patterns: (1) a 1 to 2 mm spherical distribution around the cannula tip, and (2) appreciable "axonal streaming" orthograde to the cannula tip, and (3) significant diffusion to the cerebral ventricular system.[12] Although the sites that were effective in precipitating withdrawal in the Calvino et al. (1979) and Wei et al. (1973) studies had cannulae which penetrated various aspects of the ventricular system, effective and noneffective sites are not clearly discernable on the basis of proximity to the

---

[12] In the case of dopamine, less diffusion is seen after liquid (Bondareff, Routtenberg, Narotzky, & Mc Lone (1970) than crystalline (Routtenberg et al., 1968) application. Routtenberg (1972) has suggested that this may be due to the extremely high concentration of drug applied with the crystalline method.

ventricular system. Nonetheless, diffusion to a distal site of action whether via the cerebral ventricular system or "axonal streaming" seems to be a viable explanation of the discrepant findings of these experiments and the present study. Another possibility would be that the high concentration reached after crystalline naloxone application caused nonspecific effects that resulted in the withdrawal reaction. Without controlling for actions produced by the physico-chemical properties of naloxone (i.e., comparison of the potency of active and inactive stereoisomers), these studies are difficult to interpret. It is likely, however, that the amygdaloid complex is involved in some aspect of opiate withdrawal since lesions of this area (Calvino et al., 1979) or amygdaloid kindling (Le Gal La Salle & Lagowska, 1980) attenuates the severity of the opiate withdrawal syndrome.

The importance of the periventricular gray region in the development of physical dependence on morphine was suggested by Herz et al. (1972) and Laschka et al (1976b). Using a preparation in which a ventricular "plug" was inserted in the cerebral aqueduct, a narcotic antagonist was microinjected into the lateral or fourth ventricle after the animals were made physically dependent on systemic morphine. When the antagonist challenge was restricted to the region of the lateral and third ventricles, little sign of abstinence was precipitated. Microinjections into the fourth ventricle, on the other hand, produced a marked with-

drawal syndrome which included most of the signs seen after systemic narcotic-antagonist challenge. The observation (in the present study) that infusions of morphine into the periventricular gray region produced the greatest degree of physical dependence is concordant with the Herz et al. (1972) and Laschka et al. (1976b) studies. There is, however, one important difference among these studies: their procedure may have prevented drug diffusion to the most sensitive part of the periventricular gray substance. The anatomical location of the periventricular "plug" used by Herz et al. (1972) and Laschka et al. (1976b) is difficult to determine based on their published reports. In another experiment using the same procedure, Schulz, Blasig, Laschka, and Herz (1978) illustrate the approximate location of the "plug" (see Schulz et al., 1978, Figure 1). It would appear that the ventricular "plug" used to restrict the ventricular diffusion of microinjected narcotic antagonist would effectively block drug diffusion to the rostral portion of the periventricular gray substance. Thus, the fourth ventricle injections of Laschka et al. (1976b) would reach the caudal periventricular gray substance tested in the present experiment, but the "plug" would probably prohibit appreciable diffusion to the rostral periventricular gray region found to be the most sensitive area to the chronic infusion of morphine. Similarly, injections into the lateral ventricle would fail to result in drug diffusion to the rostral periventricular gray region. Therefore, the studies using the

ventricular "plug" may have missed the most effective site in producing the opiate withdrawal symptom of escape behavior.[13]

The level of responding seen after naloxone challenge in animals chronically infused with morphine into the rostral periventricular gray region compares favorably with that seen in other physical dependence studies. Table 7.4 summarizes various studies using several methods of demonstrating physical dependence. The intensity of the jumping response seen in the present study (see Figure 7.1, PVG-R) is higher than that reported from systemic drug injections suggesting that the degree of physical dependence in these subjects is as great as that obtainable with systemic injections. This would be expected if the rostral periventricular gray region were the site of action for physical dependence resulting from systemic opiate injections.

7.3 Temporal Dissociation of Reward and Physical Dependence

The intravenous self-administration paradigm has been used extensively to study the rewarding properties of abused drugs. Work with opiates has shown that laboratory

---

[13] Whether the periventricular gray region is heterogeneous with regard to its ability to produce physical dependence on morphine has not been clearly established. Since ventricular flow in this region is in a rostral to caudal direction (Clark, 1975), it is possible that this structure is homogeneous and that the greater level of escape behavior after rostral periventricular gray infusions is the result of drug diffusion to a larger proportion of periventricular gray opiate-receptors.

TABLE 7.4

A COMPARISON OF THE SEVERITY OF WITHDRAWAL JUMPING REPORTED FOR VARIOUS PROCEDURES IN THE RAT

Investigator	Agonist Applied	Antagonist Applied	Jumps[a]	Minutes Tested
Blasig et al. (197)	systemic	systemic	30	30
Calvino et al. (1979)	systemic	systemic	8	30
Calvino et al. (1979)	systemic	amygdala	25	30
Kruszewska (1980)	systemic	systemic	13 to 23[c]	20
Laschka et al. (1976a)	systemic	systemic	17[b]	30
Laschka et al. (1976b)	systemic	4th ventricle	12	30
Pinsky et al. (1982)	systemic	systemic	7	10
Schulz et al. (1978)	systemic	4th ventricle	12	15 to 20
Wei et al. (1973)	systemic	systemic	11	10
Wei (1981)	PVG-4th ventricle[d]	systemic	25[e]	15

[a]Mean number of jumps/escapes

[b]3.3 mg dose

[c]Note variability in the same study (cf. Figures 3 & 4, p. 660)

[d]Periventricular gray-fourth ventricle region

[e]108 nmole dose

animals will reliably self-administer opiates and that physical dependence frequently accompanies this self-administration (Weeks & Collins, 1979; Young et al., 1981). Other self-administration studies have shown that animals will self-administer opiates at what appear to be sub-dependence producing doses (Deneau et al., 1969; Woods & Schuster, 1968, 1971). Upon termination of testing, these subjects do not show obvious signs of physical dependence. Nonetheless, the failure to observe marked signs of abstinence does not eliminate the possibility that some degree of physical dependence has developed in these subjects. The abstinence syndrome is accompanied by marked dysphoria in humans, and it is not clear that the autonomic effects that are usually quantified in animals are necessary for the production of this dysphoric response.[14] It seems highly probably that some degree of withdrawal distress is produced by the termination of chronic opiate intake in animals and that this subjective state is not readily detectable with the current methods used by these investigators. Therefore, relief of the withdrawal distress by subsequent opiate self-

---

[14] The withdrawal signs usually studied in monkeys include convulsions, vomiting, anorexia, and diarrhea (e.g., Killam & Deneau, 1973; Kolb & DuMez, 1931; Martin & Jasinsky, 1977; Spragg, 1940; Villarreal, 1973; Villarreal & Karbowski, 1974). Work with rats has been more quantitatively oriented using such measures as escape behavior or withdrawal jumping, teeth-chattering, wet-dog shakes, and weight loss (e.g., Blasig et al., 1973; Koga & Inukai, 1981b; Martin et al., 1963; Nakaki, Saito, Nakadate, Tokunaga, & Kato, 1981; Wei et al., 1973).

administration may not be ruled out as a factor in the control of opiate intake in any paradigm involving repeated drug administration (cf. Weeks & Collins, 1979).

To avoid the potentially confounding influence of negative reinforcement mechanisms associated with the relief of withdrawal discomfort by opiate injections, the rewarding impact of a single injection of heroin was assessed in rats. In Chapter 6, animals receiving a series of four heroin injections associated with a novel environment returned to the place where they had previously experienced the effects of these injections (Bozarth & Wise, 1981b). This conditioned place preference paradigm has been used to show the rewarding effects of several drugs including morphine (Rossi & Reid, 1976; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980a; Stapleton, Lind, Merriman, Bozarth, & Reid, 1979), heroin (Bozarth & Wise, 1981b), and amphetamine (Sherman, Roberts, Roskam, & Holman, 1980b), and it has been proposed as a measure of the affective consequences of drug administration (Rossi & Reid, 1976). The present study involved testing for a conditioned place preference after only one injection of heroin; with this procedure there was no opportunity for the animals to learn about the effects of the drug injection on any opiate withdrawal symptoms which might have occurred with repeated drug injections.

7.3.1 Method

Experimentally naive, male, Long-Evans rats (weighing 325-450 g) were adapted to a shuttle box (25 x 36 x 35 cm) for five days, and the amount of time spent on each side of the chamber was automatically recorded during 15 minute test sessions. One side of the test box had a plain plywood floor while the other side had a plywood floor covered with wire mesh. The amount of time spent on each of the two sides on the last trial of the adaptation period was used to determine the initial place preference for each animal. On the sixth day, 12 rats were subcutaneously injected with heroin (0.5 mg/kg) immediately before placement on their nonpreferred side of the shuttle box. A partition was used to confine the animals to their nonpreferred side for 30 minutes following the heroin injection. Another group of 12 rats was injected with isotonic saline (1 ml/kg, s.c.) to assess any spontaneous shifts in place preference which might occur due to habituation to their nonpreferred side. On the next day, place preference was retested during a single 15 minute trial in the drug-free state. Increases in the amount of time spent on the side of putative conditioning were interpreted as an indication of drug-induced reward.

7.3.2 Results

Figure 7.9 shows the change in place preference following a single injection of heroin. Each animal's pre-

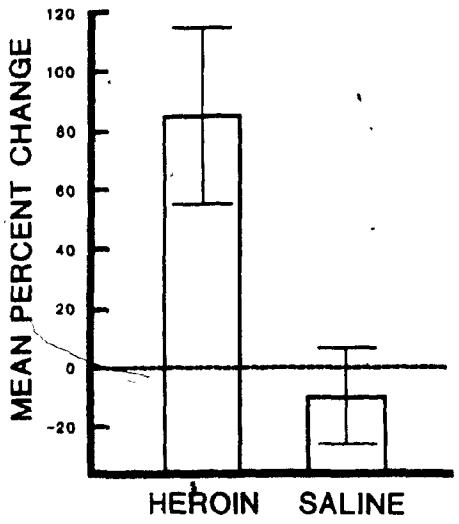

Fig. 7.9. Mean (±SEM) percentage change in place preference as a function of treatment. The difference between groups is significant {t(22)=2.65, p<.01, one-tailed} as is the increase in preference for the conditioning side displayed by the heroin conditioned group (see text).

ference during the test trial was expressed as a percentage change from its pretreatment preference. The single injection of heroin resulted in a significant shift in the animals' place preferences {t (11)=2.80, p<.01} while saline treatment did not produce a shift {t (11)=0.63, p>.25}. This place preference produced by acute heroin treatment suggests that initial heroin administration is rewarding in naive animals. Since this study involved only one injection of drug, there was no opportunity for the drug injections to relieve withdrawal distress. Thus, positive reinforcement mechanisms are sufficient to account for the place preference of these animals and opiate reward can be temporally disso-

ciated from the development of physical dependence.

Since most animals spent appreciably less than half of the 15 minute period on their nonpreferred side, increases in locomotor activity could potentially inflate their place preference scores. This pseudopreference might simply reflect a more even distribution of time spent on each side of the test chamber and be the result of increased locomotor activity (see Bozarth & Wise, 1981b). In the present study, however, no significant changes in locomotor activity for either the heroin or saline injected groups occurred {t's (11)=1.17 & 1.44, p's >.20 & .10, respectively} eliminating this potentially confounding influence of locomotor activity on place preference scores.

7.3.3 Discussion

The initial subjective effects of opiate intake are equivocal in humans. Some investigators report that the first injections of opiates are aversive and that the reinforcing properties do not emerge until appreciable experience with the addictive agent has developed (e.g., see Beecher, 1966; Criswell & Levitt, 1975; Lasagna, von Felsinger, & Beecher, 1955). Other investigators have found that opiate users experience pronounced euphorigenic effects with initial use and that chronic exposure to opiates is not necessary to produce these positive changes in affect and mood (e.g., McAuliffe, 1975; McAuliffe & Gordon, 1974; Mirin, Meyer, & McNamee, 1976). Animal studies have shown that opiates have

mixed affective consequences: the same injection of morphine is both rewarding and aversive (Gorman, De Obaldia, Scott, & Reid, 1978; Sherman et al., 1980a; White Sklar, & Amit, 1977; but see Stapleton et al., 1979, Experiment III).[15] With repeated opiate administration, however, tolerance to aversive effects develops while the rewarding properties become even more apparent (see Gorman et al., 1978).

The present study showed that the dominant effect of heroin for at least the first 30 minutes after the first injection was associated with a positive affective state. The change in place preference produced by a single injection of heroin suggests that the aversiveness of this opiate may develop some time after the initial 30 minutes of drug action since a place aversion would be expected to develop if the predominant effect of the drug were dysphoria (Stewart & Grupp, 1981). The conditioned taste aversion paradigm, which has been used to assess the aversive properties of opiates (e.g., Jacobs, Zellner, LoLordo, & Riley, 1981; Riley, Jacobs, & LoLordo, 1978; Stewart & Eikelboom, 1978; Stolerman, Pilcher, & D'Mello, 1978; Switzman, Fishman, & Amit, 1981; Van der Kooy & Phillips, 1977), would be sensitive to any aversive consequences for hours after exposure to the test substance (see Logue, 1979). Thus it seems that the initial effect of opiate administration may

[15] Wise, Yokel, and deWit (1976) have shown that intravenously self-administered apomorphine can produce a conditioned taste aversion. See Goudie (1979) for a discussion of the apparently paradoxical properties of addictive drugs.

be rewarding while the aversion that is demonstrable in the conditioned taste aversion paradigm is related to drug withdrawal or some other secondary effect of drug administration that outlasts the initial rewarding action (cf. Sklar & Amit, 1977). The fact that physical dependence is demonstrable after a single injection of morphine (Kosersky, Harris, & Harris, 1974; Ritzmann, 1981) makes this possibility viable although studies showing withdrawal symptoms after a single opiate injection involve a much higher dose than that used in conditioned taste aversion experiments.

7.4 General Discussion

Many theories of opiate addiction have focused on the physical dependence producing properties of this class of drugs. Although some theorists have suggested that opiates are taken because of their ability to alter mood and create a "general feeling of well being" (e.g., Eddy, 1973; Eddy, Halback, & Braenden, 1957; Jaffe, 1975; Martin & Sloan, 1977; McAuliffe & Gordon, 1980), several have formulated theories around the physical dependence producing properties of these drugs. This negative reinforcement model of addiction (e.g., Dole, 1980, Lindesmith, 1938, 1970, 1980; Nichols, 1965; Spragg, 1940; Wikler, 1980; Wikler, Martin, Pescor, & Eades, 1963; Wikler & Pescor, 1967) suggests that it is the relief of withdrawal distress which accompanies opiate assimilation in the dependent person that is primarily responsible for the maintenance of drug intake. This line

of thinking, however, suffers from several serious limitations. First, it fails to account for the initial use of opiates in the drug naive person (i.e., acquisition) relying on theories of personality deficits or psychosocial models of dependent personalities (e.g., the "addictive personality") to explain the acquisition phase of drug addiction.[16] Second, withdrawal distress is usually abated after a week of abstinence from drug (Jaffe, 1975), yet relapse rates for detoxified addicts are very high. Theories of protracted abstinence (e.g., Wickler & Pescor, 1967; Wickler, Pescor, Miller, & Porrell, 1971) have been postulated to account for the failure of opiate users to remain abstinent even though they may have had no access to opiates for a period of several months. Even at the period of peak intensity, however, many addicts report that the severity of their discomfort is no worse than a cold or mild flu (Jaffe, 1975).[17] Third, an increasing number of middle

---

[16] It is interesting to note that the psychodynamic theories that have been postulated to account for drug addiction generally do not require the negative reinforcing effects associated with physical dependence (e.g., Rado, 1958; Rasor, 1958; deRopp, 1957; see also Lettieri, Sayers, & Pearson, 1980, for a collection of psychodynamic and psychosocial theories). The motivation provided by the avoidance of withdrawal distress would appear to be redundant in such theories.

[17] Even though methadone effectively abates the withdrawal discomfort associated with abstinence from opiates (Cushman & Dole, 1973; Dole, 1980; Martin, 1977), addicts frequently continue to use illicit opiates during methadone treatment (Des Jarlais, Joseph, & Dole, 1981; Ling, Blakis, Holmes, Klett, & Carter, 1980; Sorensen, Hargreaves, & Weinberg, 1982a,b). Also, successful detoxification from methadone maintenance programs produces only a slightly

class people use opiates recreationally during limited periods of their lives (Zinberg, Harding, Stelmack, & Marblestone, 1978; Zinberg & Jacobson, 1976; see also Jaffe, 1975). Frequently the pattern reflects only weekend use of the drug with no illicit drug use occurring during the week (i.e., "chipping"). Fourth, several classes of drugs produce physical dependence (e.g., chlorpromazine, atropine, clonidine), but abrupt cessation of their therapeutic administration does not lead to abuse and subsequent addiction; other drugs which are addictive fail to produce appreciable physical dependence (i.e., amphetamine, cocaine, methamphetamine).[18] Fifth, the pre-

---

lower rate of relapse to opiate addiction than other treatment modalities (cf. Bale, Van Stone, Kuldau, Engelsing, Elashoff, & Zarcone, 1980; Cushman, 1981; Cushman & Dole, 1973; Des Jarlais et al., 1981; Martin, 1977; Sells, Demaree, Simpson, & Joe, 1978). Hence, the relief of withdrawal distress does not seem to result in a significantly lower recidivism rate.

[18] Withdrawal syndromes are associated with several nonaddictive drugs which are therapeutically administered for the treatment of such problems as hypertension. In particular, clonidine withdrawal has been extensively documented and studied in both animals and humans (see Thoolen, Timmermans, & van Zwieten, 1981). Chlorpromazine withdrawal also produces autonomic signs similar, at least in some respects, to opiate withdrawal, but no abuse has been reported of this drug (see Jaffe, 1975). The mixed agonist-antagonist opioids nalorphine and cyclazocine both produce physical dependence, but their withdrawal is not accompanied by drug-seeking behavior (Martin et al., 1965; Martin & Gorodetzky, 1965). Addiction to psychomotor stimulants has traditionally been viewed as independent of physical dependence (Tatum & Seevers, 1929, 1931; see also Jaffe, 1975; Petersen & Stillman, 1977) and the self-administration of these drugs by laboratory animals is not accompanied by the development of physical dependence (Deneau et al., 1969; Pickens & Thompson, 1968).

sent studies show that (i) the opiate receptor population mediating the physical dependence-producing effects of morphine are neuroanatomically dissociable from its site of rewarding action and (ii) the rewarding properties of heroin are demonstrable at a time when opportunity evolved for the subjects to have developed an association between the drug injection and relief from withdrawal distress.

The fact that opiates can serve as powerful positive reinforcers and that a state of physical dependence is not necessary for this rewarding property to occur does not mean that the tendency to use the drug is independent of personality or psychosocial factors nor does it mean that physical dependence is irrelevant to the strength of the drug taking habit. Indeed, it would seem likely that both positive and negative reinforcing effects are important in governing the behavior of opiate addicts: the opiate addict is probably under the control of the drug for a combination of its positive and negative reinforcing actions. The reported dissociation does, however, leave open the possibility that much of the rewarding impact of opiates is due to an action on the neural substrate involved in psychomotor stimulant and natural rewards (see Wise & Bozarth, 1981).

CHAPTER 8

THEORETICAL PROSPECTUS

8.1 Introduction

The development of theoretical models can serve several useful functions in science. Two of the more important are (i) its value for the integration of existing data and (ii) its heuristic value which can serve an almost paradigmatic function by separating some of the more interesting experimental hypotheses from the countless number that could be experimentally evaluated. This chapter explores some of the more theoretical aspects of the study of the neural basis of opiate reward. It is provided as a prospectus for an emerging theory integrating drug addiction with appetitive motivation and natural rewards.

The rewarding properties of both opiates and psychomotor stimulants appear to derive from their actions of brain reward mechanisms associated with positive reinforcement and appetitive motivation. These two markedly different classes of drugs produce an elevation in mood and a strong psychological dependence on their continued use. The degree that they share similar actions on behavior can be appreciated by examining the rapidity with

which addiction to opiates and amphetamines can develop and by observing the prepotent control these agents exert on behavior.

The dependence of psychomotor stimulant reward on a dopaminergic substrate is well established (for reviews, see Fibiger, 1978; Wise, 1978a, 1980, 1982). Since much more is known about the mechanism of action of psychomotor stimulants, this section will begin with a brief overview of the neural basis of psychomotor stimulant reward.

8.2 Psychomotor Stimulant Reward

Psychomotor stimulants have well documented effects on catecholamine systems. Most of their behavioral effects have been related to an action on dopaminergic rather than noradrenergic systems (see Breese, Hollister, & Cooper, 1976; Cole, 1978; Creese & Iversen, 1975; Thornburg & Moore, 1975). Amphetamine has been shown (1) to increase the release of dopamine from the presynaptic terminals, (2) to block the reuptake of dopamine, and (3) to inhibit the degradation of dopamine by monoamine oxidase; the stimulatory action of cocaine appears to result from cocaine's ability to block the reuptake of dopamine (see Cooper, Bloom, & Roth, 1978; Lewander, 1977; Wise, 1978a). Thus both of these psychomotor stimulants have neurochemical actions that suggest an enhancement of brain dopamine function.

Yokel and Wise (1975, 1976) have shown that neuro-

leptic challenge[1] of intravenous psychomotor stimulant self-administration can produce one of two patterns of responding depending on the dose of neuroleptic administered: low doses produce a dose-dependent increase in drug intake while higher doses result in an extinction pattern of responding where subjects initially emit a high rate of lever pressing (i.e., extinction burst) followed by a cessation in drug intake. The accelerated drug intake seen with lower doses of a neuroleptic parallels the increased drug intake seen when the amount of drug delivered per infusion is reduced in this paradigm. Similarly, the extinction pattern of responding seen when the subjects are challenged with higher doses of a neuroleptic parallels the behavioral response to nonreward (e.g., saline substitution) observed in the intravenous self-administration paradigm. Thus neuroleptics seem to effectively attenuate the rewarding impact of psychomotor stimulant reward, and they do so in a manner which suggests that they are acting as competitive antagonists in this preparation.[2]

---

[1]The neuroleptics used in these studies are selective blockers of dopamine receptors and have little effect on other catecholaminergic systems (e.g., pimozide; see Janssen, Niemegeers, Schellekens, Dresse, Lenaerts, Pinchard, Schaper, Van Nueten, & Verbruggen, 1968; Seeman, 1980).

[2]Yokel and Wise (1975, 1976) have shown that high doses of noradrenergic blocking agents can produce a simple decrease in the rate of responding for psychomotor stimulants, and they suggested that this pattern of

Direct-acting dopamine agonists are also self administered (e.g., apomorphine, piribedil; Roberts, Corcoran, & Fibiger, 1977; Yokel & Wise, 1978). These drugs directly activate postsynaptic dopamine receptors and have little affect on other neurotransmitter systems (Di Chiara & Gessa, 1978). The intravenous self administration of apomorphine is blocked by neuroleptic treatment, but the response pattern seen in this case is much different from that seen with amphetamine or cocaine self-administration. Animals administered a neuroleptic show a simple decrease or cessation in apomorphine self administration; neither compensatory increases in drug intake nor extinction patterns of responding are seen with neuroleptic challenge (Yokel & Wise, 1978). Nonetheless, the selective activation of dopamine receptors by apomorphine suggests that decreases in drug intake following neuroleptic challenge results from an attenuation of apomorphine reward produced by dopamine-receptor blockade.

Lesions studies have shown that the rewarding properties of psychomotor stimulants are dependent on an action in the dopamine terminal field in the nucleus accumbens (Lyness, Friedle, & Moore, 1979; Roberts et al., 1977; Roberts, Koob, Klonoff, & Fibiger, 1980). Lesions that selectively deplete dopamine in the nucleus accumbens

---

responding reflects motor sedation. Thus the importance of increased response rates to rule out simple motor sedation has been argued.

produce a simple decrease in response rates similar to the effect of neuroleptic challenge of apomorphine self administration.[3]

Further evidence that the nucleus accumbens is important in psychomotor stimulant reward comes from the demonstration that amphetamine is self-administered directly into this brain region (Monaco, Hernandez, & Hoebel, 1981). Phillips, Mora, & Rolls (1981) have reported that amphetamine is also self-administered into the dopamine terminal field in the frontal cortex. These intracranial self-administration studies suggest that amphetamine action at either dopamine terminal field (i.e., nucleus accumbens or frontal cortex) is sufficient for reward; lesion challenges of intravenous amphetamine self administration suggest that amphetamine action in these terminal fields is also necessary for reward. It should be noted that lesions of the nucleus accumbens may also produce depletions of dopamine in the frontal cortex so the effect of lesioning could be related to frontal cortical as well as nucleus accumbens dopamine terminal fields

---

[3]Roberts et al. (1980) report that about 35% of their lesioned animals show an extinction-like pattern of responding. The failure to observe extinction-like patterns or compensatory increases in drug intake in most animals suggests that the effects of nucleus accumbens lesions are more complex than just a simple reduction in the rewarding impact of the drug injections (see Lyness et al., 1979; Roberts, et al., 1977, 1980). The results of saline-substitution and neuroleptic challenge studies clearly show that nonreward usually produces an extinction burst followed by a cessation in responding for drug.

(see Roberts et al., 1980). A derivative of this possibility is that the massive depletion of dopamine in the nucleus accumbens is responsible for the reduced drug intake, but the low level of self-administration that remains after such lesions results from incomplete depletion of frontal cortical dopamine terminals.

Although the relative importance of each of these terminal fields in psychomotor stimulant reward has not been established, there is agreement regarding the dopaminergic basis of this behavior. Psychomotor stimulants have known actions on dopaminergic function, and neuroleptics attenuate the rewarding impact of psychomotor stimulant self-administration. Direct acting dopaminergic agonists are self-administered and this effect is also blocked by neuroleptic treatment. Lesions that selectively deplete dopamine produce a decrease in psychomotor stimulant self-administration. Furthermore, intracranial self administration studies suggest that psychomotor stimulants are rewarding when delivered to dopamine terminal fields in the nucleus accumbens or frontal cortex.

8.3 Opiate Reward

The actions of opiates on dopamine systems are not as well defined as that of psychomotor stimulants. The evidence reviewed in Chapter 5 suggests that opiates enhance dopaminergic function although many investigators have adopted the opposite position. The neurochemical

evidence is inconclusive but permissive of an enhanced dopaminergic function. The behavioral indices of brain dopamine function consistently point to an opiate-induced enhancement of dopaminergic function; the rotational model provides especially compelling evidence. Thus, although opiates affect many neurotransmitter systems, it is viable that opiates produce their rewarding effects through an action on dopaminergic systems.

The experiments reported in Chapter 3 and 4 show that morphine is rewarding when delivered to the ventral tegmental area. This rewarding action is not apparent from morphine infusions into other opiate receptor fields. Although the intracranial self-administration and conditioned place preference studies (reported in Chapters 3 and 4, respectively) only establish that morphine infusions into the ventral tegmental area are sufficient for reward, the recent demonstration that a narcotic antagonist infused into this region attenuates intravenous opiate reward (Britt & Wise, 1981a) suggests that opiate action in the ventral tegmental area is also necessary for the rewarding effect of opiates. In comparison, the lateral hypothalamic self-administration of morphine reported by M. Olds (1979) is likely to be the result of ventricular diffusion of morphine, since the use of large diameter cannulae appears to be necessary for intracranial self-administration into this region. Furthermore, Britt and Wise (1981c) have

shown that large lesions of the lateral hypothalamus fail to alter intravenous heroin self-administration suggesting that this region is not necessary for reward from systemic opiates. Thus opiate action at the lateral hypothalamic area appears to be neither a necessary nor sufficient condition for opiate reward.

The intravenous self-administration of opiates is attenuated by neuroleptic treatment (Ettenberg, Koob, Pettit, & Bloom, 1981; Glick & Cox, 1975; Hanson & Cimini Venema, 1972; Pozuelo & Kerr, 1972; Smith & Davis, 1973), but the response pattern does not eliminate the possibility that the decrease in drug intake is the result of nonspecific motor sedation. Like the neuroleptic challenge of apomorphine self-administration (Yokel & Wise, 1978) and lesion challenges of cocaine and amphetamine self administration (Lyness et al., 1979; Roberts et al., 1980), neuroleptics produce a simple reduction in opiate intake; accelerated drug intake has been seen only with low doses of a neuroleptic (Smith & Davis, 1973; M. Bozarth, unpublished observations). However, the experiments presented in Chapter 6 show that neuroleptics block the development of conditioned reinforcement and conditioned place preference produced by opiate administration. These studies test animals in the drug-free condition and thus eliminate any influence that neuroleptic-induced motor sedation may have on the assessment of opiate reward. Phillips, Spyraki,

and Fibiger (1982) have recently shown that the place preference produced by morphine injections into the ventral tegmental area is attenuated by neuroleptic treatment in a dose-dependent manner.

Since morphine is rewarding when infused into the ventral tegmental area and because the rewarding effects of systemically delivered opiates are dependent on dopaminergic function, it seems likely that the rewarding impact of opiate administration results from an enhancement of dopaminergic function in one of the terminal fields associated with the mesocephalic dopamine systems[4] (e.g., nucleus accumbens, frontal cortex, caudate nucleus). One of the predictions derived from this hypothesis is that lesions of the critical dopaminergic terminal field(s) should attenuate the rewarding impact of systemically administered opiates. Which dopamine terminal field is involved (or even whether more than one is critically involved) is not specified by this hypothesis. A first approach, however, might be to determine the importance

---

[4]Mesocephalic is used here to collectively refer to the dopamine systems whose cell bodies are located in the ventral tegmental area (A10) and substantia nigra (A9). This system includes what various authors have called the nigrostriatal, mesolimbic, mesocortical, mesencephalic, and mesotelecephalic dopamine systems (Bunney & Aghajanian, 1978; Lindvall & Bjorklund, 1974; Moore & Kelly, 1978; Ungerstedt, 1971a). Moore and Bloom (1978) have suggested that the term mesocortical be used to collectively refer to these systems, but this practice conflicts with another use of this term to designate specific (i.e., cortical) projections of this system (e.g., Bunney & Aghajanian, 1978; Moore & Kelly, 1978).

of dopamine terminal fields shown to be critically involved in psychomotor stimulant reward.

Few studies have assessed the effects of lesioning dopamine terminal fields on the intravenous self administration of opiates. The only published full reports come from one laboratory. Glick, Cox, and Crane (1975) have reported that lesions of the caudate nucleus decrease the self-administration of morphine, but attempts to replicate this finding have been unsuccessful (e.g., M. Britt, unpublished observations; G. Gerber, unpublished observations). Glick and Cox (1978) have also reported that lesions of the nucleus accumbens fail to alter the self-administration of morphine. In an abstract presented by Dworkin, Guerin, Lane, Cherek, and Smith (1982), however, accumbens lesions were reported to be effective in decreasing intravenous morphine self administration (cf. Smith, Co, Crenshaw, Barr, & Lane, 1981). The reason for this discrepant finding is not clear but may be related to the extent of lesioning. Roberts et al. (1980) report that lesions which deplete accumbens dopamine levels by approximately 60% produced only slight changes in psychomotor stimulant intake. Apparently, lesions need to deplete accumbens dopamine by about 90% before they effectively attenuate psychomotor stimulant self-administration.[5] This observation is not

[5]The nucleus accumbens lesions shown to be effective

surprising when one considers the compensatory changes in dopaminergic function that result from the partial destruction of this system (e.g., Creese & Snyder, 1978; Hefti, Melamed, & Wurtman, 1980; Mishra, Marshall, & Varmuza, 1980; Ungerstedt, 1971c). A similar refractoriness has been reported for other behaviors that are believed to be dependent on this dopamine system (e.g., feeding and drinking; Stricker & Zigmond, 1976; Ungerstedt, 1971d).

It would be of considerable interest to compare the effects of nucleus accumbens lesions on the self administration of psychomotor stimulants and opiates in the same preparation ensuring that the lesions were effective in attenuating psychomotor stimulant reward. This would permit a direct assessment of the involvement of the same dopaminergic terminal fields in both psychomotor stimulant and opiate reward. A preliminary report from Pettit, Ettenberg, Bloom, and Koob (1982) suggests that lesions attenuating psychomotor stimulant reward may have no effect on opiate self-administration. Additional testing is required to confirm this important observation; attention should be focused on the possible diminution of the reinforcing efficacy of drug intake after lesioning: some psychomotor stimulant self-administration remains after these lesions (Roberts et al., 1977, 1980), and the

---

by both Lyness et al. (1979) and Roberts et al. (1977) depleted nucleus accumbens dopamine to about 10% of control levels.

differential effects seen with psychomotor stimulant and opiate self-administration may simply reflect differences in the neural mechanisms through which they enhance dopamine function.

8.4 Implications

Both psychomotor stimulant and opiate rewards appear to be dependent on an action on dopaminergic systems, and this finding suggests that they may share a common neural substrate. This notion is further strengthened by the anatomical localization of the sites of action for these two classes of drugs. Psychomotor stimulants enhance dopamine function by an action at the terminal fields of these systems while opiates seem to enhance dopamine function by an action at or near the cell bodies in the ventral tegmentum. Thus both types of drug reward activate the same dopaminergic system but at different regions of this system. Opiates have a pronounced inhibitory action on GABAergic pathways (which are generally inhibitory; see Nicoll, Alger & Jahr, 1980) and this raises the possibility that the activation of the dopaminergic systems by opiates may result from disinhibition. If this were the case, then not only are the sites where psychomotor stimulants and opiates act on the mesocephalic dopamine system different, but the mechanisms of action are markedly different as well: psychomotor stimulants produce an enhancement of dopamine function through a direct action of endogenous dopamine

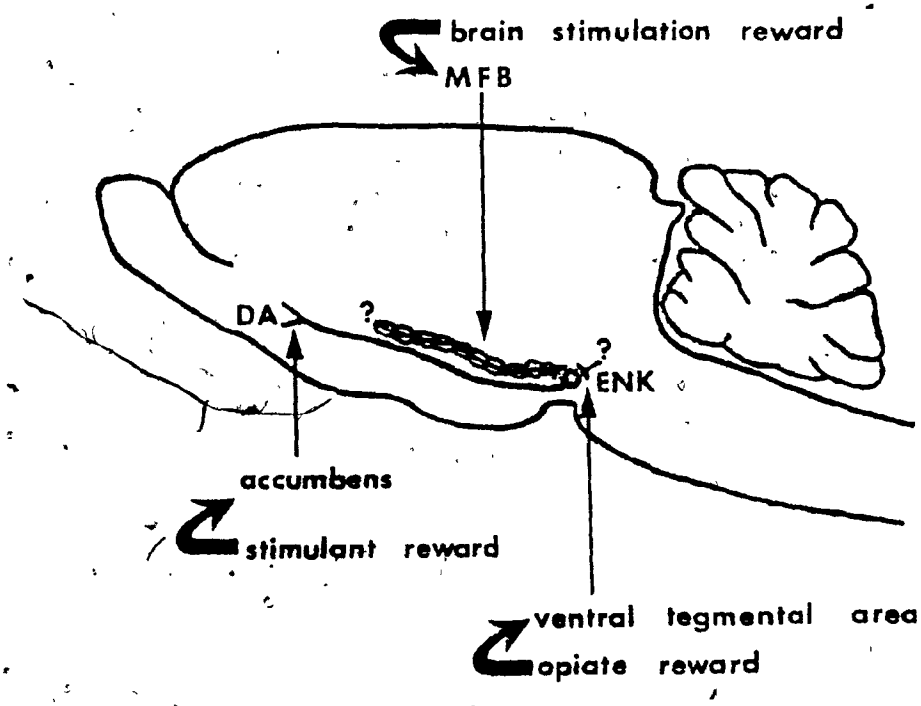

Fig. 8.1. Psychomotor stimulant reward appears to be dependent on a drug action in the nucleus accumbens, although an action in the frontal cortex is also likely. Opiate reward seems to be dependent on an action in the ventral tegmental area. Brain stimulation reward from lateral hypothalamic electrodes (MFB) appears to activate small, myelinated axons projecting to the ventral tegmental area (Shizgal, Bielajew, Corbett, Skelton, & Yeomans, 1980) which may transsynaptically activate the mesocephalic dopamine system (see Gallistel, Shizgal, & Yeomans, 1981; Wise, 1980). All three rewards are attenuated by neuroleptic treatment. Lesions of the ventral tegmental area produce adipsia and aphagia (Ungerstedt, 1971d), and neuroleptics have been shown to attenuate the rewarding impact of food and water (see Wise, 1982). These data suggest that a common dopaminergic system may be involved in the mediation of reward from a variety of sources. This does not preclude the possibility that some rewards (e.g., opiates) have additional reinforcing properties produced by an action outside this system. The ventral tegmental area, however, would seem to be a site where dopaminergic and enkephalinergic (ENK) systems interface to influence appetitive motivational processes.

while opiates produce an enhancement of dopaminergic function through a disinhibitory action at the dopamine cell bodies of this system.

Since psychomotor stimulants are thought to enhance dopaminergic function at all dopamine terminal fields whereas opiates have been shown to enhance dopaminergic function of only the mesocephalic dopamine systems, there may be differences in the anatomical distribution of the systems underlying psychomotor stimulant and opiate reward. For this reason, the present model proposes only that psychomotor stimulant and opiate rewards have partially overlapping neural substrates. The terminal projections which are necessary for this rewarding action are probably located in either the nucleus accumbens or the frontal cortex. It is possible that psychomotor stimulant reward is produced by an action in both fields while opiate reward relies on the enhancement of dopaminergic function in only one. This would be consistent with the preliminary neurochemical data suggesting that morphine may enhance dopaminergic function in the nucleus accumbens without affecting that in the frontal cortex (see Section 5.2.3).

The mesocephalic dopamine systems have also been strongly implicated in the regulation of food and water intake (Stricker & Zigmond, 1976; Teitelbaum & Epstein, 1962; Ungerstedt, 1971d). Furthermore, the reinforcing

action of brain stimulation reward, at least from some electrode placements, seems to be dependent on these same dopamine systems (Fibiger & Phillips, 1979; Wise, 1978a, 1982). Thus it seems likely that psychomotor stimulants and opiates derive their rewarding effects by pharmacologically activating the neural system involved in natural rewards. This hypothesis would suggest that these drug rewards are another instance of appetitive motivation and argues against a specialized mechanism to account for drug addiction.

The finding that morphine reward is dependent on an action in the ventral tegmental area suggests a neuroanatomical separation of the rewarding, analgesic, and physical dependence-producing effects of opiates. Analgesia has been shown to result from the direct application of morphine into the periventricular gray region (Pert & Yaksh, 1974; Sharpe, Garnett, & Cicero, 1974; Thorn-Gray, Levitt, Hill, & Ward, 1981; Yaksh, Yeung, & Rudy, 1976), although there are also spinal mechanisms that can produce morphine analgesia (Johnson & Duggan, 1981; Yaksh & Rudy, 1976, 1977). The physical dependence-producing properties of morphine also seem to result from an action at the periventricular gray region (see Chapter 7) as do the motor sedative properties of opiates (Broekkamp, 1976; Broekkamp, Van Den Bogaard, Heynen, Rops, Cools, & Van Rossum, 1976; Pert, 1978; Pert, DeWald, Liao, & Sivit, 1979; Thorn-Gray et al., 1981,

Wilcox & Levitt, 1978). The thermoregulatory effects of opiates are attributed to an action in the preoptic area of the lateral hypothalamus (Lotti, Lomax, & George, 1965; Teasdale, Bozarth, & Stewart, 1981) while hyperactivity

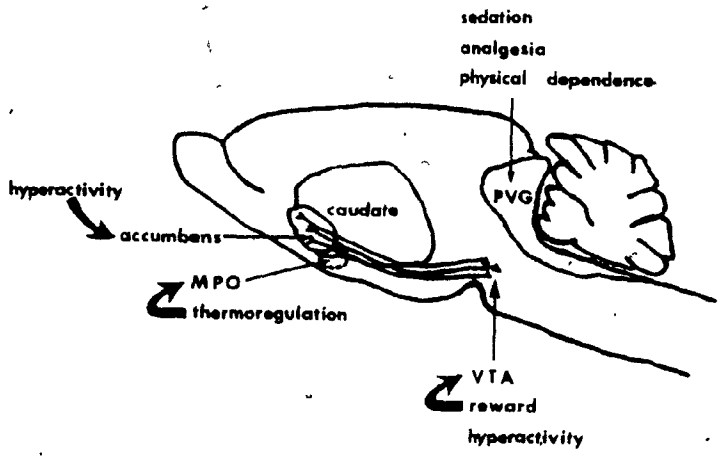

Fig. 8.2. Several of the effects of opiates can be neuroanatomically separated. Analgesia, sedation, and physical dependence seem to result from an action in the periventricular gray region (PVG). The medial preoptic area (MPO) appears to be the site of action for the thermoregulatory effects of opiates. Hyperactivity has been reported from morphine infusions into the nucleus accumbens and ventral tegmental area (VTA). The localization of the reward-relevant opiate receptors in the ventral tegmentum suggests that the acute rewarding-properties of opiates are independent of most other opiate induced effects. Opiate reinforcement seen after chronic drug intake might also result from an action in the periventricular gray region which is associated with the relief of withdrawal discomfort. The ventral tegmental action, however, is likely to still be operative and contribute significantly to the reinforcing effects of opiates.

is produced by morphine infusions into either the nucleus accumbens (Pert et al., 1979; Pert & Sivit, 1977) or ventral tegmental area (Joyce & Iversen, 1979; Kelley, Stinus, & Iversen, 1980).

The neuroanatomical separation of morphine's rewarding effect from its other actions has obvious implications for theories of opiate addiction. It appears that opiate reward can be produced by a drug action that is independent from analgesia and physical dependence. Thus physical dependence may be relatively unimportant in the development of opiate addiction. Alternatively, the reinforcing properties of opiate intake in the addict may be produced by a drug action at each of two different systems: one motivational effect may be related to the positive reinforcing properties of opiate action in the ventral tegmental area, and another motivational effect may be produced by the avoidance or termination of withdrawal discomfort resulting from a drug action in the periventricular gray region. This is likely to be the case for chronic opiate intake involving the frequent administration of large quantities of opiates. In this sense, the opiate addict may be simultaneously influenced by both positive and negative reinforcement processes and his behavior may be governed by some combination of appetitive and aversive motivation. The relative importance of these two sources of motivation has not been directly assessed in laboratory

animals, but some reports in the clinical literature suggest that the ability of opiates to relieve withdrawal discomfort may be relatively unimportant in drug addiction (see Chapter 7). Nonetheless, both appetitive and aversive motivations would seem to be operating concurrently in the opiate addict, and the resulting behavior is likely to be the product of this joint action even if appetitive motivation were more salient in this situation.

If addiction to opiates were the result of a pharmacological activation of the neural mechanism involved in appetitive motivation, it is apparent that an effective treatment of this addiction would be difficult to develop. Direct pharmacological activation of this reward substrate would be expected to produce a prepotent rewarding action, and natural rewards might have relatively little efficacy in controlling the addict's behavior. In contrast, if the motivational properties of opiate addiction were derived from a drug action on the neural mechanisms involved in aversive motivation, psychotherapeutic interventions should be effective. In this case, treatments that lessen the impact of aversive stimuli (e.g., the development of more effective coping mechanisms, restructuring of familiar environment) should produce an appreciable decrease in recidivism rates, and natural rewards (e.g., family bonds, social praise and support) should be capable of significantly affecting behavior. Unfortunately, most psychothera-

peutic interventions are relatively ineffective, and the only treatments that have consistently yielded remission rates that reflect a significant improvement in the typically dismal prognosis are pharamcological (i.e., methadone or narcotic antagonist treatment programs). Again, this outcome would be predicted from a hypothesis asserting an appetitive motivational basis of drug addiction.

Three observations combine to support the notion that opiate addiction may be a useful tool for the study of the neural substrate of basic motivational processes. First, opiates are potent agonists for endorphin receptors and endorphins have been implicated in several aspects of motivated behavior. Second, the current evidence favors a hypothesis suggesting that opiate reward is dependent on a drug action at a specialized neural system; this system is probably involved in the reinforcing action of natural rewards as well. Finally, opiates have profound effects on behavior, blunting the rewarding impact of natural reinforcers while reinforcing behaviors associated with their own ingestion. Thus the study of natural rewards can be seen to contribute to the understanding of drug addiction, and the study of drug addiction can be seen to contribute to the understanding of basic motivational processes.

REFERENCES

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

Adler, M. W. Opioid peptides. Life Sciences, 1980, 26, 497-510.

Adler, M. W., & Geller, E. B. Factors to be considered in using brain lesions to study the central sites of action of narcotics. In M. L. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

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

Aghajanian, G. K. Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. Nature, 1978, 276, 186-188.

Aghajanian, G. K. Regulation of central noradrenergic cell firing: Role of α_2-adrenoceptors and opiate receptors. In L. Starne, P. Hedqvist, H. Lagercrantz, and A. Wennmalm (Eds.), Chemical Neurotransmission: 75 Years. London: Academic Press, 1981.

Akil, H., Watson, S. J., Holman, R. B., & Barchas, J. D. Parallels between the neuromodulator mechanisms of stimulation analgesia and morphine analgesia. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

Akiyama, K., Sato, M., & Otsuki, S. Increased ^{3}H-spiperone binding sites in mesolimbic area related to methamphetamine-induced behavioral hypersensitivity. Biological Psychiatry, 1982, 17, 223-231.

Albert, D. J., & Madryga, F. J. An examination of the functionally effective spread of 4 µl of slowly infused lidocaine. Behavioral and Neural Biology, 1980, 29, 378-384.

Albus, K., Schott, M., & Herz, A. Interaction between morphine and morphine antagonists after systemic and intraventricular application. European Journal of Pharmacology, 1970, 12, 53-64.

Alexander, B. K., Beyerstein, B. L., Hadaway, P. F., & Coambs, R. B. Effect of early and later colony housing on oral ingestion of morphine in rats. Pharmacology Biochemistry & Behavior, 1981, 15, 571-576.

Alexander, B. K., Coambs, R. B., & Hadaway, P. F. The effect of housing and gender on morphine self administration in rats. Psychopharmacology, 1978, 58, 175-179.

Alper, R. H., Demarest, K. T., & Moore, K. E. Morphine differentially alters synthesis and turnover of dopamine in central neuronal systems. Journal of Neural Transmission, 1980, 48, 157-165.

Altshuler, H. L. The use of self-administration for assessing the action of the opiates. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Actions of Narcotics. New York: Raven Press, 1978.

Amir, S., Blair, R., Shizgal, P., & Amit, Z. Dual mechanisms mediating opiate effects? Science, 1979, 205, 424-425.

Amir, S., Brown, Z. W., & Amit, Z. The role of endorphins in stress: Evidence and speculations. Neuroscience and Biobehavioral Reviews, 1980, 4, 77-86.

Amir, S., Galina, Z. H., Blair, R., Brown, Z. W., & Amit, Z. Opiate receptors may mediate the suppressive but not the excitatory action of ACTH on motor activity in rats. European Journal of Pharmacology, 1980, 66, 307-313.

Amit, Z., Corcoran, M. E., Amir, S., & Urca, G. Ventral hypothalamic lesions block the consumption of morphine in rats. Life Sciences, 1973, 13, 805-816.

Andén, N. E., Dahlstrom, A., Fuxe, K., & Larsson, K. Functional role of the nigro-striatal dopamine neurons. Acta Pharmacologica et Toxicologica, 1966, 24, 263-274.

Andén, N. E., & Grabowska-Andén, M. Morphine-induced changes in striatal dopamine mechanisms not evoked from the dopamine nerve terminals. Journal of Pharmacy and Pharmacology, 1978, 30, 732-734.

Arbuthnott, G. W., & Ungerstedt, U. Turning behavior induced by electrical stimulation of the nigro neostriatal system of the rat. Experimental Neurology, 1975, 47, 162-172.

Arnt, J., & Scheel-Kruger, J. GABA in the ventral tegmental area: Differential regional effects on locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. Life Sciences, 1979, 25, 1351-1360.

Atalay, J., Bozarth, M. A., & Wise, R. A. Task-independent reward and performance shifts from pimozide. Paper presented at The 43rd Annual Meeting of the Canadian Psychological Association, Montréal, June, 1982.

Atweh, S. F., & Kuhar, M. J. Autoradiographic localization of opiate receptors in rat brain--II. The brain stem. Brain Research, 1977a, 129, 1-12.

Atweh, S. F., & Kuhar, M. J. Autoradiographic localization of opiate receptors in rat brain--III. The telencephalon. Brain Research, 1977b, 134, 393-405.

Ayhan, I. H., & Randrup, A. Role of brain noradrenaline in morphine-induced stereotyped behavior. Psychopharmacologia, 1972, 27, 203-212.

Ayhan, I. H., & Randrup, A. Behavioral and pharmacological studies on morphine-induced excitation of rats: Possible relation to brain catecholamines. Psychopharmacologia, 1973, 29, 317-328.

Babbini, M., & Davis, W. M. Time-dose relationships for locomotor activity effects of morphine after acute or repeated treatment. British Journal of Pharmacology, 1972, 46, 213-224.

Babbini, M., Gaiardi, M., & Bartoletti, M. Motility effects of methamphetamine in rats chronically treated with morphine. Neuropharmacology, 1978, 17, 979-983.

Bailey, R. C., & Jackson, D. M. A pharmacological study of changes in central nervous system receptor responsiveness after long-term dexamphetamine and apomorphine administration. Psychopharmacology, 1978, 56, 317-326.

Baldino, F., Beckman, A. L., & Adler, M. W. Actions of iontophoretically applied morphine on hypothalamic thermosensitive units. Brain Research, 1980, 196, 199-208.

Bale, R. N., Van Stone, W. W., Kuldau, J. M., Engelsing, T. M. J., Elashoff, R. M., & Zarcone, V. P. Therapeutic communities vs methadone maintenance. Archives of General Psychiatry, 1980, 37, 179-193.

Bannon, M. J., Bunney, E. B., & Roth, R. H. Mesocortical dopamine neurons: rapid transmitter turnover compared to other brain catecholamine systems. Brain Research, 1981, 218, 376-382.

Barchas, J. D., Akil, H., Elliott, G. R., Holman, R. B., & Watson, S. J. Behavioral neurochemistry: Neuroregulators and behavioral states. Science, 1978, 200, 964-973.

Barghon, R., & Costentin, J. H. Rotational behavior induced by unilateral electrical stimulations of nigro-striatal dopamine neurons: Modification by low doses of apomorphine. European Journal of Pharmacology, 1980, 64, 39-46.

Beckett, A. H. Stereochemical factors in biological activity. Progress in Drug Research, 1959, 1, 455-530.

Beecher, H. K. The use of chemical agents on the control of pain. In R. S. Knighton and P. R. Dunke (Eds.), Pain. Boston: Little, Brown, 1966.

Belluzzi, J. D., & Stein, L. Enkephalin may mediate euphoria and drive-reduction reward. Nature, 1977, 266, 556-558.

Berger, P. A., Watson, S. J., Akil, H., Elliott, G. R., Rubin, R. T., Pfefferbaum, A., Davis, K. L., Barchas, J. D., & Li, C. H. β-endorphin and schizophrenia. Archives of General Psychiatry, 1980, 37, 635-640.

Bernardi, G., Marciani, M. G., Morocutti, C., Pavone, F., & Stanzione, P. The action of dopmaine on rat caudate neurones intracellularly recorded. Neuroscience Letters, 1978, 8, 235-240.

Bhargava, H. N., & Way, E. L. Acetylcholinesterase inhibition and morphine effects in morphine tolerant and dependent mice. The Journal of Pharmacology and Experimental Therapeutics, 1972, 183, 31-40.

Bindra, D. A unified account of classical conditioning and operant training. In A. H. Black and W. F. Prokasy (Eds.), Classical Conditioning II: Current Theory and Research. New York: Appleton-Century Crofts, 1972.

Bindra, D. A motivational view of learning, performance, and behavior modification. Psychological Review, 1974, 81, 199-213.

Bindra, D. A Theory of Intelligent Behavior. New York: John Wiley & Sons, 1976.

Bindra, D., & Palfai, T. Nature of positive and negative incentive-motivational effects on general activity. Journal of Comparative and Physiological Psychology, 1967, 63, 288-297.

Blair, R., Cytryniak, H., Shizgal, P., & Amit, Z. Heroin, but not levorphanol, produces explosive motor behavior in naloxone-treated rats. Psychopharmacology, 1980, 69, 313-314.

Blair, R., Liran, U., Cytryniak, H., Shizgal, P., & Amit, Z. Explosive motor behavior, rigidity and periaqueductal gray lesions. Neuropharmacology, 1978, 17, 205-209.

Blake, P. H., & Halpern, L. M. The effects of morphine on intracranial self-stimulation behavior in rats. Pharmacologist, 1971, 13, 501.

Blasig, J., Herz, A., Reinhold, K., & Zieglgansberger, S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated withdrawal syndrome in rats. Psychopharmacologia, 1973, 33, 19-38.

Blasig, J., Hollt, V., Herz, A., & Paschelke, G. Comparison of withdrawal precipitating properties of various morphine antagonists and partial agonists in relation to their stereospecific binding to brain homogenates. Psychopharmacologia, 1976, 46, 41-51.

Bloom, F. E. The role of cyclic nucleotides in central synaptic function. Review of Physiology and Biochemical Pharmacology, 1975, 74, 1-103.

Bondareff, W., Routtenberg, A., Narotzky, R., & McLone, D. G. Intrastriatal spreading of biogenic amines. Experimental Neurology, 1970, 28, 213-229.

Blumberg, H., & Ikeda, C. Naltrexone, morphine and cocaine interactions in mice and rats. Journal of Pharmacology and Experimental Therapeutics, 1978, 206, 303-310.

Blundell, C., Crossman, A. R., & Slater, P. The effect of morphine on turning behavior in rats and mice with unilateral 6-hydroxydopamine lesions. British Journal of Pharmacology, 1976, 57, 456.

Bolles, R. C. Theory of Motivation. New York: Harper & Row, 1975.

Bolles, R. C., & Fanselow, M. S. Endorphins and behavior. Annual Review of Psychology, 1982, 33, 87-101.

Bozarth, M. A. Intracranial self-stimulation as an index of opioid addiction liability: An evaluation. Unpublished Master's Thesis, Rensselaer Polytechnic Institute, 1978.

Bozarth, M. A. Opiate reward mechanisms mapped by intracranial self-administration. In J. E. Smith and J. D. Lane (Eds.), The Neurobiology of Opiate Processes. Amsterdam: Elsevier Biomedical Press, 1983 (in press).

Bozarth, M. A., Gerber, G. J., & Wise, R. A. Intracranial self-stimulation as a technique to study the reward properties of drugs of abuse. Pharmacology Biochemistry & Behavior, 1980, 13, Suppl. 1, 245-247.

Bozarth, M. A., & Reid, L. D. Addictive agents and intracranial stimulation (ICS): Naloxone blocks morphine's acceleration of pressing for ICS. Bulletin of the Psychonomic Society, 1977, 10, 478-480.

Bozarth, M. A., & Wise, R. A. Electrolytic microinfusion transducer system: An alternative method of intracranial drug application. Journal of Neuroscience Methods, 1980a, 2, 273-275.

Bozarth, M. A., & Wise, R. A. Intracranial self administration of morphine into the ventral tegmental area. Paper presented at The 88th Annual Meeting of the American Psychological Association, Montréal, September, 1980b.

Bozarth, M. A., & Wise, R. A. Intracranial self administration of morphine into various brain regions. Society for Neuroscience Abstracts, 1980c, 6, 309.

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

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

Bozarth, M. A., & Wise, R. A. Dopaminergic mediation of opiate reward. Society for Neuroscience Abstracts, 1981c, 7, 50.

Bozarth, M. A., & Wise, R. A. Localization of the reward relevant opiate receptors. In L. S. Harris (Ed.), Problems of Drug Dependence, 1981. Rockville, Maryland: National Institute on Drug Abuse, 1982.

Brady, L. S., & Holtzman, S. G. Locomotor activity in morphine-dependent and post-dependent rats. Pharmacology Biochemistry & Behavior, 1981, 14, 361-370.

Brandt, M., Traber, J., Glaser, T., & Hamprecht, B. Opioid action on cultured neuroblastoma x glioma hybrid cells. In M. Adolphe (Ed.), Advances in Pharmacology and Therapeutics, Vol. 10: Chemotherapy. New York: Pergamon Press, 1978.

Breese, G. R., Hollister, A. S., & Cooper, B. R. Role of monoamine neural pathways in d-amphetamine- and methylphenidate-induced locomotor activity. In E. H. Ellinwood and M. M. Kilbey (Eds.), Cocaine and Other Stimulants. New York: Plenum Press, 1976.

Breese, G. R., & Traylor, T. D. Effect of 6-hydroxydopamine on brain norepinephrine and dopmaine: Evidence for selective degeneration of catecholamine neurons. The Journal of Pharmacology and Experimental Therapeutics, 1970, 174, 413-420.

Breese, G. R., & Traylor, T. D. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. British Journal of Pharmacology, 1971, 42, 88-99.

Briggs, C. A., & McAfee, D. A. Proving a role for cyclic AMP in synaptic transmission. Trends in Pharmacological Sciences, 1982, 3, 241-244.

Britt, M. D., & Wise, R. A. Attenuation of intravenous heroin reward by ventral tegmental opiate receptor blockade. Society for Neuroscience Abstracts, 1981a, 7, 607.

Britt, M. D., & Wise, R. A. Kainic acid spares fibers of the dorsal noradrenergic bundle. Brain Research Bulletin, 1981b, 7, 437-440.

Britt, M. D., & Wise, R. A. Opiate rewarding action: independence of the cells of the lateral hypothalamus. Brain Research, 1981c, 222, 213-217.

Broekkamp, C. L. E. The Modulation of Rewarding Systems in the Animal Brain by Amphetamine, Morphine, and Apomorphine. Druk: Stichting Studentenpers Nijmgen, 1976.

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

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

Bunney, B. S. The electrophysiological pharmacology of midbrain dopaminergic systems. In A. S. Horn, J. Korf, and B. H. C. Westerink (Eds.), The Neurobiology of Dopamine. New York: Academic Press, 1979.

Bunney, B. S., & Aghajanian, G. K. d-Amphetamine-induced inhibition of central dopaminergic neurons: Mediation by a striato-nigral feedback pathway. Science, 1976, 192, 391-393.

Bunney, B. S., & Aghajanian, G. K. Mesolimbic and mesocortical dopaminergic systems: Physiology and pharmacology. In M. A. Lipton, A. Di Mascio, and K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Bush, H. D., Bush, M. F., Miller, M. A., & Reid, L. D. Addictive agents and intracranial self-stimulation. Physiological Psychology, 1976, 4, 79-85.

Calvino, B., Lagowska, J., & Ben-Ari, Y. Morphine withdrawal syndrome: Differential participation of structures located within the amygdaloid complex and striatum of the rat. Brain Research, 1979, 177, 19-34.

Campbell, D. T., & Stanley, J. C. Experimental and Quasi-Experimental Designs for Research. Chicago: Rand McNally College Publishing Company, 1966.

Cannon, W. B., & Rosenblueth, A. Autonomic Neuro-effector Systems. New York: MacMillan, 1937.

Carenzi, A. Role of the cyclic nucleotide system in opiate actions. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

Carenzi, A., Frigeni, V., & Bella, D. D. Synaptic localization of opiate receptors in rat striatum. In E. Costa and M. Trabucchi (Eds.), Advances in Biomedical Psychopharmacology, Vol. 18. New York: Raven Press, 1978.

Carlson, K. R., & Almasi, J. Behavioral supersensitivity to apomorphine following chronic narcotic treatment in the guinea pig. Psychopharmacology, 1978, 57, 273-277.

Carlson, K. R., & Seeger, T. F. Interaction of opiates with dopamine receptors: Receptor binding and behavioral assays. Pharmacology Biochemistry & Behavior, 1982, 16, 119-124.

Carlsson, A. Does dopamine have a role in schizophrenia? Biological Psychiatry, 1978, 13, 3-21.

Carlsson, A., & Lindqvist, M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharmacologica et Toxicologica, 1963, 20, 140-144.

Carroll, B. J., & Sharp, P. T. Monoamine mediation of the morphine-induced activation of mice. British Journal of Pharmacology, 1972, 46, 124-139.

Carroll, M. E., & Meisch, R. A. Etonitazene as a reinforcer: Oral intake of etonitazene by rhesus monkeys. Psychopharmacology, 1978, 59, 225-229.

Caruso, T. P., Larson, D. L., Portoghese, P. S., & Takemori, A. E. Pharmacological studies with an alkylating narcotic agonist, chloroxymorphamine, and antagonist, chlornaltrexamine. The Journal of Pharmacology and Experimental Therapeutics, 1980, 213, 539-544.

Cervo, L., Rochat, C., Romandini, S., & Samanin, R. Evidence of a preferential role of brain serotonin in the mechanisms leading to naloxone-precipitated compulsive jumping in morphine-dependent rats. Psychopharmacology, 1981, 74, 271-274.

Chan, S. H. H. Central neurotransmitter systems in the morphine suppression of jaw-opening reflex in rabbits: The dopaminergic system. Experimental Neurology, 1979, 65, 526-534.

Charness, M. E., Amit, Z., & Taylor, M. Morphine induced stereotypic behavior in rats. Behavioral Biology, 1975, 13, 71-80.

Cheramy, A., Leviel, V., & Glowinski, J. Dendritic release of dopamine in the substantia nigra. Nature, 1981, 289, 537-542.

Chesselet, M. F., Cheramy, A., Reisine, T. D., & Glowinski, J. Morphine and δ-opiate agonists locally stimulate in vivo dopamine release in cat caudate nucleus. Nature, 1981, 291, 320-322.

Christensen, A. V., & Nielsen, I. M. Dopaminergic supersensitivity: Influence of dopamine agonists, cholinergics, anticholinergics, and drugs used for the treatment of tardive dyskinesia. Psychopharmacology, 1979, 62, 111-116.

Clark, R. G. Manter and Gatz's Essentials of Clinical Neuroanatomy and Neurophysiology. Philadelphia: F. A. Davis, 1975.

Clouet, D. H. Possible roles of catecholamines in the action of narcotic drugs. In A. J. Friedhoff (Ed.), Catecholamines and Behavior, Vol. 2: Neuropsychopharmacology. New York: Plenum Press, 1975.

Clouet, D. H., Johnson, J. C., Ratner, M., Williams, N., & Gold, G. J. The effect of morphine on rat brain catecholamines: Turnover in vivo and uptake in isolated synaptosomes. In E. Usdin and S. H. Snyder (Eds.), Frontiers in Catecholamine Research. London: Pergamon Press, 1973.

Clouet, D. H., & Ratner, M. Catecholamine biosynthesis in brains of rats treated with morphine. Science, 1970, 168, 854-855.

Cole, S. O., Brain mechanisms of amphetamine-induced anorexia, locomotion, and stereotypy: A review. Neuroscience and Biobehavioral Reviews, 1978, 2, 89-100.

Collaer, M., Magnuson, D., & Reid, L. Addictive agents and intracranial stimulation (ICS): Pressing for ICS before and after self-administration of sweetened morphine solutions. Physiological Psychology, 1977, 5, 425-428.

Collier, H. O. J. Consequences of interaction between opioid molecule and specific receptor. In R. F. Beers and E. G. Bassett (Eds.), Mechanisms of Pain and Analgesic Compounds. New York: Raven Press, 1979.

Collier, H. O. J. Cellular site of opiate dependence. Nature, 1980, 283, 625-629.

Collu, R., Taché, Y., & Charpenet, G. Role of biogenic amines in opioid peptide-induced secretion of pituitary hormones. Pharmacological Research Communications, 1980, 12, 403-409.

Colpaert, F. C. Discriminative stimulus properties of narcotic analgesic drugs. Pharmacology Biochemistry & Behavior, 1978, 9, 863-887.

Colpaert, F. C., Lal, H., Niemegeers, C. J. E., & Janssen, P. A. J. Investigations on drug produced and subjectively experienced discriminative stimuli. I. The fentanyl cue, a tool to investigate subjectively experienced narcotic drug actions. Life Sciences, 1975, 16, 705-716.

Colpaert, F. C., Niemegeers, C. J. E., & Janssen, P. A. J. Effects of α-methyl-p-tyrosine, adrenolytic compounds, pimozide and other neuroleptics, on the narcotic cue. Archives of Internationales de Pharmacodynamie et de Thérapie, 1977, 225, 308-316.

Committee on Problems of Drug Dependence. United States procedures for screening drugs: Testing for dependence liability in animals and man. Bulletin on Narcotics, 1970, 12, 11-17.

Contreras, G., Contreras, E., Gonzalez, C., & Concha, J. Acute and chronic effects of morphine on transmembrane potential and on short-circuit current in isolated toad skin: Apparent morphine dependence. Neuropharmacology, 1980, 19, 1081-1086.

Conway, P. G., & Uretsky, N. J. Role of striatal dopaminergic receptors in amphetamine-induced behavioral facilitation. The Journal of Pharmacology and Experimental Therapeutics, 1982, 221, 650-655.

Cooper, J. R., Bloom, F. E., & Roth, R. H. The Biochemical Basis of Neuropharmacology. New York: Oxford University Press, 1978.

Corrigall, W. A., & Linseman, M. A. A specific effect of morphine on evoked activity in the rat hippocampal slice. Brain Research, 1980, 192, 227-238.

Costa, E., Carenzi, A., Guidotti, A., & Revuelta, A. Narcotic analgesics and the regulation of neuronal catecholamine stores. In E. Usdin and S. H. Snyder (Eds.), Frontiers in Catecholamine Research. Oxford: Pergamon Press, 1973.

Costa, E., Cheney, D. L., Racagni, G., & Zsilla, G. An analysis at synaptic level of the morphine action in striatum and N. accumbens: Dopamine and acetylcholine interactions. Life Sciences, 1975, 17, 1-8.

Costall, B., Fortune, D. H., Naylor, R. J. Involvement of mesolimbic and extrapyramidal nuclei in the motor depressant action of narcotic drugs. Journal of Pharmacy and Pharmacology, 1978, 30, 566-572.

Costall, B., & Naylor, R. J. Neuroleptic and non neuroleptic catalepsy. Arzneimittel Forschung, 1973, 23, 674-683.

Costall, B., & Naylor, R. J. The importance of the ascending dopaminergic systems to the extra-pyramidal and mesolimbic brain areas for the cataleptic action of the neuroleptic and cholinergic agents. Neuropharmacology, 1974a, 13, 353-364.

Costall, B., & Naylor, R. J. A role for the amygdala in the development of the cataleptic and stereotypic actions of the narcotic agonists and antagonists in the rat. Psychopharmacologia, 1974b, 35, 203-213.

Costall, B., Naylor, R. J., & Olley, J. E. Catalepsy and circling behavior after intracerebral injections of neuroleptic, cholinergic, and anticholinergic agents into the caudate-putamen, globus pallidus, and substantia nigra of rat brain. Neuropharmacology, 1972, 11, 645-663.

Cox, B., Ary, M., & Lomax, P. Changes in sensitivity to apomorphine during morphine dependence and withdrawal in rats. Journal of Pharmacology and Experimental Therapeutics, 1976, 196, 637-641.

Cox, B. M., Opheim, K. E., Teschemacher, H., & Goldstein, A. A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties. Life Sciences, 1975, 16, 1777-1782.

Craviso, G. L., & Musacchio, J. M. Opiate receptor: Irreversible inactivation by an alkylating local anesthetic. Life Sciences, 1976, 18, 821-828.

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

Creese, I., & Snyder, S. H. Behavioral and biochemical properties of the dopamine receptor. In M A. Lipton, A. DiMascio, and K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Criado, M., Aguilar, J. S., & De Robertis, E. Action of detergents and pre- and postsynaptic localization of ^{3}H-Naloxone binding in synaptosomal membranes. A structural approach. Journal of Neurobiology, 1981, 12, 259-267.

Criswell, H. E. A simple chronic microinjection system for use with chemitrodes. Pharmacology Biochemistry & Behavior, 1977, 6, 237-238.

Criswell, H. E., & Levitt, R. A. The narcotic analgesics. In R. A. Levitt (Ed.), Psychopharmacology: A Biological Approach. Washington, D.C.: Hemisphere, 1975.

Cronin, M. J. The role and direct measurement of the dopamine receptor(s) in the anterior pituitary. In E. E. Muller and R. M. MacLeod (Eds.), Neuroendocrine Perspectives Vol. 1. Amsterdam: Elsevier Biomedical Press, 1982.

Crow, T. J., & Deakin, J. F. W. Brain reinforcement centers and psychoactive drugs. In Y. Israel (Ed.), Research Advances in Alcohol and Drug Problems, Vol. 4. New York: Plenum Press, 1978.

Crowder, W. F., Smith, S. G., Davis, W. M., Noel, J. T., & Coussens, W. R. Effect of morphine dose size on the conditioned reinforcing potency of stimuli paired with morphine. Psychological Record, 1972, 22, 441-448.

Cushman, P. Detoxification after methadone maintenance treatment. Annals of the New York Academy of Sciences, 1981, 362, 217-230.

Cushman, P., & Dole, V. P. Detoxification of rehabilitated methadone-maintained patients. The Journal of the American Medical Association, 1973, 226, 747-752.

Cytawa, J., & Jurkowlaniec, E. Noradrenergic stimulation of the lateral hypothalamus as a reinforcement in T maze learning in rats. Acta Neurobiologiae Experimentalis, 1978, 38, 209-213.

Cytawa, J., & Jurkowlaniec, E. Intrahypothalamic microinjections of noradrenaline with and without induction of the alimentary drive as reward in a T maze learning in rats. Acta Neurobiologiae Experimentalis, 1979, 39, 41-47.

Cytawa, J., Jurkowlaniec, E., & Bialowas, J. Positive reinforcement produced by noradrenergic stimulation of the hypothalamus in rats. Physiology & Behavior, 1980, 25, 615-619.

Dafny, N., Brown, M., Burks, T. F., & Rigor, B. M. Morphine tolerance and dependence: sensitivity of caudate nucleus neurons. Brain Research, 1979, 162, 363-368.

Davis, G. C., & Bunney, W. E. Psychopathology, and endorphins. In E. Costa and M. Trabucchi (Eds.), Neural Peptides and Neuronal Communication. New York: Raven Press, 1980.

Davis, W. M., Babbini, M., & Khalsa, J. H. Antagonism by α-methyltyrosine of morphine induced motility in non-tolerant and tolerant rats. Research Communications in Chemical Pathology and Pharmacology, 1972, 4, 267-279.

Davis, W. M., & Smith, S. G. Role of conditioned reinforcers in the initiation, maintenance and extinction of drug-seeking behavior. Pavlovian Journal, 1976, 11, 222-236.

Deitrich, R., & Erwin, V. Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and tryptolines (β-Carbolines). Annual Review of Pharmacology and Toxicology, 1980, 20, 55-80.

Demarest, K. T., & Moore, K. E. Disruption of 5-hydroxytryptaminergic neuronal function blocks the action of morphine on tuberoinfundibular dopaminergic neurons. Life Sciences, 1981, 28, 1345-1351.

Deneau, G., Yanagita, T., & Seevers, M. H. Self administration of psychoactive substances by the monkey: A measure of psychological dependence. Psychopharmacologia, 1969, 16, 30-48.

De Ryck, M., Schallert, T., & Teitelbaum, P. Morphine versus haloperidol catalepsy in the rat: A behavioral analysis of postural support mechanisms. Brain Research, 1980, 201, 143-172.

Des Jarlais, D. C., Joseph, H., & Dole, V. P. Long-term outcomes after termination from methadone maintenance treatment. Annals of the New York Academy of Sciences, 1981, 362, 231-238.

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

Di Giulio, A. M. Groppetti, A., Cattabeni, F., Galli, C. L., Maggi, A., Algeri, S., & Ponzio, F. Significance of dopamine metabolites in the evaluation of drugs acting on dopaminergic neurons. European Journal of Pharmacology, 1978, 52, 201-207.

Dixon, W. R., Mosimann, W. F., & Weiner, N. The role of presynaptic feedback mechanisms in regulation of norepinephrine release by nerve stimulation. The Journal of Pharmacology and Experimental Therapeutics, 1979, 209, 196-204.

Dole, V. P. Addictive behavior. Scientific American, 1980, 243, 138-154.

Dominic, J. A., & Moore, K. E. Acute effects of α-methyltyrosine on brain catecholamine levels and on spontaneous and amphetamine-stimulated motor activity in mice. Archives Internationales de Pharmacodynamie et de Therapie, 1969, 178, 166-176.

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

Downs, D. A., & Woods, J. H. Naloxone: Behavioral paradigms of aversive control. Pharmacologist, 1973, 15, 236.

Downs, D. A., & Woods, J. H. Morphine, pentazocine and naloxone effects on responding under a multiple schedule of reinforcement in rhesus monkeys and pigeons. The Journal of Pharmacology and Experimental Therapeutics, 1976, 196, 298-306.

Duka, T., Schubert, P., Wuster, M., Stoiber, R., & Herz, A. A selective distribution pattern of different opiate receptors in certain areas of rat brain as revealed by in vitro autoradiography. Neuroscience Letters, 1981, 21, 119-124.

Dunstan, R., Broekkamp, C. L., & Lloyd, K. G. Involvement of caudate nucleus, amygdala or reticular formation in neuroleptic and narcotic catalepsy. Pharmacology Biochemistry & Behavior, 1981, 14, 169-174.

Durham, H. D., & Frank, G. B. Dual action of meperidine on the frog neuromuscular junction: A prejunctional, opiate receptor-mediated depression of transmitter release and a postjunctional, nonopiate receptor effect on the endplate. Archives Internationales de Pharmacodynamie et de Thérapie, 1981, 251, 150-165.

Dworkin, S. I., Guerin, G. F., Lane, J. D., Cherek, D. R., & Smith, J. E. Effects of kainic acid lesions of the nucleus accumbens on responding maintained by a concurrent food, water and morphine schedule. Society for Neuroscience Abstracts, 1982, 8, 591.

Eddy, N. B. Prediction of drug dependence and abuse liability. In L. Goldberg and F. Hoffmeister (Eds.), Psychic Dependence: Definition, Assessment in Animals and Man, Theoretical and Clinical Implications. New York: Springer-Verlag, 1973.

Eddy, N. B., Halbach, H., & Braenden, O. J. Synthetic substances with morphine-like effect--clinical experience: Potency, side-effects, addiction liability. Bulletin of the World Health Organization, 1957, 17, 569-863.

Eidelberg, E. Possible actions of opiates upon synapses. Progress in Neurobiology, 1976, 6, 81-102.

Eidelberg, E., & Erspamer, R. Dopaminergic mechanisms of opiate actions in brain. The Journal of Pharmacology and Experimental Therapeutics, 1975, 192, 50-57.

Eidelberg, E., & Schwartz, A. S. Possible mechanisms of action of morphine on brain. Nature, 1970, 225, 1152-1153.

Engberg, G., Elam, M., & Svensson, T. H. Clonidine withdrawal: Activation of brain noradrenergic neurons with specifically reduced a_2-receptor sensitivity. Life Sciences, 1982, 30, 235-243.

Enjalbert, A., Ruberg, M., Arancibia, S., Priam, M., & Kordon, C. Endogenous opiates block dopamine inhibition of prolactin secretion in vitro. Nature, 1979, 280, 595-597.

Epstein, R., & Skinner, B. F. Resurgence of responding after the cessation of response-independent reinforcement. Proceedings of the National Academy of Science U.S.A., 1980, 77, 6251-6253.

Esposito, R., & Kornetsky, C. Morphine lowering of self stimulation thresholds: Lack of tolerance with long-term administration. Science, 1977, 195, 189-191.

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

Ettenberg, A., Koob, G. F., Pettit, H. O., & Bloom, F. E. Opiate and stimulant intravenous self-administration in rats: Mediation by separate neural systems. Society for Neuroscience Abstracts, 1981, 7, 263.

Ezrin-Walters, C., Muller, P., & Seeman, P. Catalepsy induced by morphine or haloperidol: Effects of apomorphine and anticholinergic drugs. Canadian Journal of Physiology and Pharmacology, 1976, 54, 516-519.

Fantozzi, R., Mullikin-Kilpatrick, D., & Blume, A. J. Irreversible inactivation of the opiate receptors in the neuroblastoma x glioma hybrid NG108-15 by chlornaltrexamine. Molecular Pharmacology, 1981, 20, 8-15.

Feigenbaum, J. J. I., Moon, B. H., & Klawans, H. L. The effect of morphine on apomorphine induced stereotypy in rats. Society for Neuroscience Abstracts, 1978, 4, 422.

Fennessy, M. R., & Laska, F. J. Comparison of opiate agonists and their N-Allyl derivatives in the production of physical dependence in the rat. Psychopharmacology, 1979, 64, 277-283.

Fibiger, H. C. Drugs and reinforcement mechanisms: A critical review of the catecholamine theory. Annual Reviews of Pharmacology and Toxicology, 1978, 18, 37-56.

Fibiger, H. C., & Phillips, A. G. Dopamine and the neural mechanisms of reinforcement. In A. S. Horn, B. H. C. Westerink, and J. Korf (Eds.), The Neurobiology of Dopamine. New York: Academic Press, 1979.

Fielding, S., Wilker, J., Hynes, M., Szewczak, M., Novick, W. J., & Lal, H. A comparison of clonidine with morphine for antinociceptive and antiwithdrawal actions. The Journal of Pharmacology and Experimental Therapeutics, 1978, 207, 899-905.

Fields, H. L. An endorphin-mediated analgesia system: Experimental and clinical observations. In J. B. Martin, S. Reichlin, and K. L. Bick (Eds.), Neurosecretion and Brain Peptides. New York: Raven Press, 1981

File, S. E., & Silverstone, T. Naloxone changes self ratings but not performance in normal subjects. Psychopharmacology, 1981, 74, 353-354.

Fingl, E., & Woodbury, D. M. General principles. In L. S. Goodman and A. Gilman (Eds,), The Pharmacological Basis of Therapeutics. New York: MacMillan Publishing, 1975.

Fink, J. S., & Smith, G. P. Abnormal pattern of amphetamine locomotion after 6-OHDA lesion of anteromedial caudate. Pharmacology Biochemistry & Behavior, 1979a, 11, 23-30.

Fink, J. S., & Smith, G. P. Decreased locomotor and investigatory exploration after denervation of catecholamine terminal fields in the forebrain of rats. Journal of Comparative and Physiological Psychology, 1979b, 93, 34-65.

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

Fink, J. S., & Smith, G. P. Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Research, 1980b, 199, 359-384.

Finnerty, E. P., & Chan, S. H. H. Morphine suppression of substantia nigra zona reticulata neurons in the rat: Implicated role for a novel striatonigral feedback mechanism. European Journal of Pharmacology, 1979, 59, 307-310.

Finnerty, E. P., & Chan, S. H. H. The participation of substantia nigra zona compacta and zona reticulata neurons in morphine suppression of caudate spontaneous neuronal activities in the rat. Neuropharmacology, 1981, 20, 241-246.

Fisher, A. E. Maternal and sexual behavior induced by intracranial chemical stimulation. Science, 1956, 124, 228-229.

Fisher, R. A. 1935. Cited in Harold R. Lindman, Analysis of Variance in Complex Experimental Designs. San Francisco: W. H. Freeman, 1974.

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

Fouriezos, G., & Wise, R. A. Pimozide-induced extinction of intracranial self-stimulation: response patterns rule out motor or performance dificits. Brain Research, 1976, 103, 377-380.

Frank, G. B. Opiate drug receptors on excitable cell membranes. Archives of Internationales de Pharmacodynamie et de Thérapie, 1975, 217, 4-17.

Frank, G. B., & Marwaha, J. An investigation of the activity of opiate drug receptors located on frog skeletal muscle fibre membrane. Canadian Journal of Physiology and Pharmacology, 1978, 56, 501-508.

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

Franklin, K. B. J., & McCoy, S. N. Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. Pharmacology Biochemistry & Behavior, 1979, 11, 71-75.

Franz, D. N., Hare, B. D., & McCloskey, K. L. Spinal sympathetic neurons: Possible sites of opiate withdrawal suppression by clonidine. Science, 1982, 215, 1643-1645.

Frederickson, R. C. A., & Norris, F. H. Enkephalin-induced depression of single neurons in brain areas with opiate receptors--antagonism by naloxone. Science, 1976, 194, 440-442.

Frederickson, R. C. A., & Norris, F. H. Enkephalins as inhibitory neurotransmitters modulating nociception. In R. Ryall and J. S. Kelly (Eds.), Iontophoresis and Transmitter Mechanisms in the Mammalian Central Nervous System. Amsterdam: Elsevier, 1978.

French, E. D., & Siggins, G. R. An iontophoretic survey of opioid peptide actions in the rat limbic system: In search of opiate epileptogenic mechanisms. Regulatory Peptides, 1980, 1, 127-146.

Friedhoff, A. J. Catecholamines and Behavior, Vol. 1: Basic Neurobiology. New York: Plenum Press, 1975.

Fuchs, V., & Coper, H. Modification of different morphine actions by 6-hydroxydopamine and 6-hydroxydopamine plus desmethylimipramine. Psychopharmacology, 1980, 67, 181-188.

Gahwiler, B. H. Excitatory action of opioid peptides and opiates on cultured hippocampal pyramidal cells. Brain Research, 1980, 194, 193-203.

Gahwiler, B. H., & Maurer, R. Involvement of μ-receptors in the opioid-induced generation of bursting discharges in hippocampal pyramidal cells. Regulatory Peptides, 1981, 2, 91-96.

Gallistel, C. R., Shizgal, P., & Yeomans, J. S. A portrait of the substrate for self-stimulation. Psychological Review, 1981, 88, 228-273.

Gardner, E. L., Zukin, R. S., & Makman, M. H. Modulation of opiate receptor binding in striatum and amygdala by selective mesencephalic lesions. Brain Research, 1980, 194, 232-239.

Gauchy, C., Agiel, Y., Glowinski, J., & Cheramy, A. Acute effects of morphine on dopamine synthesis and release and tyrosine metabolism in the rat striatum. European Journal of Pharmacology, 1973, 22, 311-319.

Gebhart, G. F. Opiate and opioid peptide effects on brain stem neurons: Relevance to nociception and antinociceptive mechanisms. Pain, 1982, 12, 93-140.

Gerber, G. J., Bozarth, M. A., & Wise, R. A. Small-dose intravenous heroin facilitates hypothalamic self stimulation without response suppression in rats. Life Sciences, 1981, 28, 557-562.

Gerber, G. J., Sing, J., & Wise, R. A. Pimozide attenuates lever pressing for water reinforcement in rats. Pharmacology Biochemistry & Behavior, 1981, 14, 201-205.

Gerber, G. J., & Stretch, R. Drug-induced reinstatement of extinguished self-administration behavior in monkeys. Pharmacology Biochemistry & Behavior, 1975, 3, 1055-1061.

Gero, A. The action of opioid drugs on human plasma cholinesterase. Life Sciences, 1979, 25, 201-208.

Gessa, G. L., Paglietti, E., & Quarantotti, B. P. Induction of copulatory behavior in sexually inactive rats by naloxone. Science, 1979, 204, 203-205.

Ghetti, B., Horoupian, D. S., & Wisniewsky, H. M. Transsynaptic response of the lateral geniculate nucleus and the pattern of degeneration of the nerve terminals in the rhesus monkey after eye enucleation. Brain Research, 1972, 45, 31-48.

Glick, S. D., & Cox, R. D. Dopaminergic and cholinergic influences on morphine self administration in rats. Research Communications in Chemical Pathology and Pharmacology, 1975, 12, 17-24.

Glick, S. D., & Cox, R. D. Changes in morphine self administration after brainstem lesions in rats. Psychopharmacology, 1977, 52, 151-156.

Glick, S. D., & Cox, R. D. Nocturnal rotation in normal rats: correlation with amphetamine-induced rotation and effects of nigrostriatal lesions. Brain Research, 1978a, 150, 149-161.

Glick, S. D., & Cox, R. D. Changes in morphine self administration after tel-diencephalic lesions in rats. Psychopharmacology, 1978b, 57, 283-288.

Glick, S. D., Cox, R. D., & Crane, A. M. Changes in morphine self-administration and morphine dependence after lesions of the caudate nucleus in rats. Psychopharmacologia, 1975, 41, 219-224.

Glick, S. D., Cox, R. D., & Meibach, R. C. Selective effect of reinforcing doses of morphine in striatum. Brain Research, 1980, 190, 298-300.

Glick, S. D., Jerussi, T. P., & Fleisher, L. N. Turning in circles: The neuropharmacology of rotation. Life Sciences, 1976, 18, 889-896.

Glick, S. D., Weaver, L. M., & Meibach, R. C. Amphetamine induced rotation in normal cats. Brain Research, 1981, 298, 227-229.

Glowinski, J., Cheramy, A., & Giorguieff, M. F. In-vivo and in-vitro release of dopamine. In A. S. Horn, J. Korf, and B. H. C. Westerink (Eds.) The Neurobiology of Dopamine. New York: Academic Press, 1979.

Glowinski, J., Michelot, R., & Cheramy, A. Role of stiato-nigral substance P in the regulation of the activity of the nigro-striatal dopaminergic neurons. In E. Costa and M. Trabucchi (Eds.), Neural Peptides and Neuronal Communication. New York: Raven Press, 1980.

Goeders, N. E., & Smith, J. E. Intracranial cocaine self administration. Society for Neuroscience Abstracts, 1982, 8, 1029.

Gold, M. S., & Pottash, A. C. The neurobiological replications of clonidine HCL. Annals of the New York Academy of Sciences, 1981, 362, 191-202.

Gold, M. S., Pottash, A. L. C., Sweeney, D. R., & Kleber, H. D. Efficacy of clonidine in opiate withdrawal: A study of thirty patients. Drug and Alcohol Dependence, 1980, 6, 201-208.

Gold, M. S., Redmond, D. E., Donabedian, R. K., Goodwin, F. K., & Extein, I. Increase in serum prolactin by exogenous and endogenous opiates: Evidence for antidopamine and antipsychotic effects. American Journal of Psychiatry, 1978, 135, 1415-1416.

Gold, M. S., Redmond, D. E., & Kleber, H. D. Clonidine in opiate withdrawal. The Lancet, 1978, 1, 929-930.

Goldberg, S. R., Hoffmeister, F., & Schlichting, U. U. Morphine antagonists: Modification of behavioral effects by morphine dependence. In J. M. Singh, L. Miller, and H. Lal (Eds.), Drug Addiction: I. Experimental Pharmacology. Mount Kisco, New York: Futura Publishing, 1972.

Goldberg, S. R., Hoffmeister, F., Schlichting, U., & Wuttke, W. Aversive properties of nalorphine and naloxone in morphine-dependent rhesus monkeys. The Journal of Pharmacology and Experimental Therapeutics, 1971, 179, 268-276.

Goldberg, S. R., Woods, J. H., & Schuster, C. R. Nalorphine induced changes in morphine self-administration in rhesus monkeys. Journal of Pharamcology and Experimental Therapeutics, 1971, 176, 464-471.

Goldstein, A. Opiate receptors. Life Sciences, 1974, 14, 615-623.

Goldstein, A., & Cox, B. M. The opioid peptides (endorphins): A progress report. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

Goldstein, A., Lowney, L. I., & Pal, B. K. Stereospecific and nonspecific interactions of morphine congener levorphanol into subcellular fractions of mouse brain. Proceedings of the National Academy of Science, 1971, 68, 1742-1747.

Goldstein, D. B. Speculations on membrane lipid adaptation as a mechanism for drug tolerance and dependence. In C. W. Sharp and L. G. Abood (Eds.), Membrane Mechanisms of Drugs of Abuse. New York: Alan R. Liss, 1979.

Goldstein, D. B., & Chin, J. H. Interaction of ethanol with biological membranes. Federation Proceedings, 1981, 40, 2073-2076.

Goldstein, M., Lew, J. Y., Asano, T., & Ueta, K. Alterations in dopamine receptors: Effect of lesion and haloperidol treatment. Communications in Psychopharmacology, 1980, 4, 21-25.

Gorman, J. E., De Obaldia, R. N., Scott, R. C., & Reid, L. D. Morphine injections in the taste aversion paradigm: Extent of aversions and readiness to consume sweetened morphine solutions. Physiological Psychology, 1978, 6, 101-109.

Goudie, A. J. Aversive stimulus properties of drugs. Neuropharmacology, 1979, 18, 971-979.

Greengard, P. Possible role for cyclic nucleotides and phosphorylated membrane proteins in postsynaptic actions of neurotransmitters. Nature, 1976, 160, 101-108.

Greengard, P. Intracellular signals in the brain. In D. M. Marcus (Ed.), The Harvey Lectures, Series 75. New York: Academic Press, 1981.

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

de Groot, J. The rat forebrain in stereotaxic coordinates. Verhandelingen der Koninkluke Nederlandse Akademie Van Wetenschappen, Afd. Natuurkunde, 1959, 527, 1-40.

Grossman, S. P. Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. American Journal of Physiology, 1962, 202, 872-882.

Gunne, L. M. Effects of amphetamines in humans. In W. R. Martin (Ed.), Drug Addiction II: Amphetamine, Psychotogen, and Marihuana Dependence. New York: Springer-Verlag, 1977.

Gysling, K., & Wang, R. Morphine facilitates the activity of dopaminergic neurons in the rat ventral tegmental area. Society for Neuroscience Abstracts, 1982, 8, 777.

Haber, S., & Elde, R. The distribution of enkephalin immunoreactive fibers and terminals in the monkey central nervous system: An immunohistochemical study. Neuroscience, 1982, 7, 1049-1095.

Haertzen, C. A. Development of scales based on patterns of drug effects, using the Addiction Research Center Inventory (ARCI). Psychological Record, 1966, 18, 163-194.

Hall, G., Channell, S., & Pearce, J. M. The effects of a signal for free or for earned reward: Implications for the role of response-reinforcer associations in instrumental performance. Quarterly Journal of Experimental Psychology, 1981, 33B, 95-107.

Hall, R. D., Bloom, F. E., & Olds, J. (Eds.), Neuronal and neurochemical substrates of reinforcement. Neuroscience Research Program Bulletin, 1977, 15, 133-313.

Hamprecht, B. Opioids and cyclic nucleotides. In A. Herz (Ed.), Developments in Opiate Research. New York: Marcel Dekker, 1978.

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

Hartley, E. J., Spuhler, K., Prasad, K. N., & Seeman, P. A neuroblastoma clone which has dopamine-sensitive adenylate cyclase but which lacks stereospecific binding sites for the neuroleptic ^{3}H-spiperone. Brain Research, 1980, 190, 574-577.

Hattori, T., & Fibiger, H. C. On the use of lesions of afferents to localize neurotransmitter receptor sites in the striatum. Brain Research, 1982, 238, 245-250.

Havemann, U., & Kuschinsky, K. Further characterization of opioid receptors in the striatum mediating muscular rigidity in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 1981, 317, 321-325.

Havemann, U., & Kuschinsky, K. Neurochemical aspects of the opioid-induced "catatonia." Neurochemistry International, 1982, 4, 199-215.

Havemann, U., Winkler, M., Genç, E., & Kuschinsky, K. Opioid actions on motility: Possible actions on GABA-ergic and dopaminergic neurons. Brain Research Bulletin, 1980, 5, 891-896.

Havemann, U., Winkler, M., Genç, E., & Kuschinsky, K. Effects of striatal lesions with kainic acid on morphine-induced "catatonia" and increase of striatal dopamine turnover. Naunyn-Schmiedeberg's Archives of Pharmacology, 1981, 317, 44-50.

Havemann, U., Winkler, M., & Kuschinsky, K. Opioid receptors in the caudate nucleus can mediate EMG-recorded rigidity in rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 1980, 313, 139-144.

Hayashi, T., Ohashi, K., & Tadokoro, S. Conditioned drug effects to d-amphetamine- and morphine-induced motor acceleration in mice: Experimental approach for placebo effect. Japanese Journal of Pharmacology, 1980, 30, 93-100.

Hazum, E., Chang, K. J., Cuatrecasas, P., Pasternak, G. W. Naloxazone irreversibly inhibits the high affinity binding of {^{125}I}D-ala^{2}-D-leu^{5}-enkephalin. Life Sciences, 1981 28, 2973-2979.

Hefti, F., Melamed, E., & Wurtman, R. J. Partial lesions of the dopaminergic nigrostriatal system in rat brain: Biochemical characterization. Brain Research, 1980, 195, 123-137.

Herkenham, M., & Pert, C. B. In vitro autoradiography of opiate receptors in rat brain suggests loci of "opiatergic" pathways. Proceedings of the National Academy of Science U.S.A., 1980, 77, 5532-5536.

Hernandez, L. L., Holohean, A. M., & Appel, J. B. Effects of opiates on the discriminative stimulus properties of dopamine agonists. Pharmacology Biochemistry & Behavior, 1978, 9, 459-463.

Herrling, P. L., & Hull, C. D. Iontophoretically applied dopamine depolarizes and hyperpolarizes the membrane of cat caudate neurons. Brain Research, 1980, 192, 441-462.

Herz, A. Role of endorphins in addiction. Modern Problems of Pharmacopsychiatry, 1981, 17, 175-180.

Herz, A., Blasig, J., & Papeschi, R. Role of catecholaminergic mechanisms in the expression of the morphine abstinence syndrome in rats. Psychopharmacologia, 1974, 39, 121-143.

Herz, A., Hollt, V., & Przewtocki, R. Endogenous opioids and addiction. In W. Wuttke, A. Weindl, K. H. Voigt, and R. R. Dries (Eds.), Brain and Pituitary Peptides. Basel: S. Karger, 1980.

Herz, A., Teschemacher, H., Albus, K., & Zieglgansberger, S. Morphine abstinence syndrome in rabbits precipitated by injection of morphine antagonists into the ventricular system and restricted parts of it. Psychopharmacologia, 1972, 26, 219-235.

Hill, H. E., Haertzen, C. A., Wolbach, A. B., & Miner, E. J. The Addiction Research Center Inventory: Standardization of scales which evaluate subjective effects of morphine, amphetamine, pentobarbital, alcohol, LSD-25, pyrahexyl and chlorpromazine. Psychopharmacologia, 1963, 4, 167-183.

Hill, R. G. The status of naloxone in the identification of pain control mechanisms operated by endogenous opioids. Neuroscience Letters, 1981, 21, 217-222.

Himmelsbach, C. K. With reference to physical dependence. Federation Proceedings, 1943, 2, 201-203.

Hinson, R. E., & Poulos, C. X. Sensitization to the behavioral effects of cocaine: Modification by Pavlovian conditioning. Pharmacology Biochemistry & Behavior, 1981, 15, 559-562.

Ho, B. T., & McKenna, M. Discriminative stimulus properties of central stimulants. In B. T. Ho, D. W. Richards, and D. L. Chute (Eds.), Drug Discrimination and State Dependent Learning. New York: Academic Press, 1978.

Ho, B. T., Richards, D. W., & Chute, D. L. (Eds.), Drug Discrimination and State Dependent Learning. New York: Academic Press, 1978.

Ho, I. K., & Harris, R. A. Mechanism of action of barbiturates. Annual Review of Pharmacology and Toxicology, 1981, 21, 83-111.

Hoch, P. H., & Zubin, J. Problems of Addiction and Habituation. New York: Grune & Stratton, 1958.

Hodgman, C. D., Weast, R. C., Shankland, R. S., & Selby, S. M. (Eds.), Handbook of Chemistry and Physics: A Ready-Reference Book of Chemical and Physical Data. Cleveland, Ohio: The Chemical Rubber Publishing, 1962.

Hokfelt, T., Elde, R., Johansson, D., Terenius, L., & Stein, L. Distribution of enkephalin-like immunoreactivity in the rat central nervous system. Neuroscience Letters, 1977, 5, 25-31.

Hollister, L. E., Johnson, K., Boukhabza, D., & Gillespie, H. K. Aversive effects of naltrexone in subjects not dependent on opiates. Drug and Alcohol Dependence, 1981, 8, 37-41.

Holtzman, S. G. Effects of narcotic antagonists on fluid intake in the rat. Life Sciences, 1975, 16, 1465-1470.

Holtzman, S. G. Suppression of appetitive behavior in the rat by naloxone: Lack of effect of prior morphine dependence. Life Sciences, 1979, 24, 219-226.

Hong, J. S., Yang, H. Y. T., Fratta, W., & Costa, E. Determination of methionine enkephalin in discrete regions of rat brain. Brain Research, 1977, 134, 383-386.

Hornykiewicz, O. Dopamine and its physiological significance in brain function. In G. H. Bourne (Ed.), The Structure and Function of Nervous Tissue, Vol. 6. New York: Academic Press, 1972.

Houde, R. W. Analgesic effectiveness of the narcotic agonist-antagonists. British Journal of Clinical Pharmacology, 1979, 7, 297S-308S.

Huang, J. T., Yano, I., & Takemori, A. E. Effect of various central biogenic amine-modifiers and ambient temperature on the naloxone-induced jumping in morphine-dependent mice. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

Huffman, R. D., & Felpel, L. P. A microiontophoretic study of morphine on single neurons in the rat globus pallidus. Neuroscience Letters, 1981, 22, 195-199.

Hughes, J. Isolation of an endogenous compound from the brain with properties similar to morphine. Brain Research, 1975, 88, 295-308.

Hughes, J., Smith, T. W., Kosterlitz, H. W., Fothergill, L. A., Morgan, B. A., & Morris, H. R. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature, 1975, 258, 577-579.

Hume, D. Treatise of Human Nature, 1739. Cited in R. C. Bolles, Theory of Motivation. New York: Harper & Row, 1975.

Innes, I. R., & Nickerson, M. Norepinephrine, epinephrine, and the sympathomimetic amines. In L. S. Goodman and A. Gilman (Eds.), The Pharmacological Basis of Therapeutics. New York: MacMillan Publishing, 1975.

Iwamoto, E. T., Loh, H. H., & Way, E. L. Circling behavior after narcotic drugs and during naloxone-precipitated abstinence in rats with unilateral nigral lesions. Journal of Pharmacology and Experimental Therapeutics, 1976, 197, 503-516.

Iwamoto, E. T., & Way, E. L. Circling behaviour and stereotypy induced by intranigral opiate microinjections. Journal of Pharmacology and Experimental Therapeutics. 1977, 203, 347-359.

Iwamoto, E. T., & Way, E. L. Opiate actions and catecholamines. In H. H. Loh and D. H. Ross (Eds.), Neurochemical Mechanisms of Opiates and Endorphins. New York: Raven Press, 1979.

Iwatsubo, K. Narcotic analgesics and nigrostriatal dopaminergic neurons. Trends in Pharmacological Sciences, 1982, 3, 64-66.

Iwatsubo, K., & Clouet, D. H. Effects of morphine and haloperidol on the electrical activity of rat nigrostriatal neurons. Journal of Pharmacology and Experimental Therapeutics, 1977, 202, 429-436.

Izquierdo, I. Effect of naloxone and morphine on various forms of memory in the rat: Possible role of endogenous opiate mechanisms in memory consolidation. Psychopharmcology, 1979, 66, 199-203.

Jacob, J. J. C., Michaud, G. M., Tremblay, E. C. Mixed agonist-antagonist opiates and physical dependence. British Journal of Clinical Pharmacology, 1979, 7, 291S-296S.

Jacobowitz, D. M., & Palkovits, M. Topagraphic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. I. Forebrain (telencephalon, diencephalon). Journal of Comparative Neurology, 1974, 157, 13-28.

Jacobs, W. J., Zellner, D. A., LoLordo, V. M., & Riley, A. L. The effect of post-conditioning exposure to morphine on the retention of a morphine-induced conditioned taste aversion. Pharmacology Biochemistry & Behavior, 1981, 14, 779-785.

Jacquet, Y. F. Intracerebral administration of opiates. In S. Ehrenpreis and A. Neidle (Eds.), Methods of Narcotics Research. New York: Dekker, 1975.

Jacquet, Y. F. Opiate effects after adrenocorticotropin or β-endorphin injection in the periaqueductal gray matter of rats. Science, 1978, 201, 1032-1034.

Jacquet, Y. F. Excitatory and inhibitory effects of opiates in the rat vas deferens: A dual mechanism of opiate action. Science, 1980, 210, 95-97.

Jacquet, Y. F., & Lajtha, A. Morphine action at central nervous system sites in rat: analgesia or hyperalgesia depending on site and dose. Science, 1973, 182, 490-492.

Jacquet, Y. F., & Lajtha, A. Paradoxical effects after microinjection of morphine in the periaqueductal gray matter in the rat. Science, 1974, 185, 1055-1057.

Jaffe, J. H. Drug addiction and drug abuse. In L. S. Goodman & A. Gilman (Eds.), The Pharmacological Basis of Therapeutics. New York: MacMillan, 1975.

Jaffe, J. H., & Martin, W. R. Narcotic analgesics and antagonists. In L. S. Goodman and A. Gilman (Eds.), The Pharmacological Basis of Therapeutics. New York: MacMillan, 1975.

Jalowiec, J. E., Panksepp, J., Zolovick, A. J., Najam, N., & Herman, B. H. Opioid modulation of ingestive behavior. Pharmacology Biochemistry & Behavior, 1981, 15, 477-484.

Janssen, P. A. J., Niemegeers, C. J. E., & Schellekens, K. H. L. Is it possible to predict the clinical effects of neuroleptic drugs (major tranquillizers) from animals data? Arzneimittel-Forschung, 1965, 15, 104-117.

Janssen, P. A., Niemegeers, S., Schellekens, K. H. L., Dreese, A., Lenaerts, F. M., Pinchard, A., Schaper, W. K. A., Van Nueten, J. M., & Verbruggen, F. J. Pimozide, a chemically novel, highly potent and orally long-acting neuroleptic: Part I. The comparative pharmacology of pimozide, haloperidol and chlorpromazine. Arzneimittle Forschung, 1968, 18, 261-279.

Jasinski, D. R. Human pharmacology of narcotic antagonists. British Journal of Pharmacology, 1979, 7, 287S-209S.

Jensen, T. S., & Smith, D. F. Dopaminergic effects on tail-flick response in spinal rats. European Journal of Pharmacology, 1982, 79, 129-133.

Jerussi, T. P., & Glick, S. D. Drug-induced rotation in rats without lesions: behavioral and neurochemical indices of a normal asymmetry in nigro-striatal function. Psychopharmacology, 1976, 47, 249-260.

Johnson, J. H. Release of prolactin in response to microinjection of morphine into mesencephalic dorsal raphe nucleus. Neuroendocrinology, 1982, 35, 169-172.

Johnson, R. P., Sar, M., & Stumpf, W. E. 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, 1980, 194, 566-571.

Johnson, S. M., & Duggan, A. W. Evidence that the opiate receptors of the substantia gelatinosa contribute to the depression, by intravenous morphine, of the spinal transmission of impulses in unmyelinated primary afferents. Brain Research, 1981, 207, 223-228.

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

Jonsson, G. Chemical neurotoxins as denervation tools in neurobiology. Annual Review of Neurobiology, 1980, 3, 169-187.

Joyce, E. M., & Iversen, S. D. The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neuroscience Letters, 1979, 14, 207-212.

Joyce, E. M., Koob, G. F., Strecker, R., Iversen, S. D., & Bloom, F. E. The behavioral effects of enkephalin analogues injected into the ventral tegmental area and globus pallidus. Brain Research, 1981, 221, 359-370.

Kaplan, G. P., Harman, B. K., & Creveling, C. R. Immunohistochemical demonstration of catechol-o-methyltransferase in mammalian brain. Brain Research, 1979, 167, 241-250.

Karras, P. J., & North, R. A. Inhibition of neuronal firing by opiates: Evidence against the involvement of cyclic nucleotides. British Journal of Pharmacology, 1979, 65, 647-652.

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

Kebabian, J. W., & Greengard, P. Dopamine-sensitive adenyl cyclase: Possible role in synaptic transmission. Science, 1971, 174, 1346-1349.

Kebabian, J. W., Zatz, M., & O'Dea, R. F. Modulation of receptor sensitivity in the pineal: The roles of cyclic nucleotides. In W. M. Cowan and J. A. Ferrendelli (Eds.), Society for Neuroscience, Vol. 2: Approaches to the Cell Biology of Neurons. Bethesda, Maryland: Society for Neuroscience, 1977.

Kehne, J. H., Sant, W. W., & Sorenson, C. A. The effects of radio-frequency lesions of the nucleus accumbens on d-amphetamine-induced locomotor and rearing behavior in rats. Psychopharmacology, 1981, 75, 363-367.

Kehr, W. 3-Methoxytyramine as an indicator of impulse induced dopamine release in rat brain in vivo. Naunyn-Schmiedeberg's Archives of Pharmacology, 1976, 293, 209-215.

Kelleher, R. T., & Goldberg, S. R. Effects of naloxone on schedule-controlled behavior in monkeys. In E. Usdin, W. E. Bunney, and N. S. Kline (Eds.), Endorphins in Mental Health Research. London: The MacMillan Press, 1979.

Kelley, A. E., Stinus, L., & Iversen, S. D. Interactions between d-ala-met-enkephalin, A10 dopaminergic neurones, and spontaneous behaviour in the rat. Behavioral Brain Research, 1980, 1, 3-24.

Kelley, K. L., & Reid, L. D. Addictive agents and intracranial stimulation: Morphine and thresholds for positive intracranial reinforcement. Bulletin of the Psychonomic Society, 1977, 10, 298-300.

Kelly, P. H., Seviour, P. W., & Iversen, S. D. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research, 1975, 94, 507-522.

Kerlinger, F. N. Foundations of Behavioral Research. New York: Holt, Rinehart and Winston, 1973.

Khavari, K. A., & Risner, M. E. Opiate dependence produced by ad libitum drinking of morphine in water, saline, and sucrose vehicles. Psychopharmacologia, 1973, 30, 291-302.

Kilbey, M. M., & Ellinwood, E. H. Reverse tolerance to stimulant-induced abnormal behavior. Life Sciences, 1977, 20, 1063-1076.

Kilbey, M. M., Ellinwood, E. H., & Easler, M. E. The effects of chronic cocaine oretreatment on kindled seizures and behavioral stereotypies. Experimental Neurology, 1979, 64, 306-314.

Killam, K. F., & Deneau, G. A. A study of morphine dependence in four species of subhuman primates. Proceedings of the Western Pharmacological Society, 1973, 16, 1-3.

Klawans, H., & Margolin, D. Amphetamine-induced dopaminergic hypersensitivity in guinea pigs. Archives of General Psychiatry, 1975, 32, 725-732.

Klee, W. A. Drug actions mediated by changes in adenylate cyclase activity. In C. W. Sharp and L. G. Abood (Eds.), Membrane Mechanisms of Drugs of Abuse. New York: Alan R. Liss, 1979.

Klemm, W. R. Opiate mechanisms: Evaluation of research involving neuronal action potentials. Progress in Neuro-Psychopharmacology, 1981, 5, 1-33.

Klemm, W. R., & Mallari, C. G. Effects of morphine and naloxone on the responsiveness (unit and field potential) of three opiate-relevant brain areas during electrical stimulation of the substantia nigra. Progress in Neuro-Psychopharmacology, 1980, 4, 1-12.

Koenig, J., Mayfield, M. A., Coppings, R. J., McCann, S. M., & Krulich, L. Role of central nervous system neurotransmitters in mediating the effects of morphine on growth hormone- and prolactin-secretion in the rat. Brain Research, 1980, 197, 453-468.

Koffer, K. B., Berney, S., & Hornykiewicz, O. The role of the corpus striatum in neuroleptic and narcotic induced catalepsy. European Journal of Pharmacology, 1978, 47, 81-86.

Koga, Y., & Inukai, T. Assessment of physical dependence inducing capacity of narcotic agonists and antagonists in rats by intermittent infusion technique. Psychopharmacology, 1981, 73, 236-239.

Koga, Y., & Inukai, T. Characterization of withdrawal syndrome of morphine-dependent rats prepared by intermittent infusion technique. Psychopharmacology, 1981, 73, 230-235.

Kolb, L. Pleasure and deterioration from narcotic addiction. Mental Hygiene, 1925, 9, 699-724.

Kolb, L., & DuMez, A. G. Experimental addiction of animals to opiates. U.S. Public Health Reports, 1931, 46, 698-726.

Konig, J. F. R., & Klippel, R. A. The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem. Baltimore: Williams & Wilkins, 1963.

Korf, J. Electrical stimulation as a tool for the study of biochemical aspects of dopamine neurotransmission. In A. S. Horn, J. Korf, and B. H. C. Westerink (Eds.), The Neurobiology of Dopamine. New York: Academic Press, 1979.

Korf, J., Grasdijk, L., & Westerink, B. H. C. Effects of electrical stimulation of the nigrostriatal pathway of the rat on dopamine metabolism. Journal of Neurochemistry, 1976, 26, 579-584.

Korf, J., Zieleman, M., & Westerink, B. H. C. Dopamine release in substantia nigra? Nature, 1976, 260, 257-258.

Kornetsky, C., & Esposito, R. U. Euphorigenic drugs: Effects on the reward pathways of the brain. Federation Proceedings, 1979, 38, 2473-2476.

Korf, J., Bunney, B. S., & Aghajanian, G. K. Noradrenergic neurons: Morphine inhibition of spontaneous activity. European Journal of Pharmacology, 1974, 25, 165-169.

Kosersky, D. S., Harris, R. A., & Harris, L. S. Naloxone precipitated jumping activity in mice following the acute administration of morphine. European Journal of Pharmacology, 1974, 26, 122-124.

Kosterlitz, H. W. Possible functions of the enkephalins. In E. Costa and M. Trabucchi (Eds.), Neural Peptides and Neuronal Communication. New York: Raven Press, 1980.

Kostowski, W., & Trzaskowska, E. Effects of lesion of the locus coeruleus and clonidine treatment on ethanol withdrawal syndrome in rats. Polish Journal of Pharmacology and Pharmacy, 1980, 32, 617-623.

Krauthamer, G. M. Catecholamines in behavior and sensorimotor integration: The neostriatal system. In A. J. Friedhoff (Ed.), Catecholamines and Behavior, Vol. 1: Basic Neurobiology. New York: Plenum Press, 1975.

Kuczenski, R., & Leith, N. J. Chronic amphetamine: Is dopamine a link in or a mediator of the development of tolerance and reverse tolerance? Pharmacology Biochemistry & Behavior, 1981, 15, 405-413.

Kuschinsky, K. Dopamine receptor sensitivity after repeated morphine administration to rats. Life Sciences, 1975, 17, 43-48.

Kuschinsky, K. Psychic dependence on opioids: mediated by dopaminergic mechanisms in the striatum? Trends in Pharmacological Sciences, 1981, 2, 287-289.

Kuschinsky, K., & Hornykiewicz, O. Morphine catalepsy in the rat: Relation to striatal dopamine metabolism. European Journal of Pharmacology, 1972, 19, 119-122.

Kuschinsky, K., & Hornykiewicz, O. Effects of morphine on striatal dopamine metabolism: Possible mechanism of its opposite effect on locomotor activity in rats and mice. European Journal of Pharmacology, 1974, 26, 41-50.

Labrie, F., Cusan, L., Dupont, A., Ferland, L., & Lemay, A. Opioids and anterior pituitary hormone secretion. In J. M. Van Ree and L. Terenius (Eds.), Characteristics and Function of Opioids. Amsterdam: Elsevier/North Holland Biomedical Press, 1978.

Lagowska, J., Calvino, B., & Ben-Ari, Y. Intra-amygdaloid applications of naloxone elicits severe withdrawal signs in morphine dependent rats. Neuroscience Letters, 1978, 8, 241-245.

Lal, H. Narcotic dependence, narcotic action and dopamine receptors. Life Sciences, 1975, 17, 483-496.

Lal, H. (Ed.), Discriminative Stimulus Properties of Drugs. New York: Plenum Press, 1977.

Lal, H., Gianutsos, G., & Puri, S. K. A comparison of narcotic analgesics with neuroleptics on behavioral measures of dopaminergic activity. Life Sciences, 1975, 17, 29-37.

Lal, S., Nair, N. P. V., Iskandar, H. L., Etienne, P., Wood, P. L., Schwartz, G., & Guyda, H. Effect of domperidone on apomorphine-induced growth hormone secretion in normal men. Journal of Neural Transmission, 1982, 54, 75-84.

Langer, G., Sachar, E. J., Gruen, P. H., & Halpern, F. S. Human prolactin responses to neuroleptic drugs correlate with antischizophrenic potency. Nature, 1977, 266, 639-640.

Lasagna, L., van Felsinger, J. M., & Beecher, H. K. Drug-induced mood changes in man. 1. Observations on healthy subjects, chronically ill patients, and "postaddicts." Journal of the American Medical Association, 1955, 157, 1006-1020.

Laschka, E., Gramsch, C., Blasig, J., & Herz, A. On the role of dopamine in precipitated morphine withdrawal in rats. In M. W. Adler, L. Manara, and R. Samanin (Eds.), Factors Affecting the Action of Narcotics. New York: Raven Press, 1978.

Laschka, E., Herz, A., & Blasig, J. Sites of action of morphine involved in the development of physical dependence in rats. I. Comparison of precipitated morphine withdrawal after intraperitoneal and intraventricular injection of morphine antagonists. Psychopharmacologia, 1976, 46, 133-139.

Laschka, E., Teschemacher, H., Mehraein, P., & Herz, A. Sites of action of morphine involved in the development of physical dependence in rats: II. Morphine withdrawal precipitated by application of morphine antagonists into restricted parts of the ventricular system and by microinfusion into various brain areas. Psychopharmacologia, 1976, 46, 141-147.

Laska, F. J., & Fennessy, M. R. Induction of dependence on cyclazocine and pentazocine in the rat. European Journal of Pharmacology, 1978, 48, 57-65.

Le Gal La Salle, G., & Lagowska, J. Amygdaloid kindling procedure reduces severity of morphine withdrawal syndrome in rats. Brain Research, 1980, 184, 239-242.

Leslie, J. C., Boakes, R. A., Linaza, J., & Ridgers, A. Autoshaping using visual stimuli in the rat. The Psychological Record, 1979, 29, 523-546.

Lettieri, D. J., Sayers, M., & Pearson, H. W. (Eds.), Theories on Drug Abuse: Selected Contemporary Perspectives (NIDA Research Monograph 30). Rockville, Maryland: National Institute on Drug Abuse, 1980.

Lewander, T. Effects of amphetamine in animals. In W. R. Martin (Ed.), Drug Addiction II. New York: Springer Verlag, 1977.

Lewis, J. W., Caldecott-Hazard, S., Cannon, J. T., & Liebeskind, J. C. Possible role of opioid peptides in pain inhibition and seizures. In J. B. Martin, S. Reichlin, and K. L. Bick (Eds.), Neurosecretion and Brain Peptides. New York: Raven Press, 1981.

Libet, B. Which postsynaptic action of dopamine is mediated by cyclic AMP? Life Sciences, 1979, 24, 1043-1058.

Lindesmith, A. R. A sociological theory of drug addiction. American Journal of Sociology, 1938, 43, 593-613.

Lindesmith, A. Psychology of addiction. In W. G. Clark and J. del Giudice (Eds.), Principles of Psychopharmcology. New York: Academic Press, 1970.

Lindesmith, A. R. A general theory of addiction to opiate-type drugs. In D. J. Lettieri, M. Sayers, and H. W. Pearson (Eds.), Theories on Drug Abuse: Selected Contemporary Perspectives. Rockville, Maryland: National Institute on Drug Abuse, 1980.

Lindman, H. R. Analysis of Variance in Complex Experimental Designs. San Francisco: W. H. Freeman, 1974.

Lindvall, O., & Bjorklund, A. The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. Acta Physiologica Scandinavica, 1974, 412, 1-48.

Ling, W., Blakis, M., Holmes, E. D., Klett, J., & Carter, W. E. Restabilization with methadone after methadyl acetate maintenance. Archives of General Psychiatry, 1980, 37, 194-196.

Linton, M., & Gallo, P. S. The Practical Statistician: Simplified Handbook of Statistics. Monterey, California: Brooks/Cole Publishing, 1975.

Llorens-Cortes, C., Pollard, H., & Schwartz, J. C. Localization of opiate receptors in substantia nigra evidence by lesion studies. Neuroscience Letters, 1979, 12, 165-170.

Lloyd, K. G., & Hornykiewicz, O. Catecholamines in regulation of motor function. In A. J. Friedhoff (Ed.), Catecholamines and Behavior, Vol. 1: Basic Neurobiology. New York: Plenum Press, 1975.

Login, I. S., & MacLeod, R. M. Failure of opiates to reverse dopamine inhibition of prolactin secretion in vitro. European Journal of Pharmacology, 1979, 60, 253-255.

Logue, A. W. Taste aversion and the generality of the laws of learning. Psychological Bulletin, 1979, 86, 276-296.

Lomax, P. The distribution of morphine following intracerebral microinjection. Experientia, 1966, 22, 249-250.

Lomax, P. Investigation of the central effects of morphine on body temperature. Archivos de Biologia y Medicina Experimentales, 1967, 4, 119-124.

Lorens, S. A., & Mitchell, C. L. Influence of morphine on lateral hypothalamic self-stimulation in the rat. Psychopharmacologia, 1973, 32, 271-277.

Lotti, V. J., Lomax, P., & George, R. Temperature responses in the rat following intracerebral microinjections of morphine. Journal of Pharmacology and Experimental Therapeutics, 1965, 150, 135-139.

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

Marchais, D., & Bockaert, J. Is there a connection between high affinity ^{3}H-spiperone binding sites and DA-sensitive adenylate cyclase in corpus striatum? Biochemical Pharmacology, 1980, 29, 1331-1336.

Marcus, R., & Kornetsky, C. Negative and positive intracranial reinforcement thresholds: Effects of morphine. Psychopharmacologia, 1974, 38, 1-13.

Martin, W. R. Assessment of the dependence producing potentiality of narcotic analgesics. In C. Radouco Thomas and L. Lasagna (Eds.), International Encyclopedia of Pharmacology and Therapeutics, Vol. 1. Glasgow: Pergamon Press, 1966.

Martin, W. R. Opioid antagonists. Pharmacological Reviews, 1967, 19, 463-521.

Martin, W. R. Assessment of the abuse potentiality of amphetamines and LSD-like hallucinogens in man and its relationship to basic animal assessment programs. In L. Goldberg and F. Hoffmeister (Eds.), Psychic Dependence. New York: Springer, 1973.

Martin, W. R. Chemotherapy of narcotic addiction. In W. R. Martin (Ed.), Drug Addiction I. New York: Springer-Verlag, 1977.

Martin, W. R. History and development of mixed opioid agonists, partial agonists and antagonists. British Journal of Clinical Pharmacology, 1979, 7, 273S-279S.

Martin, W. R., & Frazer, H. F. A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in post-addicts. Journal of Pharmacology and Experimental Therapeutics, 1961, 133, 388-399.

Martin, W. R., Fraser, H. F., Gorodetzky, C. W., & Rosenberg, D. E. Studies of the dependence producing potential of the narcotic antagonist 2-cyclopropylmethyl-2'-hydroxy-5,9-dimethyl-6,7-benzomorphan (cyclazocine, win-20,740,arc II-C-3). The Journal of Pharmacology and Experimental Therapeutics, 1965, 150, 426-436.

Martin, W. R., & Gorodetzky, C. W. Demonstration of tolerance to and physical dependence of n-allylnormorphine (nalorphine). The Journal of Pharmacology and Experimental Therapeutics, 1965, 150, 437-442.

Martin, W. R., & Jasinski, D. R. Assessment of abuse potential of narcotic analgesics in animals. In W. R. Martin (Ed.), Drug Addiction I. New York: Springer-Verlag, 1977.

Martin, W. R., & Sloan, J. W. Neuropharmacology and neurochemistry of subjective effects, analgesia, tolerance and dependence produced by narcotic analgesics. In W. R. Martin (Ed.), Drug Addiction I. New York: Springer-Verlag, 1977.

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

Maslow, A. Motivation and Personality. New York: Harper & Row, 1954.

Mason, S. T., & Fibiger, H. C. Anxiety: The locus coeruleus disconnection. Life Sciences, 1979, 25, 2141-2147.

Mason, S. T., Roberts, D. C. S., & Fibiger, H. C. Noradrenergic influences on catalepsy. Psychopharmacology, 1978, 60, 53-57.

Matthew, R. T., & German, D. C. Electrophysiological evidence for morphine excitation of ventral tegmental area dopamine neurons. Society for Neuroscience Abstracts, 1982, 8, 777.

McAuliffe, W. E. A second look at first effects of opiates on nonaddicts. Journal of Drug Issues, 1975, fall, 369-399.

McAuliffe, W. E., & Gordon, R. A. A test of Lindesmith's theory of addiction: The frequency of euphoria among long-term addicts. American Journal of Sociology, 1974, 79, 795-840.

McAuliffe, W. E., & Gordon, R. A. Reinforcement and the combination of effects: Summary of a theory of opiate addiction. In D. J. Lettieri, M. Sayers, and H. W. Pearson (Eds.), Theories on Drug Abuse: Selected Contemporary Perspectives. Rockville, Maryland: National Institute on Drug Abuse, 1980.

McCarten, M. D., & Lal, H. Attenuation of the discriminative stimulus strength of morphine by haloperidol. Neuropharmacology, 1979, 18, 465-467.

McCarthy, P. S., Metcalf, G., & Howe, S. J. Continuous infusion in rats as a method of assessing morphine like-physical dependence for opiates. Pharmacology Biochemistry & Behavior, 1982, 16, 725-729.

McGeer, E. G., & McGeer, P. L. Neurotoxins as tools in neurobiology. International Review of Neurobiology, 1981, 22, 173-204.

McGeer, P. L., & McGeer, E. G. Kainic acid: The neurotoxic breakthrough. CRC Critical Reviews in Toxicology, 1982, 10, 1-26.

McGilliard, K. L., & Takemori, A. E. The effect of dopaminergic modifiers on morphine-induced analgesia and respiratory depression. European Journal of Pharmacology, 1979, 54, 61-68.

McMillan, D. E., & Morse, W. H. Some effects of morphine and morphine antagonists on schedule-controlled behavior. The Journal of Pharmacology and Experimental Therapeutics, 1967, 157, 175-184.

Medgett, I. C., McCulloch, M. W., & Rand, M. J. Partial agonist action of clonidine on prejunctional and postjunctional α-adrenoceptors. Naunyn Schmiedeberg's Archives of Pharmacology, 1978, 304, 215-221.

Meites, J., Bruni, J. F., Van Vugt, D. A., & Smith, A. F. Relation of endogenous opiod peptides and morphine to neuroendocrine functions. Life Sciences, 1979, 24, 1325-1336.

Mendelson, J. H., Ellingboe, J., Keuhnle, J. C., & Mello, N. K. Effects of naltrexone on mood and neuroendocrine function in normal adult males. Psychopharmacology, 1979, 3, 231-236.

Menon, M. K., Tseng, L. F., Loh, H. H., & Clark, W. G. An electromyographic method for the assessment of naloxone-induced abstinence in morphine-dependent rats. Naunyn-Schmiedeberg's Archives of Pharmacology, 1980, 312, 43-49.

Meyer, D. R., & Sparber, S. B. Clonidine antagonizes body weight loss and other symptoms used to measure withdrawal in morphine pelleted rats given naloxone. Pharmacologist, 1976, 18, 236.

Miksic, S., Shearman, G., & Lal, H. Generalization study with some narcotic and nonnarcotic drugs in rats trained for morphine-saline discrimination. Psychopharmacology, 1978, 60, 103-104.

Miller, N. E. Experiments on motivation: Studies combining psychological, physiological, and pharmacological techniques. Science, 1957, 126, 1271-1278.

Miller, N. E. Chemical coding of behavior in the brain. Science, 1965, 148, 328-338.

Mirin, S. M., Meyer, R. E., & McNamee, H. B. Psychopathology and mood during heroin use. Archives of General Psychiatry, 1976, 33, 1503-1508.

Mishra, R. K., Marshall, A. M., & Varmuza, S. L. Supersensitivity in rat caudate nucleus: Effects of 6-hydroxydopamine on the time course of dopamine receptor and cyclic AMP changes. Brain Research, 1980, 200, 47-57.

Mitro, A., & Palkovits, M. Morphology of the Rat Brain Ventricles, Epindyma, and Periventricular Structures. Basel: Karger, 1981.

Mohrland, J. S., & Gebhart, G. F. Effects of focal electrical stimulation and morphine microinjection in the periaqueductal gray of the rat mesencephalon on neuronal activity in the medullary reticular formation. Brain Research, 1980, 201, 23-37.

Moleman, P. Modulation of dopaminergic neurotransmission by morphine in the rat. Unpublished doctoral dissertation, Erasmus University, 1977.

Moleman, P., Versluis, D. J., & Bruinvels, J. Is morphine induced catalepsy related to activation of dopaminergic neurones? Psychopharmacology, 1978, 60, 35-39.

Monaco, A. P., Hernandez, L., & Hoebel, B. G. Nucleus accumbens: Site of amphetamine self-injection: Comparison with the lateral ventricle. In R. B. Chronister and J. F. DeFrance (Eds.), The Neurobiology of the Nucleus Accumbens. Brunswick, Maine: Haer Institute for Electrophysiological Research, 1981.

Moore, K. E., & Johnston, C. A. The median eminence: aminergic control mechanisms. In E. E. Muller and R. M. MacLeod (Eds.), Neuroendocrine Perspectives Vol. 1. Amsterdam: Elsevier Biomedical Press, 1982.

Moore, K. E., & Kelly, P. H. Biochemical pharmacology of mesolimbic and mesocortical dopaminergic neurons. In M. A. Lipton, A. DiMascio, and K. F. Killam (Eds.), Psychopharmcology: A Generation of Progress. New York: Raven Press, 1978.

Moore, K. E., & Wuerthele, S. M. Regulation of nigrostriatal and tuberoinfundibular-hypophyseal dopaminergic neurons. Progress in Neurobiology, 1979, 13, 325-359.

Moore, R. Y., & Bloom, F. E. Central catecholamine neuron systems: Anatomy and physiology of the dopamine systems. Annual Review of Neuroscience, 1978, 1, 129-169.

Moore, R. Y., & Bloom, F. E. Central catecholamine neuron systems: Anatomy and physiology of the norepinephrine and epinephrine systems. Annual Review of Neuroscience, 1979, 2, 113-168.

Mora, F., Sanguinetti, A. M., Rolls, E. T., & Shaw, S. G. Differential effects on self-stimulation and motor behaviour produced by microintracranial injections of a dopamine-receptor blocking agent. Neuroscience Letters, 1975, 1, 1979-1984.

Morgane, P. J. Reinforcing effects of self-injected cholinergic agents into hypothalamic "drinking" areas in rats. Federation Proceedings, 1962, 21, 352.

Morley, J. E. The neuroendocrine control of appetite: The role of the endogenous opiates, cholecystokinin, TRH, gamma-amino-butyric-acid and the diazepam receptor. Life Sciences, 1980, 27, 355-368.

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

Mucha, R. F., Volkovskis, C., & Kalant, H. Conditioned increases in locomotor activity produced with morphine as an unconditioned stimulus, and the relation of conditioning to acute morphine effect and tolerance. Journal of Comparative and Physiological Psychology, 1981, 95, 351-362.

Muller, E. E., Locatelli, V., Cocchi, D., Spampinato, S., Apud, J., Ferri, S., & Racagni, G. Neural factors involved in the central regulation of prolactin. In R. M. MacLeod and U. Scapagnini (Eds.), Central and Peripheral Regulation of Prolactin Function. New York: Raven Press, 1980.

Muller, P., & Seeman, P. Dopaminergic supersensitivity after neuroleptics: Time-course and specificity. Psychopharmacology, 1978, 60, 1-11.

Mundl, W. J. Constant-current source for an electrolytic microinfusion transducer system. Journal of Neuroscience Methods, 1981, 4, 287-290.

Muraki, T., Tokunaga, Y., Nakadate, T., & Kato, R. Inhibition by cholinergic agonists of the prolactin release induced by morphine. Naunyn-Schmiedeberg's Archives of Pharmacology, 1979, 308, 249-254.

Murphy, M. R. Methadone reduces sexual performance and sexual motivation in the male Syrian golden hamster. Pharmacology Biochemistry & Behavior, 1981, 14, 561-567.

Murrin, L. C., Coyle, J. T., & Kuhar, M. J. Striatal opiate receptors: Pre- and postsynaptic localization. Life Sciences, 1980, 27, 1175-1183.

Murring, L. C., & Roth, R. H. Dopaminergic neurons: Effects of electrical stimulation on dopamine biosynthesis. Molecular Pharmacology, 1976, 12, 463-475.

Myers, B. M., & Baum, M. J. Facilitation of copulatory performance in male rats by naloxone: Effects of hypophysectomy, 17 alpha-entradial, and lateininzing hormone releasing hormone. Pharmacology Biochemistry & Behavior, 1980, 12, 365-370.

Myers, R. D. An intracranial chemical stimulation system for chronic or self-infusion. Journal of Applied Physiology, 1963, 18, 221-223.

Myers, R. D. Methods for chemical stimulation of the brain. In R. D. Myers (Ed.), Methods in Psychobiology, Vol. 1. New York: Academic Press, 1972.

Myers, R. D. Handbook of Drug and Chemical Stimulation of the Brain. New York: Van Nostrand Reinhold, 1974.

Myers, R. D., & Hoch, D. B. ^{14}C-dopamine microinjected into the brain-stem of the rat: Dispersion kinetics, site content and functional dose. Brain Research Bulletin, 1978, 3, 601-609.

Myslobodsky, M. S., & Braun, H. Postural asymmetry and directionality of rotation in rats. Pharmacology Biochemistry & Behavior, 1980, 13, 743-745.

Nakaki, T., Saito, M., Nakadate, T., Tokunaga, Y., & Kato, R. Acute physical dependence induced by continuous intravenous infusion of morphine or meperidine in the rat. Psychopharmacology, 1981, 73, 215-218.

Nakamura, K., Kuntzman, R., Maggio, A., & Conney, A. H. Decrease in morphine's analgesic action and increase in its cataleptic action by 6-hydroxydopamine injected bilaterally into caudate and putamen areas; Partial restoration by L-dopa plus decarboxylase inhibition. Neuropharmacology, 1973, 12, 1153-1160.

Neff, N. H., Parenti, M., Gentlemen, S., & Olianas, M. C. Modulation of dopamine receptors by opiates. In G. L. Gessa and G. U. Corsini (Eds.), Apomorphine and Other Dopaminergics, Vol. 1: Basic Pharmacology. New York: Raven Press, 1981.

Nichols, J. R. How opiates change behavior. Scientific American, 1965, 212, 80-88.

Nicolaou, N. M. Acute and chronic effects of neuroleptics and acute effects of apomorphine and amphetamine on dopamine turnover in corpus striatum and substantia nigra of the rat brain. European Journal of Pharmacology, 1980, 64, 123-132.

Nicoll, R. A., Alger, B. E., & Jahr, C. E. Enkephalin blocks inhibitory pathways in the vertebrate CNS. Nature, 1980, 287, 22-25.

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

North, R. A. Opiates, opioid peptides and single neurones. Life Sciences, 1979, 24, 1527-1546.

North, R. A., & Vitek, L. V. A study of the role of cyclic adenosine 3',5'-monophosphate in the depression by opiates and opioid peptides of excitatory junction potentials in the mouse vas deferens. British Journal of Pharmcology, 1980, 71, 307-313.

Oldendorf, W. H., Hyman, S., Braun, L., & Oldendorf, S. Z. Blood-brain barrier: Penetration of morphine, codeine, heroin, and methadone after carotid injection. Science, 1972, 178, 984-986.

Olds, J. Hypothalamic substrates of reward. Physiological Review, 1962, 42, 554-604.

Olds, J. Drives and Reinforcements: Behavioral Studies of Hypothalamic Functions. New York: Raven Press, 1977.

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

Olds, J., & Olds, M. E. Positive reinforcement produced by stimulating hypothalamus with iproniazid and other compounds. Science, 1958, 127, 1175-1176.

Olds, J., & Travis, R. P. Effects of chlorpromazine, meprobamate, pentobarbital and morphine on self stimulation. Journal of Pharmacology and Experimental Therapeutics, 1960, 128, 397-404.

Olds, J., Yuwiler, A., Olds, M. E., & Yun, C. Neurohumors in hypothalamic substrates of reward. American Journal of Physiology, 1964, 207, 242-254.

Olds, M. E. Comparative effects of amphetamine, scopolamine, chlordiazepoxide, and diphenylhydantoin on operant and extinction behavior with brain stimulation and food reward. Neuropharmacology, 1970, 9, 519-532.

Olds, M. E. Hypothalamic substrate for the positive reinforcing properties of morphine in the rat. Brain Research, 1979, 168, 351-360.

Olson, G. A., Olson, R. D., Kastin, A. J., & Coy, D. H. Endogenous opiates: 1979. Peptides, 1980, 1, 365-379.

Ostrowski, N. L., Chiodo, L. A., Keller, R., & Caggiula, A. R. Morphine produces different effects on subtypes of mesencephalic dopamine systems. In H. Takagi (Ed.), Advances in Endogenous and Exogenous Opioids: Proceedings of INRC. New York: Raven Press, 1981.

Ostrowski, N. L., Foley, T. L., Lind, M. D., & Reid, L. D. Naloxone reduces fluid intake: Effects of water and food deprivation. Pharmacology Biochemistry & Behavior, 1980, 12, 431-435.

Ostrowski, N. L., Rowland, N., Foley, T. L., Nelson, J. L., & Reid, L. D. Morphine antagonists and consummatory behaviors. Pharmacology Biochemistry & Behavior, 1981, 14, 549-559.

Palkovits, M. Isolated removal of hypothalamic or other brain nuclei of the rat. Brain Research, 1973, 59, 449-450.

Palkovits, M., & Jacobowitz, D. M. Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). Journal of Comparative Neurology, 1974, 157, 29-42.

Palmer, M. R., & Hoffer, B. J. Catecholamine modulation of enkephalin-induced electrophysiological responses in cerebral cortex. The Journal of Pharmacology and Experimental Therapeutics, 1980, 213, 205-215.

Panksepp, J., Herman, B. H., Vilberg, T., Bishop, P., & DeEskinazi, F. G. Endogenous opioids and social behavior. Neuroscience and Biobehavioral Reviews, 1978, 4, 473-487.

Panksepp, J., Zolovick, A. J., Najam, N., & Herman, B. H. Opioid modulation of ingestive behavior. Pharmacology Biochemistry & Behavior, 1981, 15, 477-484.

Parker, M., & Atkinson, J. Withdrawal syndromes following cessation of treatment with antihypertensive drugs. General Pharmacology, 1982, 13, 79-85.

Pasternak, G. W., Childers, S. R., & Snyder, S. H. Opiate analgesia: Evidence for mediation by a subpopulation of opiate receptors. Science, 1980, 208, 514-516.

Pasternak, G. W., Goodman, R., & Snyder, S. H. An endogenous morphine-like factor in mammalian brain. Life Sciences, 1975, 16, 1765-1769.

Pellegrino, L. J., Pellegrino, A. S., & Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press, 1979.

Perry, D. C., Mullis, K. B., Oie, S., & Sadée, W. Opiate antagonist receptor binding in vivo: Evidence for a new receptor binding model. Brain Research, 1980, 199, 49-61.

Pert, A. Effects of opiates on rewarding and aversive brain stimulation in the rat. Problems of Drug Dependence, 1975, 963-973.

Pert, A. The effects of opiates on nigrostriatal dopaminergic activity. In J. M. Van Ree and L. Terenius (Eds.), Characteristics and Function of Opioids. Amsterdam: Elsevier/North Holland Biomedical Press, 1978.

Pert, A., DeWald, L. A., Liao, H., & Sivit, C. Effects of opiates and opioid peptides on motor behaviors: Sites and mechanisms of action. In E. Usdin, W. E. Bunney, and N. S. Kline (Eds.), Endorphins in Mental Health Research. London: MacMillan, 1979.

Pert, A., & Sivit, C. Neuroanatomical focus for morphine and enkephalin-induced hypermotility. Nature, 1977, 265, 645-647.

Pert, A., & Yaksh, T. Site of morphine induced analgesia in the primate brain: Relation to pain pathways. Brain Research, 1974, 80, 135-140.

Pert, C. B., Kuhar, M. J., & Snyder, S. H. Autoradiographic localization of the opiate receptor in rat brain. Life Sciences, 1975, 16, 1849-1854.

Pert, C. B., Kuhar, M. J., & Snyder, S. H. The opiate receptor: autoradiographic localization in rat brain. Proceedings of the National Academy of Science, U.S.A., 1976, 73, 3729-3733.

Pert, C. B., Snowman, A. M., & Snyder, S. H. Localization of opiate receptor binding in synaptic membranes of rat brain. Brain Research, 1974, 70, 184-188.

Pert, C. B., & Snyder, S. H. Identification of opiate receptor binding in intact animals. Life Sciences, 1975, 16, 1623-1634.

Pert, C. B., Taylor, D. P., Pert, A., Herkenham, M. A. & Kent, J. L. Biochemical and autoradiographical evidence for Type 1 and Type 2 opiate receptors. In E. Costa and M. Trabucchi (Eds.), Neural Peptides and Neuronal Communication. New York: Raven Press, 1980.

Petersen, R. C., & Stillman, R. C. (Eds.), Cocaine: 1977. Rockville, Maryland: National Institute on Drug Abuse, 1977.

Peterson, G. M., & Moore, R. Y. Selective effects of kainic acid on diencephalic neurons. Brain Research, 1980, 202, 165-182.

Pettit, H. O., Ettenberg, A., Bloom, F. E., & Koob, G. F. Nucleus accumbens lesions selectively attenuate cocaine but not heroin self-administration in rats. Society for Neuroscience Abstracts, 1982, 8, 1029.

Phillips, A. G., & Fibiger, H. C. The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum, nucleus accumbens, and medial prefrontal cortex. Canadian Journal of Psychology, 1978, 32, 58-66.

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

Phillips, A. G., Mora, F., & Rolls, E. T. Intracerebral self-administration of amphetamine by rhesus monkeys. Neuroscience Letters, 1981, 24, 81-86.

Phillips, A. G., Spyraki, C., & Fibiger, H. C. Conditioned place preference with amphetamine and opiate as reward stimuli: Attenuation by haloperidol. In B. G. Hoebel and D. Novin (Eds.), The Neural Basis of Feeding and Reward. Brunswick, Maine: Haer Institute for Electrophysiological Research, 1982.

Pickens, R., Meisch, R. A., & Thompson, T. Drug self administration: An analysis of the reinforcing effects of drugs. In L. L. Iversen, S. D. Iversen, and S. H. Snyder (Eds.), Handbook of Psychopharmacology, Vol. 12. New York: Plenum Press, 1978.

Pickens, R., & Thompson, T. Cocaine-reinforced behavior in rats: effects of reinforcement magnitude and fixed-ratio size. Journal of Pharmacology and Experimental Therapeutics, 1968, 161, 122-129.

Pickens, R., & Thompson, T. Characteristics of stimulant drug reinforcement. In T. Thompson and R. Pickens (Eds.), Stimulus Properties of Drugs. New York: Appleton-Century-Crofts, 1971.

Pinching, A. J., & Powell, T. P. S. Ultrastructural features of transneuronal cell degeneration in the olfactory system. Journal of Cell Science, 1971, 8, 253-268.

Pinsky, C., Dua, A. K., & LaBella, F. S. Peptidase inhibitors reduce opiate narcotic withdrawal signs, including seizure activity, in the rat. Brain Research, 1982, 243, 301-307.

Pollard, H., Llorens, D., Bonnet, J. J., Costentin, J., & Schwartz, J. C. Opiate receptors on mesolimbic dopaminergic neurons. Neuroscience Letters, 1977, 7, 295-299.

Pollard, H., Llorens-Cortes, C., & Schwartz, J. C. Enkephalin receptors on dopaminergic neurones in rat striatum. Nature, 1977, 268, 745-747.

Post, R. M., Cutler, N. R., Jimerson, D. C., & Bunney, W. E. Dopamine agonists in affective illness: Implications for underlying receptor mechanisms. In G. U. Corsini and G. L. Gessa (Eds.), Apomorphine and Other Dopaminomimetics. Vol. 2: Clinical Pharmacology. New York: Raven Press, 1981.

Post, R. M., Lockfeld, A., Squillace, K. M., & Contel, N. R. Drug-environment interaction: Context dependency of cocaine-induced behavioral sensitization. Life Sciences, 1981, 28, 755-760.

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

Price, M. T. C., & Fibiger, H. C. Ascending catecholamine systems and morphine analgesia. Brain Research, 1975, 99, 189-193.

Prout, C. T., White, M. A., & Charry, J. Current issues in addiction research. In P. H. Hoch and J. Zubin (Eds.), Problems of Addiction and Habituation. New York: Grune & Stratton, 1958.

Puri, S. K., & Lal, H. Effects of morphine, haloperidol, apomorphine and benztropine on dopamine turnover in rat corpus striatum: Evidence showing morphine induced rotation in CNS' DA activity. Federation Proceedings, 1973, 32, 3065.

Puri, S. K., Reddy, C., & Lal, H. Blockade of dopaminergic receptors by morphine: Effect of haloperidol, apomorphine or benztropine. Research Communications in Chemical Pathology and Pharmacology, 1973, 5, 389-401.

Pycock, C. J. Turning behavior in animals. Neuroscience, 1980, 5, 461-514.

Rado, S. Narcotic bondage: A general theory of the dependence on narcotic drugs. In P. H. Hoch and J. Zubin (Eds.), Problems of Addiction and Habituation. New York: Grune & Stratton, 1958.

Ranck, J. B. Which elements are excited in electrical stimulation of mammalian central nervous system: A review. Brain Research, 1975, 98, 417-440.

Ranck, J. B. Extracellular stimulation. In M. M. Patterson and R. P. Kesner (Eds.), Electrical Stimulation Research Techniques. New York: Academic Press, 1981.

Randrup, A., & Munkvad, I. Biochemical, anatomical, and psychological investigations of stereotyped behavior induced by amphetamines. In E. Costa and S. Garattini (Eds.), Amphetamines and Related Compounds. New York: Raven Press, 1970.

Randrup, A., Munkvad, I., Fog, R., & Ayhan, I. H. Catecholamines in activation, stereotypy, and level of mood. In A. J. Friedhoff (Ed.), Catecholamines and Behavior, Vol. 1: Basic Neurology. New York: Plenum Press, 1975.

Rasor, R. W. Narcotic addicts: Personality characteristics and hospital treatment. In P. H. Hoch and J. Zubin (Eds.), Problems of Addiction and Habituation. New York: Grune & Stratton, 1958.

Redmond, D. E., & Huang, Y. H. Current concepts: II. New evidence for a locus coeruleus-norepinephrine connection with anxiety. Life Sciences, 1979, 25, 2149-2162.

Redmond, D. E., Huang, Y. H., & Gold, M. S. Anxiety: the locus coeruleus connection. Society for Neuroscience Abstracts, 1977, 3, 258.

Redmond, D. E., Huang, Y. H., Snyder, D. R., & Maas, J. W. Behavioral effects of stimulation of the locus coeruleus in the stumptail monkey (Macaca arctoides). Brain Research, 1976, 116, 502-510.

Reid, L. D. Reinforcement from the direct stimulation of the brain. Unpublished doctoral dissertation, University of Utah, 1967.

Reid, L. D., & Bozarth, M. A. Addictive agents and pressing for intracranial stimulation (ICS): The effects of various opioids on pressing for ICS. Problems of Drug Dependence, 1978, 729-741.

Reid, L. D., Lind, M. D., Bozarth, M. A., Merriman, V. J., & Stapleton, J. M. Small doses of morphine sulfate and pressing for hypothalamic intracranial stimulation (ICS) in rats. Society for Neuroscience Abstracts, 1978, 4, 501.

Renner, K. E. Delay of reinforcement: A historical review. Psychological Bulletin, 1964, 61, 341-361.

Rigter, H. Attenuation of amnesia in rats by systemically administered enkephalins. Science, 1978, 200, 83-85.

Riley, A. L., Jacobs, W. J., & LoLordo, V. M. Morphine induced taste aversions: A consideration of parameters. Physiological Psychology, 1978, 6, 96-100.

Riley, A. L., Zellner, D. A., & Duncan, H. J. The role of endorphins in animal learning and behavior. Neuroscience and Biobehavioral Reviews, 1980, 4, 69-76.

Ritchie, J. M., & Cohen, P. J. Cocaine; procaine and other synthetic local anesthetics. In L. S. Goodman and A. Gilman (Eds.), The Pharmacological Basis of Therapeutics. New York: MacMillan, 1975.

Ritzmann, R. F. Opiate dependence following acute injections of morphine and naloxone: The assessment of various withdrawal signs. Pharmacology Biochemistry & Behavior, 1981, 14, 575-577.

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

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

Roberts, D. C. S., Mason, S. T., & Fibiger, H. C. 6-OHDA lesion to the dorsal noradrenergic bundle alters morphine-induced locomotor activity and catalepsy. European Journal of Pharmacology, 1978, 52, 209-214.

Roberts, D. C. S., Zis, A. P., & Fibiger, H. C. Ascending catecholamine pathways and amphetamine-induced locomotor activity: importance of dopamine and apparent noninvolvement of norepinephrine. Brain Research, 1975, 93, 441-454.

Robertson, J., Weston, R., Lewis, M. J., & Barasi, S. Evidence for the potentiation of the antinociceptive action of morphine by bromocriptine. Neuropharmacology, 1981, 20, 1029-1032.

Robinson, J. H., & Deadwyler, S. A. Morphine excitation: Effects on field potentials recorded in the in vitro hippocampal slice. Neuropharmacology, 1980, 19, 507-514.

Roffman, M., Bernard, P. S., Dawson, K. M., Sobiski, R. E., & Saelens, J. K. The effects of haloperidol and clozapine on circling induced by electrical stimulation of the substantia nigra and the ventromedial tegmentum. Neuropharmacology, 1978, 17, 943-946.

deRopp, R. S. Drugs and the Mind. New York: St. Martin's, 1957.

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

Rossier, J., & Bloom, F. Central neuropharmacology of endorphins. In H. H. Loh and D. H. Ross (Eds.), Neurochemical Mechanisms of Opiates and Endorphins. New York: Raven Press, 1979.

Roth, R. H., Salzman, P. H., & Nowycky, M. C. Impulse flow and short-term regulation of transmitter biosynthesis in central catecholaminergic neurons. In M. A. Lipton, A. DiMascio, and K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Routtenberg, A. Intracranial chemical injection and behavior: A critical review. Behavioral Biology, 1972, 7, 601-642.

Routtenberg, A., Sladek, J., & Bondareff, W. Histochemical fluorescence after application of neurochemicals to caudate nucleus and septal area in vivo. Science, 1968, 161, 272-274.

Samanin, R., Cervo, L., Rochat, C., Poggesi, E., & Mennini, T. Reduction in the number of serotonin receptors in the brainstem of morphine dependent rats: Relation to blockade of naloxone precipitated jumping by serotonin agonists. Life Sciences, 1980, 27, 1141-1146.

Sanberg, P. R. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature, 1980, 284, 472-473.

Sanberg, P. R., Pisa, M., & Fibiger, H. C. Kainic acid injections in the striatum alter the cataleptic and locomotor effects of drugs influencing dopaminerg and cholingergic systems. European Journal of Pharmacology, 1981, 74, 347-357.

Sanchez-Blazquez, P., Garzon, J., & Del Rio, J. The role of dopaminergic mechanisms in the effect of (D-ala^2)-met-enkephalinamide on locomotor activity of mice. IRCS Medical Science: Biochemistry; Drug Metabolism and Toxicology; Nervous System; Pharmacology; Psychology and Psychiatry, 1980, 8, 703-704.

Sanger, D. J. Endorphinergic mechanisms in the control of food and water intake. Appetite: Journal for Intake Research, 1981, 2, 193-208.

Sar, M., Stumpf, W. E., Miller, R. J., Chang, K. J., & Cuatrecasas, P. Immunohistochemical localization of enkephalin in rat brain and spinal cord. The Journal of Comparative Neurology, 1978, 182, 17-37.

Saranak, J., & Goldfarb, J. Effects of electrolytic and 6-hydroxydopamine lesions of the lateral hypothalamus on rotation evoked by electrical stimulation of the substantia nigra in rats. Brain Research, 1981, 208, 81-95.

Sasame, H. A., Perez-Cruet, J., Di Chiara, G., Tagliamonte, A., Tagliamonte, P., & Gessa, G. L. Evidence that methadone blocks dopamine receptors in the brain. Journal of Neurochemistry, 1972, 19, 1953-1957.

Sawynok, J., Pinsky, C., & LaBella, F. S. Minireview on the specificity of naloxone as an opiate antagonist. Life Sciences, 1979, 25, 1621-1632.

Schechter, M. D., & Cook, P. G. Dopaminergic mediation of the interoceptive cue produced by d-amphetamine in rats. Psychopharmacologia, 1975, 42, 185-193.

Schiff, S. R. Conditioned dopaminergic activity. Biological Psychiatry, 1982, 17, 135-154.

Schreier, W. A., & Burks, T. F. Suppression of morphine withdrawal diarrhea by clonidine. Proceedings of the Western Pharmacological Society, 1981, 24, 341-345.

Schubert, P., Teschemacher, H., Kreutzberg, G. W., & Herz, A. Intracerebral distribution pattern of radioactive morphine and morphine-like drugs after intraventricular and intrathecal injection. Histochemie, 1970, 22, 277-288.

Schulz, R., Blasig, J., Laschka, E., & Herz, A. Site of naloxone-precipitated opiate withdrawal dissociates from that at which apomorphine reinitiates this phenomenon. Naunyn-Schmiedeberg's Archives of Pharmacology, 1978, 305, 1-4.

Schulz, R., Wuster, M., & Herz, A. Centrally and peripherally mediated inhibition of intestinal motility by opioids. Naunyn-Schmiedeberg's Archives of Pharmacology, 1979, 308, 255-260.

Schuster, C. R. Self-administration of drugs. In L. Goldberg and F. Hoffmeister (Eds.), Psychic Dependence: Definition, Assessment in Animals and Man, Theoretical and Clinical Implications. New York: Springer-Verlag, 1973.

Schuster, C. R., & Woods, J. H. The conditioned reinforcing effects of stimuli associated with morphine reinforcement. International Journal of Addiction, 1968, 3, 223-230.

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

Schwartz, J. C. Opiate receptors on catecholaminergic neurones in brain. Trends in NeuroScience, 1979, 2, 137-139.

Schwartz, J. C., Pollard, H., Llorens, C., Malfroy, B., Gros, C., Pradelles, P., & Dray, F. Endorphins and endorphin receptors in striatum: Relationships with dopaminergic neurons. In E. Costa and M. Trabucchi (Eds.), Advances in Biological Psychopharmacology, Vol. 18. New York: Raven Press, 1978.

Seeger, T. F., Thal, L., & Gardner, E. L. Behavioral and biochemical aspects of neuroleptic-induced dopaminergic supersensitivity: Studies with chronic clozapine and haloperidol. Psychopharmacology, 1982, 76, 182-187.

Seeman, P. Brain dopamine receptors. Pharmacological Review, 1980, 32, 229-313.

Seeman, P., Chau-Wong, M., & Moyyen, S. The adsorption and conduction-block of opiate narcotics on nerve membranes: Identical effects of levo- and dextro-enantiomers. Canadian Journal of Physiology and Pharmacology, 1972, 50, 1181-1192.

Seevers, M. H. Laboratory evaluation for drug dependence. In E. L. Way (Ed.), New Concepts in Pain and Its Clinical Management. Philadelphia: Davis, 1967.

Segal, D., & Mandell, A. Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. Pharmacology Biochemistry & Behavior, 1974, 2, 249-255.

Segal, D. S., Weinberger, S. B., Cahill, J., & McCunney, S. J. Multiple daily amphetamine administration: Behavioral and neurochemical alterations. Science, 1980, 207, 904-907.

Seiden, L. S., & Dykstra, L. A. Psychopharmcology: A Biochemical and Behavioral Approach. New York: Van Nostrand Reinhold, 1977.

Sells, S. B., Demaree, R. G., Simpson, D. D., & Joe, G. W. Evaluation of present treatment modalities: Research with DARP admissions, 1969-1973. Annals of the New York Academy of Sciences, 1978, 311, 270-281.

Shannon, H., & Holtzman, S. Evaluation of the discriminative effects of morphine in the rat. Journal of Pharmacology and Experimental Therapeutics, 1976, 198, 54-65.

Shannon, H. E., & Holtzman, S. G. Morphine training dose: A determinant of stimulus generalization to narcotic antagonists in the rat. Psychopharmacology, 1979, 61, 239-244.

Sharpe, L. G., Garnett, J. E., & Cicero, T. J. Analgesia and hyperactivity produced by intracranial microinjections of morphine into the periaqueductal gray matter of the rat. Behavioral Biology, 1974, 11, 303-313.

Sheridan, C. L. Methods in Experimental Psychology. New York: Holt, Rinehart and Winston, 1979.

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

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

Shizgal, P., Bielajew, C., Corbett, D., Skelton, R., & Yeomans, J. Behavioral methods for inferring anatomical linkage between rewarding brain stimulation sites. Journal of Comparative and Physiological Psychology, 1980, 94, 227-237.

Siggins, G. R. Electrophysiological role of dopamine in striatum: Excitatory or inhibitory? In M. A. Lipton, A. DiMascio, and K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Simon, E. J., & Hiller, J. M. The opiate receptors. Annual Review of Pharmacology and Toxicology, 1978, 18, 371-394.

Siviy, S. M., Calcagnetti, D. J., & Reid, L. D. Opioids and palatability. In B. G. Hoebel and D. Novin (Eds.), The Neural Basis of Feeding and Reward. Brunswick, Maine: Haer Institute for Electrophysiological Research, 1982.

Skinner, B. F. Science and Human Behavior. New York: The Free Press, 1953.

Sklar, L. S., & Amit, Z. Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs. Neuropharmacology, 1977, 16, 649-655.

Smee, M. L., & Overstreet, D. H. Alterations in the effects of dopamine agonists and antagonists on general activity in rats following chronic morphine treatment. Psychopharmacology, 1976, 49, 125-130.

Smith, J. E., Co, C., Crenshaw, C. M., Barr, T. S., & Lane, J. D. The effects of kainic acid lesions of the nucleus accumbens on intravenous morphine self administration. Society for Neuroscience Abstracts, 1981, 7, 51.

Smith, J. E., Co, C., Freeman, M. E., & Lane, J. D. Brain neurotransmitter turnover correlated with morphine seeking behavior of rats. Pharmacology Biochemistry & Behavior, 1982, 16, 509-519.

Smith, J. E., Co, C., Freeman, M. E., Sands, M. P., & Lane, J. D. Neurotransmitter turnover in rat striatum is correlated with morphine self-administration. Nature, 1980, 287, 152-154.

Smith, J. R., & Simon, E. J. Endorphins, opiate receptors, and their evolving biology. In H. L. Ioachim (Ed.), Pathobiology Annual 1981, Vol. 11. New York: Raven Press, 1981.

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

Smith, S. G., & Davis, W. M. A method for chronic intravenous drug adminstration in the rat. In S. Ehrenpreis and A. Neidle (Eds.), Methods in Narcorics Research. New York: Marcel Dekker, 1975.

Snyder, S. H., Banerjee, S. P., Yamamura, H. I., & Greenberg, D. Drugs, neurotransmitters, and schizophrenia: Phenothiazines, amphetamines, and enzymes synthesizing psychotomimetic drugs aid schizophrenia research. Science, 1974, 184, 1243-1252.

Snyder, S. H., & Matthysse, S. Opiate Receptor Mechanisms: Neurochemical and Neurophysiological Processes in Opiate Drug Action and Addiction. Cambridge: Massachusetts Institute of Technology Press, 1975.

Sokoloff, L. The deoxyglucose method for the measurement of local glucose utilization and the mapping of local functional activity in the central nervous system. International Review of Neurobiology, 1981, 22, 287-333.

Sorensen, J. E., Hargreaves, W. A., & Weinberg, J. A. Withdrawal from heroin in three or six weeks: Comparison of methadyl acetate and methadone. Archives of General Psychiatry, 1982, 39, 167-171.

Sorensen, J. L., Hargreaves, W. A., & Weinberg, J. A. Heroin addict responses to six weeks of detoxification with LAAM. Drug and Alcohol Dependence, 1982, 9, 79-87.

Sparber, S. B., Gellert, V. F., & Fossom, L. On the use of operant behavior to study the neuropsychopharmacology of opiates with special reference to morphine and its relationship to dopamine in the central nervous system. In H. H. Loh and D. H. Ross (Eds.), Neurochemical Mechanisms of Opiates and Endorphins. New York: Raven Press, 1979.

Sparber, S. B., & Meyer, D. R. Clonidine antagonizes naloxone-induced suppression of conditioned behavior and body weight loss in morphine-dependent rats. Pharmacology Biochemistry & Behavior, 1978, 9, 319-325.

Spealman, R. D., & Goldberg, S. R. Drug self-administration by laboratory animals: Control by schedules of reinforcement. Annual Review of Pharmacology and Toxicology, 1978, 18, 313-339.

Spindler, J. The effect of central dopamine cell body lesions on stimulation-induced feeding. Unpublished Master's Thesis, Concordia University, 1975.

Spragg, S. D. S. Morphine addiction in chimpanzees. Comparative Psychology Monographs, 1940, 15, 1-132.

Stanley, M. E., & Glick, S. D. Interaction of drug effects with testing procedures on the measurement of catalepsy. Neuropharmacology, 1976, 15, 393-394.

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

Stefano, G. B., Catapane, E. J., & Kream, R. M. Characterization of the dopamine stimulated adenylate cyclase in the pedal ganglia of mytilus edulis: Interactions with etorphine, β-endorphin, DALA, and methionine enkephalin. Cellular and Molecular Neurobiology, 1981, 1, 57-68.

Stein, E. A., & Olds, J. Direct intracerebral self administration of opiates in the rat. Society for Neuroscience Abstracts, 1977, 3, 302.

Stein, E. A., & Rodd, D. A new injection technique for intracerebral drug administration in behaving animals. Pharmacology Biochemistry & Behavior, 1980, 12, 815-817.

Stein, L. Reward transmitters: Catecholamines and opioid peptides. In M. A. Lipton, A. DiMascio and K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Stein, L. The chemistry of reward. In A. Routtenberg (Ed.), Biology of Reinforcement. New York: Academic Press, 1980.

Stein, L., & Belluzzi, J. D. Brain endorphins and the sense of well-being: A psychobiological hypothesis. In E. Costa and M. Trabucchi (Eds.), Advances in Biochemical Psychopharmacology, Vol. 18. New York: Raven Press, 1978.

Stein, L., & Belluzzi, J. D. Brain endorphins: Possible role in reward and memory formation. Federation Proceedings, 1979, 38, 2468-2472.

Stewart, J., & Eikelboom, R. Pre-exposure to morphine and the attenuation of conditioned taste aversion in rats. Pharmacology Biochemistry & Behavior, 1978, 9, 639-645.

Stewart, J. J., Weisbrodt, N. W., & Burks, T. F. Central and peripheral actions of morphine on intestinal transit. The Journal of Pharmacology and Experimental Therapeutics, 1978, 205, 547-555.

Stewart, R. B., & Grupp, L. A. An investigation of the interaction between the reinforcing properties of food and ethanol using the place preference paradigm. Progress in Neuro-Psychopharmacology, 1981, 5, 609-613.

Stinus, L., Koob, G. F., Ling, N., Bloom, F. E., & Le Moal, M. Locomotor activation induced by infusion of endorphins into the ventral tegmental area: Evidence for opiate-dopamine interactions. Proceedings of the National Academy of Science, 1980, 77, 2323-2327.

Stolerman, I. P., Pilcher, C. W. T., & D'Mello, G. D. Stereospecific aversive property of narcotic antagonists in morphine-free rats. Life Sciences, 1978, 22, 1755-1762.

Stretch, R., & Gerber, G. J. Drug-induced reinstatement of amphetamine self-administration behaviour in monkeys. Canadian Journal of Psychology, 1973, 27, 168-177.

Stricker, E. M., & Zigmond, M. J. Recovery of function following damage to central catecholamine-containing neurons: A neurochemical model for the lateral hypothalamic syndrome. In J. M. Sprague and A. E. Epstein (Eds.), Progress in Physiological Psychology and Psychobiology. New York: Academic Press, 1976.

Sun, A. Y., & Seaman, R. N. Physicochemical approaches to the alcohol-membrane interaction in brain. Neurochemical Research, 1980, 5, 537-545.

Takimoto, G. S., & Weiner, N. Evidence for the transsynaptic activation of tyrosine hydroxylase in rabbit protal vein mediated through a cyclic AMP-independent mechanism. The Journal of Pharmacology and Experimental Therapeutics, 1981, 219, 97-106.

Tallarida, R. J., Cowan, A., & Adler, M. W. pA_2 and receptor differentiation: A statistical analysis of competitive antagonism. Life Sciences, 1979, 25, 637-654.

Tallarida, R. J., & Jacob, L. S. The Dose-Response Relation in Pharmacology. New York: Springer-Verlag, 1979.

Tarpy, R. M., & Sawabini, F. L. Reinforcement delay: A selective review of the last decade. Psychological Bulletin, 1974, 81, 984-997.

Tatum, A. L., and Seevers, M. H. Experimental Cocaine Addiction. The Journal of Pharmacology and Experimental Therapeutics, 1929, 36, 401-410.

Tatum, A. L., & Seevers, M. H. Theories of drug addiction. Physiological Reviews, 1931, 11, 107-121.

Tatum, A. L., Seevers, M. H., & Collins, K. H. Morphine addiction and its physiological interpretation based on experimental evidences. Journal of Pharmacology and Experimental Therapeutics, 1929, 36, 447-475.

Teasdale, J. A. P., Bozarth, M. A., & Stewart, J. Body temperature responses to microinjections of morphine in brain sites containing opiate receptors. Society for Neuroscience Abstracts, 1981, 7, 799.

Teiger, D. G. Induction of physical dependence on morphine, codeine and meperidine in the rat by continuous infusion. The Journal of Pharmacology and Experimental Therapeutics, 1974, 190, 408-415.

Teitelbaum, P., & Epstein, A. N. The lateral hypothalamic syndrome: Recovery of feeding and drinking after lateral hypothalamic lesions. Psychological Review, 1962, 69, 74-90.

Terenius, L. Endogenous peptides and analgesia. Annual Review of Pharmacology and Toxicology, 1978, 18, 189-204.

Terenius, L., & Wahlstrom, A. Search for an endogenous ligand for the opiate receptor. Acta Physiologica Scandinavica, 1975, 94, 74-81.

Teschemacher, H., Opheim, K. E., Cox, B. M., & Goldstein, A. A peptide-like substance from pituitary that acts like morphine. I. Isolation. Life Sciences, 1975, 16, 1771-1776.

Thompson, T. Drugs as reinforcers: Experimental addiction. International Journal of Addictions, 1968, 3, 199-206.

Thompson, T., & Pickens, R. Stimulus Properties of Drugs. New York: Appleton-Century-Crofts, 1971.

Thompson, T., & Schuster, C. R. Morphine self administration, food-reinforced and avoidance behaviors in rhesus monkeys. Psychopharmacologia, 1964, 5, 87-94.

Thoolen, M. J. M. C., Timmermans, P. B. M. W. M., and Van Zwieten, P. A. The clonidine withdrawal syndrome. Its reproduction and evaluation in laboratory animal models. General Pharmacology, 1981, 12, 303-308.

Thornburg, J. E., & Moore, K. E. The relative importance of dopaminergic and noradrenergic neuronal systems for the stimulation of locomotor activity induced by amphetamine and other drugs. Neuropharmacology, 1973, 12, 853-866.

Thornburg, J. E., & Moore, K. E. Supersenstivity to dopaminergic agonists induced by haloperidol. In B. K. Bernard (Ed.), Aminergic Hypotheses of Behavior: Reality or Cliche? Rockville, Maryland: National Institute on Drug Abuse, 1975.

Thorn-Gray, B. E., Levitt, R. A., Hill, J., & Ward, K. A neuroanatomical study of analgesia and catatonia induced by etorphine in the rat. Neuropharmacology, 1981, 20, 763-767.

Tissari, A. H., & Gessa, G. L. New evidence for dopamine receptors in synaptosomes controlling dopamine synthesis. In G. L. Gessa and G. U. Corsini (Eds.), Apomorphine and Other Dopaminomimetics, Vol. 1: Basic Pharmacology. New York: Raven Press, 1981.

Tremblay, E. C., & Charton, G. Anatomical correlates of morphine-withdrawal syndrome: Differential participation of structures located within the limbic system and striatum. Neuroscience Letters, 1981, 23, 137-142.

Turski, W., Czuczwar, S. J., Turski, L., & Kleinrok, Z. Bilateral injection of kainic acid into the rat striatum potentiates morphine, arecoline and pilocarpine but not haloperidol catalepsy. Methods and Findings in Experimental and Clinical Pharmacology, 1982, 4, 287-291.

Tye, N. C., Horsman, L., Wright, F. C., & Pullar, I. A. Differential enhancement of locomotor activity by dopamine agonists following chronic morphine treatment. Psychopharmacology, 1979, 63, 313-315.

Uhl, G. R., Goodman, R. R., Kuhar, M. J., Childer, S. R., & Snyder, S. H. Immunohistochemical mapping of enkephalin-containing cell bodies, fibers and nerve terminals in the brainstem of the rat. Brain Research, 1979, 166, 75-94.

Uhl, G. R., Goodman, R. R., Kuhar, M. J., & Snyder, S. H. Enkephalin and neurotensin: Immunohistochemical localization and identification of an amygdalofugal pathway. In E. Costa and M. Trabucchi (Eds.), Advances in Biochemical Psychopharmacology, Vol. 18. New York: Raven Press, 1978.

Ungerstedt, U. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiologica Scandinavica, 1971a, Suppl. 367, 1-48.

Ungerstedt, U. Striatal dopamine release after amphetamine or nerve degeneration revealed by rotational behavior. Acta Physiologica Scandinavica, 1971b, Suppl. 367, 49-68.

Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiologica Scandinavica, 1971c, Suppl. 367, 69-93.

Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiologica Scandinavica, 1971d, Suppl. 367, 95-122.

Ungerstedt, U. Use of intracerebral injection of 6-hydroxydopamine as a tool for morphological and functional studies on central catecholamine neurons. In T. Malmfors and H. Thoenen (Eds.), 6-Hydroxydopamine and Catecholamine Neurons. New York: Elsevier, 1971e.

Ungerstedt, U. The role of dopamine as a neurotransmitter in the central and the autonomic nervous system. Acta Endocrinologica, 1978, 216, 13-26.

Ungerstedt, U., Butcher, L. L., Butcher, S. G., Andén, N. E., & Fuxe, K. Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat. Brain Research, 1969, 14, 461-471.

Valenstein, E. S. Problems of measurement and interpretation with reinforcing brain stimulation. Psychological Review, 1964, 71, 415.

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

Van der Kooy, D., & Phillips, A. Temporal analysis of naloxone attenuation of morphine-induced taste aversion. Pharmacology Biochemistry & Behavior, 1977, 6, 637-641.

Van Dongen, P. A. M., Broekkamp, C. L. E., & Cools, A. R. Locus coeruleus and substantia nigra: Involvement in morphine-induced behavior. Brain Research Bulletin, 1979, 4, 307-311.

Van Loon, G. R., DeSouza, E. B., Ho, D., & Shin, S. H. β-endorphin-induced prolactin secretion is mediated by suppression of release of newly synthesized hypothalamic dopamine. Canadian Journal of Physiology and Pharmacology, 1980, 58, 436-439.

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

Vedernikov, Y. P. The influence of single and chronic morphine administration on some central effects of amphetamine and apomorphine. Psychopharmacologia, 1970, 17, 283-288.

Villarreal, J. E. The effects of morphine agonists and antagonists on morphine-dependent rhesus monkeys. In H. W. Kosterlitz, H. O. J. Collier, and J. E. Villarreal (Eds.), Agonist and Antagonist Actions of Narcotic Analgesic Drugs. Baltimore: University Park Press, 1973.

Villarreal, J. E., Guzman, M., & Smith, C. B. A comparison of the effects of d-amphetamine and morphine upon the locomotor activity of mice treated with drugs which alter brain catecholamine content. Journal of Pharmacology and Experimental Therapeutics, 1973, 187, 1-18.

Villarreal, J. E., & Karbowski, M. G. The actions of narcotic antagonists in morphine-dependent rhesus monkeys. In M. C. Braude, L. S. Harris, E. L. May, J. P. Smith, and J. E. Villarreal (Eds.), Narcotic Antagonists: Advances in Biochemical Psychopharmacology, Vol. 8. New York: Raven Press, 1974.

Waldmeier, P. C., Lauber, J., Blum, W., & Richter, W. J. 3-Methoxytyramine: Its suitability as an indicator of synaptic dopamine release. Naunyn-Schmiedeberg's Archives of Pharmacology, 1981, 315, 219-225.

Walinder, J., Balldin, J., Bokstrom, K., Karlsson, I., & Lundstrom, B. Clonidine suppression of the alcohol withdrawal syndrome. Drug and Alcohol Dependence, 1981, 8, 345-348.

Wand, P., Kuschinsky, K., & Sontag, K. H. Morphine-induced muscular rigidity in rats. European Journal of Pharmacology, 1973, 24, 189-193.

Washton, A. M., & Resnick, R. B. Clonidine for opiate detoxification: Outpatient clinical trials. The American Journal of Psychiatry, 1980, 137, 1121-1122.

Washton, A. M., & Resnick, R. B. Clonidine in opiate withdrawal: Review and appraisal of clinical findings. Pharmacotherapy, 1981a, 1, 140-146.

Washton, A. M., & Resnick, R. B. The clinical use of clonidine in outpatient detoxification from opiates. In H. Lal and S. Fielding (Eds.), Psychopharmacology of Clonidine. New York: Alan R. Liss, 1981b.

Watson, S. J., Akil, H., Berger, P. A., & Barchas, J. D. Some observations on the opiate peptides and schizophrenia. Archives of General Psychiatry, 1979, 36, 35-41.

Watson, S. J., & Barchas, J. D. Anatomy of the endogenous opioid peptides and related substances: The enkephalins, β-endorphin, β-lipotropin, and ACTH. In R. F. Beers and E. G. Bassett (Eds.), Mechanisms of Pain and Analgesic Compounds. New York: Raven Press, 1979.

Waud, D. R. Analysis of dose-response curves. In E. E. Daniel and D. M. Paton (Eds.), Methods in Pharmacology, Vol. 3: Smooth Muscle. New York: Plenum Press, 1975.

Wauquier, A., & Rolls, E. T. (Eds.), Brain-stimulation Reward. New York: American Elsevier, 1976.

Way, E. L., Loh, H. H., & Shen, F. H. Simultaneous quantitative assessment of morphine tolerance and physical dependence. The Journal of Pharmacology and Experimental Therapeutics, 1969, 167, 1-8.

Wayner, M. J., Ono, T., & Nolley, D. Effects of ethyl alcohol on central neurons. Pharmacology Biochemistry & Behavior, 1975, 3, 499-506.

Webb, E. J., Campbell, D. T., Schwartz, R. D., & Sechrest, L. Unobtrusive-Measures: Nonreactive Research in the Social Sciences. Chicago: Rand McNally College Publishing, 1966.

Weeks, J. R. Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. Science, 1962, 138, 143-144.

Weeks, J. R., & Collins, R. J. Patterns of intravenous self-injection by morphine-addicted rats. Research Publication of The Association for Nervous and Mental Disorders, 1968, 46, 288-298.

Weeks, J. R., & Collins, R. J. Dose and physical dependence as factors in the self-administration of morphine in rats. Psychopharmacology, 1979, 65, 171-177.

Wei, E. T. Enkephalin analogs and physical dependence. Journal of Pharmacology and Experimental Therapeutics, 1981, 216, 12-18.

Wei, E., & Loh, H. Physical dependence on opiate-like peptides. Science, 1976, 193, 1262-1263.

Wei, E., Loh, H. H., & Way, E. L. Brain sites of precipitated abstinence in morphine-dependent rats. The Journal of Pharmacology and Experimental Therapeutics, 1973, 185, 108-115.

Welzl, H., Flack, H. G., & Huston, J. P. Contraversive circling and facilitation of the perioral biting reflex by injection of Substance P or d-Ala^2-Met-Enkephalinamide into the substantia nigra. Behavioral and Neural Biology, 1982, 34, 104-108.

Westerink, B. H. C. Effect of centrally acting drugs on regional dopamine metabolism. In P. J. Roberts, G. N. Woodruff, and L. L. Iversen (Eds.), Advances in Biochemical Psychopharmacology, Vol. 19. New York: Raven Press, 1978.

Westerink, B. H. C. Further studies on the sequence of dopamine metabolism in the rat brain. European Journal of Pharmacology, 1979, 56, 313-322.

Westerink, B. H. C., & Korf, J. Regional rat brain levels of 3,4-dihydroxyphenylacetic acid and homovanillic acid: Concurrent fluorometric measurement and influence of drugs. European Journal of Pharmacology, 1976, 38, 281-291.

Westerink, B. H. C., & Spaan, S. J. Estimation of the turnover of 3-methoxytyramine in the rat striatum by HPLC with electrochemical detection: Implications for the sequence in the cerebral metabolism of dopamine. Journal of Neurochemistry, 1982a, 38, 342-347.

Westerink, B. H. C., & Spaan, S. J. On the significance of endogenous 3-methoxytyramine for the effects of centrally acting drugs on dopamine release in the rat brain. Journal of Neurochemistry, 1982b, 38, 680-686.

White, N., Sklar, L., & Amit, Z. The reinforcing action of morphine and its paradoxical side effect. Psychopharmacology, 1977, 52, 63-66.

Wikler, A. A theory of opioid dependence. In D. J. Lettieri, M. Sayers, and H. W. Pearson (Eds.), Theories on Drug Abuse: Selected Contemporary Perspectives. Rockville, Maryland: National Institute on Drug Abuse, 1980.

Wikler, A., Fraser, H. F., & Isbell, H. N-allylnormorphine: Effects of single doses and precipitation of acute "abstinence syndromes" during addiction to morphine, methadone or heroin in man (post-addicts). The Journal of Pharmacology and Experimental Therapeutics, 1953, 107, 8-20.

Wikler, A., Martin, W. R., Pescor, F. T., & Eades, C. A. Factors regulating oral consumption of an opioid (etonitazine) by morphine-addicted rats. Psychopharmacologia, 1963, 5, 55-76.

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

Wikler, A., Pescor, F. T., Miller, D., & Porrell, H. Persistent potency of a secondary (conditioned) reinforcer following withdrawal of morphine from physically dependent rats. Psychopharmcologia, 1971, 20, 103-117.

Wilcox, R. E., & Levitt, R. A. Naloxone reversal of morphine catatonia: Role of caudate and periaqueductal gray. Pharmacology Biochemistry & Behavior, 1978, 9, 425-428.

Winer, B. Statistical Principles in Experimental Design. New York: McGraw-Hill, 1971.

Wise, R. A. Lateral hypothalamic electrical stimulation: Does it make animals "hungry"? Brain Research, 1974, 67, 187-209.

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

Wise, R. A. Neuroleptic attenuation of intracranial self stimulation: Reward or performance deficits? Life Sciences, 1978b, 22, 535-542.

Wise, R. A. Action of drugs of abuse on brain reward systems. Pharmacology Biochemistry & Behavior, 1980, 13, 213-223.

Wise, R. A. Brain dopamine and reward. In S. J. Cooper (Ed.), Theory in Psychopharmacology, Vol. 1. New York: Academic Press, 1981.

Wise, R. A. Neuroleptics and operant behavior: The anhedonia hypothesis. The Behavioral and Brain Sciences, 1982, 5, 39-88.

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

Wise, R. A., Spindler, J., deWit, H., & Gerber, G. J. Neuroleptic-induced "anhedonia" in rats: Pimozide blocks reward quality of food. Science, 1978, 201, 262-264.

Wise, R. A., Spindler, J., & Legault, L. Major attenuation of food reward with performance-sparing doses of pimozide in the rat. Canadian Journal of Psychology, 1978, 32, 77-85.

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

deWit, H., & Stewart, J. Reinstatement of cocaine reinforced responding in the rat. Psychopharmacology, 1981, 75, 134-143.

deWit, H., & Wise, R. A. 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, 1977, 31, 195-203.

Wollemann, M. Endogenous opioids and cyclic AMP. Progress in Neurobiology, 1981, 16, 145-154.

Wood, G. Fundamentals of Psychological Research. Boston: Little, Brown, 1974.

Wood, P. L., Nair, N. P. V., & Bozarth, M. A. Striatal 3-methoxytyramine as an index of dopamine release: Effects of electrical stimulation. Neuroscience Letters, 1982, 32, 291-294.

Wood, P. L., Stotland, M., Richard, J. W., & Rackham, A. Actions of mu, kappa, sigma, delta and agonist/antagonist opiates on striatal dopaminergic function. The Journal of Pharmacology and Experimental Therapeutics, 1980, 215, 697-703.

Woods, J. H. Behavioral pharmacology of drug self administration. In M. A. Lipton, A. DiMascio, & K. F. Killam (Eds.), Psychopharmacology: A Generation of Progress. New York: Raven Press, 1978.

Woods, J. H., & Schuster, C. R. Reinforcement properties of morphine, cocaine and SPA as a function of unit dose. International Journal of Addiction, 1968, 3, 231-237.

Woods, J. H., & Schuster, C. R. Opiates as reinforcing stimuli. In T. Thompson & R. Pickens (Eds.), Stimulus Properties of Drugs. New York: Appleton Century-Crofts, 1971.

Yaksh, T. L., & Rudy, T. A. Analgesia mediated by a direct spinal action of narcotics. Science, 1976, 192, 1357-1358.

Yaksh, T. L., & Rudy, T. A. Studies on the direct spinal action of narcotics in the production of analgesia in the rat. Journal of Pharmacology and Experimental Therapeutics, 1977, 202, 411-428.

Yaksh, T. L., Yeung, J. C., & Rudy, T. A. Systematic examination in the rat of brain sites sensitive to the direct application of morphine: Observation of differential effects within the periaqueductal gray. Brain Research, 1976, 114, 83-103.

Yamamoto, B. K., & Freed, C. R. The trained circling rat: A model for inducing unilateral caudate dopamine metabolism. Nature, 1982, 298, 467-468.

Yamamoto, B. K., Lane, R. F., & Freed, C. R. Normal rats trained to circle show asymmetric caudate. Life Sciences, 1982, 30, 2155-2162.

Yang, H. Y., Hong, J. S., & Costa, E. Regional distribution of leu- and met-enkephalin in rat brain. Neuropharmacology, 1977, 16, 303-307.

Yang, H. Y. T., Hong, J. S., Fratta, W., & Costa, E. Rat brain enkephalins: Distribution and biosynthesis. In E. Costa and M. Trabucchi (Eds.), Advances in Biochemical Psychopharmacology, Vol. 18. New York: Raven Press, 1978.

Yates, F. E. Use of the minipump for prolonged, continuous microinfusions into the brains of small animals. ALZET Technical Bulletin, 1977, 1, 1-3.

Yates, F. E. A new ALZET osmotic minipump: Model 2001. New features at new low prices. ALZET Technical Bulletin, 1979, 3, 1-2.

Yates, F. E. Advance information on a new ALZET product--The model 2ML1 osmotic pump. ALZET Technical Bulletin, 1981, 5, 1-2.

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

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

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

York, D. H. The neurophysiology of dopamine receptors. In A. S. Horn, J. Korf, and B. H. C. Westerink (Eds.), The Neurobiology of Dopamine. New York: Academic Press, 1979.

Young, A. M., Swain, H. H., & Woods, J. H. Comparison of opioid agonists in maintaining responding and in suppressing morphine withdrawal in rhesus monkeys. Psychopharmacology, 1981, 74, 329-335.

Young, A. M., & Woods, J. H. Behavior maintained by intravenous injection of codeine, cocaine, and etorphine in the rhesus macaque and the pigtail macaque. Psychopharmacology, 1980, 70, 263-271.

Young, P. T. The role of affective processes in learning and motivation. Psychological Review, 1959, 66, 104-125.

Zarevics, P., & Setler, P. E. Simultaneous rate-independent and rate-dependent assessment of intracranial self stimulation: Evidence for the direct involvement of dopamine in brain reinforcement mechanisms. Brain Research, 1979, 169, 499-512.

Zieglgansberger, W., French, E. D., Siggins, G. R., & Bloom, F. E. Opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurons. Science, 1979, 205, 415-417.

Zieglgansberger, W., Siggins, G., French, E., & Bloom, F. E. Effects of opioids on single unit activity. In J. M. Van Ree and L. Terenius (Eds.), Characteristics and Function of Opioids. Amsterdam: Elsevier/North Holland Biomedical Press, 1978.

Zinberg, N. E., Harding, W. M., Stelmack, S. M., & Marblestone, R. A. Patterns of heroin use. Annals of The New York Academy of Sciences, 1978, 311, 10-24.

Zinberg, N. E., & Jacobson, R. C. The natural history of "chipping." American Journal of Psychiatry, 1976, 133, 37-40.

Zis, A. P., & Fibiger, H. C. Neuroleptic-induced deficits in food and water regulation: Similarities to the lateral hypothalamic syndrome. Psychopharmacologia, 1975, 43, 63-68.