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Neural Mechanisms Underlying a Conditioned Place Preference Induced by Morphine

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April, 1995

A Thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Doctor of Philosophy

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"There is no subject

which has been the occasion

of so much controversy

as the modus operandi of opium."

H. Handy (1791)
"An Inaugural Dissertation on Opium"

ABSTRACT

The present study used the conditioned place preference (CPP) paradigm to examine the neural mechanisms underlying morphine's rewarding effect in the rat. Of thirteen sites tested with intra-cerebral morphine injections, only the ventral tegmental area (VTA) and periaqueductal gray (PAG) produced a CPP, suggesting that morphine's rewarding effect is initiated by an action at these sites. The CPPs induced by intra-VTA and intra-PAG morphine may be produced by different mechanisms because animals conditioned with these two injections exhibited different patterns of behaviour during testing. Injections of a quaternary opioid antagonist into either the VTA or PAG blocked a CPP to systemic morphine, confirming that opiate-induced reward is mediated via opioid receptors in these sites. Lesions of the pedunculopontine tegmental nucleus (PPTg), ventral striatum (VS), PAG, or fornix reduced a CPP to morphine, although PAG and fornix lesioned animals displayed a CPP when tested in a drugged state. These findings suggest that PPTg and VS lesions reduce the rewarding effect of morphine, and that PAG and fornix lesions disrupt the ability to retrieve information about the relationship between conditioned and unconditioned stimuli.

RESUME

Dans cette étude, les mécanismes neuronaux à la base de l'effet renforçant de la morphine ont été examinés à l'intérieur du paradigme de préférence positionelle conditionneé (PPC). Parmi les treize emplacements étudiés par injections intra-cérébrales de morphine, seulement les injections aux sites *area tegmenti ventraiis* (VTA) et *substantia grisea periventricularis* (PGA) ont démontré une PPC, ce qui suggère que l'effet renforçant de la morphine relèvent de ces structures. Les PPCs induits par injections de morphine à ces deux emplacements sont probablement produits par différents mécanismes puisque les animaux conditionnés par ces deux types d'injections démontraient différents patrons de comportement. L'injection d'un antagoniste opioïde, soit au VTA ou au PGA, a bloqué l'acquisition d'une PPC à la morphine, ce qui confirme que l'effet de récompense induit par les opiacés est médié par les récepteurs opioïdes de ces structures. Des lésions aux sites *nucleus tegmenti peduculopontinus* (PPTg), *neostriatum ventralis* (VS), PGA ou au fornix ont reduit la PPC à la morphine; cependant, les animaux avec lésions au PGA et au fornix démontraient une PPC lorsqu'ils étaient sous l'influence de la drogue étudiée. Ces résultats suggèrent que les lésions au PPTg et au VS réduient l'effet renforçant de la morphine, et que les lésions au PAG et au fornix rompent notre habileté de cueillir l'information portant sur la relation entre les stimuli conditionnels et inconditionnels.

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To the memory of George Allen Jr.

LIST OF ABBREVIATIONS

5-HT	Serotonin
6-OHDA	6-hydroxydopamine
ACh	Aceytlcholine
CNS	Central Nervous System
CPP	Conditioned Place Preference
CPu	Caudate Putamen
DA	Dopamine
DHBA	Dihdroxybenzylamine Hydrochloride
DMI	Desmethylimipramine
LA	Lateral Nucleus of the Amygdala
LH	Lateral Hypothalamus
LV	Lateral Ventricles
MFC	Medial Frontal Cortex
MLR	Mesencephalic Locomotor Region
NA	Noreadrenaline
NAS	Nucleus Accumbens Septi
NM	Naloxone Methiodide
NMDA	N-methyl-D-aspartate
OT	Olfactory Tubercle
PAG	Periacqueductal Gray
PH	Posterior Hypothalamus
PPTg	Pedunculopontine Tegmental Nucleus
SN	Substantia Nigra
VP	Ventral Pallidum
VS	Ventral Striatum
VTA	Ventral Tegmental Area

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STATEMENT OF ORIGINAL CONTRIBUTION

Previous evidence has indicated that opiates produce their rewarding effect by activating endogenous systems in the CNS. The present series of studies attempted to elucidate the neural mechanisms underlying morphine's rewarding effect using the conditioned place preference (CPP) paradigm as a measure of reward.

Experiment 1 examined where morphine's rewarding effect is initiated by testing whether microinjections of morphine into discrete brain regions produced a CPP. Previous studies have suggested that morphine's rewarding effect may be produced through an action at a number of neural sites, including the ventral tegmental area (VTA), the ventral striatum (VS), the periacqueductal gray (PAG), the lateral hypothalamus (LH), and the hippocampus. Results from many of these studies have been challenged, however, because doses producing the effect were much higher than those required to produce an effect with intra-ventricular or intra-VTA injections. The present study addressed this issue by comparing behavioural effects of all microinjections to those produced by injections into the lateral ventricles (LV). In the present study, only the VTA and the PAG were identified as sites where morphine's rewarding effect is initiated. Although both of these results confirm previous evidence, the present study extended the findings by analyzing and comparing behavioural responding during CPP testing of animals conditioned with intra-VTA and intra-PAG morphine injections. Based on this analysis, it was suggested that the two injections produce a CPP through different mechanisms.

Experiment 2 tested whether the rewarding effect of systemically administered morphine is mediated through an action at opioid receptors in these

two positively identified sites. Injections of an opioid receptor antagonists into either the VTA or the PAG prior to conditioning with systemic morphine blocked the development of a CPP. This is the first report that blockade of opioid receptors in the VTA or the PAG reduces morphine's rewarding effect in the CPP paradigm.

Experiments 3 and 4 examined which neural structures are necessary for morphine's rewarding effect by testing whether lesions of different brain regions blocked the development of a CPP to morphine. The first region to be tested was the mesolimbic dopamine (DA) system. Some reports indicate that morphine's rewarding effect is mediated through this system, whereas others suggest that it is not. One study reported that lesions of the mesolimbic DA system block an opiate-induced CPP (Spyraki et al., 1983) but this study used an biased CPP paradigm, the results of which may be confounded by non-specific factors including stress and anxiety. During the course of the present research project, another report that mesolimbic DA lesions block a morphine-induced CPP in a balanced CPP paradigm was published (Shippenberg et al., 1993). Despite the fact that DA depletion in Experiment 3 was greater than in the Shippenberg et al. (1993) study, these lesions had no affect on the development of a CPP to morphine. Because the results failed to replicate previous evidence, they are included in the present investigation. Previous evidence has also shown that lesions of the pedunculopontine tegmental nucleus (PPTg) block a morphine-induced CPP. The effect of PPTg lesions on a morphine-induced CPP was re-examined in the present study in order to assess changes in behavioural responding during CPP testing in greater detail than has been done in previous studies. PPTg lesions completely eliminated a CPP in both drug free and state dependent tests

supporting previous evidence that the region is critically involved in morphine's rewarding effect.

The present investigation also examined the role of six other neural sites in morphine's rewarding effect. It is the first study to test whether lesions of the VS, the ventral pallidum (VP), the PAG, the hippocampus, the lateral amygdala (LA), or the caudate-putamen (CPu) block a morphine-induced CPP. Lesions of the VS reduced, but did not eliminate, a preference for the morphine-paired compartment suggesting that this region is involved in, but is not necessary for, morphine's rewarding effect. Lesions of the fornix or the PAG blocked a morphine-induced CPP although lesioned animals displayed a CPP when they were tested in a drugged state. These findings suggest that PAG and fornix lesions disrupt the ability to retrieve information about the relationship between conditioned and unconditioned stimuli. One of the most unexpected findings was that lesions of the LA did not block a morphine-induced CPP. This finding is contrary to the abundance of evidence indicating that the LA is involved in the formation of stimulus-reward associations.

Finally, the present investigation is the first to undertake a detailed and extended analysis of behavioural responding during CPP testing. It suggests that opiate-induced reward may be mediated through more than one neural substrate, that the development of a CPP may be produced through more than one mechanism, and that the development of a CPP may be affected by factors that are not necessarily related to the rewarding properties of the drug.

GENERAL INTRODUCTION

The first undisputed reference to opium is by the Greek writer Theophrastus in his treatise on plants from the third century B.C. (Hort, 1916). It is likely, however, that the opium plant was cultivated and ingested by humans prior to this period, at least as far back as the Bronze Age (Merlin, 1984). Writings from the classical Greek era indicate that the opium poppy was valued for its medicinal, nutritive, and psychoactive properties (Latimer & Golberg, 1981). Over the centuries opium use has continued largely for the same reasons.

The German physicist Sertürner isolated an active alkaloid from the opium poppy in 1806 (Jaffe & Martin, 1993). Because of its strong ability to induce sleep, he named the substance morphine, after Morpheus the Greek god of dreams. Subsequently, the alkaloids codeine, papaverine, and thebaine were also isolated. Alkaloids of the opium poppy may be chemically modified to produce other substances such as heroin, nalorphine, levorphanol, and naloxone. This entire class of drugs is called opiates; the term refers to the alkaloids derived from the opium poppy and their synthetic congeners. In contrast, opioids are substances with opiate-like action that are chemically unrelated to morphine and not derived from the opium poppy. This class includes the synthetic drugs methadone, meperidine, and fentanyl, as well as the endogenous neuropeptides whose pharmacological properties are similar to those of opiates.

Opiate administration induces a variety of effects including drowsiness, mental cloudiness, analgesia, and physiological changes in respiration, body temperature, and gastrointestinal functioning (Jaffe & Martin, 1993). Opiates may also produce euphoric effects in humans (McAuliffe & Gordon, 1974; Woods, Smith, Mezihradsky, & Swain, 1979), although initial administration to naive

subjects is reported to be dysphoric (Abbott et al., 1992; Smith & Beecher, 1959, 1962). One of the most pronounced effects of opiates, and that which has fascinated scientists and lay people for centuries, is their ability to motivate behaviour. This topic is the focus of the present investigation.

Reward and Reinforcement

The question of how opiates produce their motivational effects relates to their role in processes of reward and reinforcement. The terms reward and reinforcement are often used interchangeably by psychologists, but this practice has been criticized (White, 1989; Wise, 1989). A clear definition of the two terms is necessary if their role in motivational processes is to be discussed and examined.

In the vernacular, the term reward refers to a renumeration or recompense for work. Its first use in the psychological literature is by Thorndike (1898), who retained the vernacular definition. In his studies of operant conditioning, a reward was the stimulus presented to animals when they correctly performed a task. Yerkes (1907) and his colleagues (Grindley, 1929; Hoge & Stocking, 1912) also used reward as a nominative reference to a stimulus, but recognized that some stimuli are capable of influencing the response that produces them. Reward, therefore, was defined as a stimulus which increases the probability that the behaviour leading to its presentation will be repeated.

Central to the work of Thorndike and Yerkes was the notion that rewards increase operant responding because they produce pleasurable affective states. This premise is based on the view expounded by the adaptive hedonists in the nineteenth century (most notably Bain, Spencer, and Bentham) that behaviour is

controlled by the desire to maximize pleasure and minimize pain. The idea can be traced further back in history to the fifth century B.C.: Aristipuss (c.a. 435-356 B.C.), Epicurus (341-270 B.C.), and their followers emphasized that happiness in life is to be attained through striving for pleasure. To Thorndike, the belief that rewarding stimuli have hedonic value was fundamental to the issues he was investigating: "How are pleasurable results able to burn in and render predominant the association which led to them. This is perhaps the greatest problem of both human and animal psychology" Thorndike, 1898, p. 103).

In the early part of this century, psychologists in the American School of Behaviourism emphasized that behaviour should be described in terms of observable events. They avoided terms implying affective states or internal causes that could not be measured directly. For this reason, Skinner objected to Thorndike's use of pleasurable, satisfying, or rewarding, and adopted the term reinforcer to describe stimuli that increase the probability of a behavioural response (Skinner, 1935). It is perhaps at this point that the distinction between reward and reinforcement became blurred.

Skinner may have advocated the use of reinforcement in scientific writings, but the first psychologist to use the term was probably William James. In an 1876 essay on free will as a determinant of behaviour, James suggested that internal forces of deliberation oppose each other and compete to control behaviour until "one representation recurs with such a degree of reinforcement that the tumult ceases" (James, 1920, p. 31). Volition is the outcome. Although James made no attempt to specify the mechanisms of internal opposition, he did speculate on whether the intensity or degree of reinforcement that determines behaviour could

be known. His use of reinforcement, therefore, suggests that the process involves a strengthening; it reflects the etymology of the term which is re-enforce, or enforce again.

Few psychologist consider James' reference to reinforcement to be the origin of its contemporary usage. Rather, it is through Pavlov's work that the term became familiar (Wise, 1989). In the translation of his lectures (Pavlov, 1928), reinforcement refers to a process whereby the association between unconditioned and conditioned stimuli is strengthened. This definition may have been based on Sherington's (1906) use of the term: reinforcement describes how reflex arcs with a common output can strengthen each other when they are simultaneously activated. While accepting that reinforcement reflects a strengthening, Skinner restricted its usage to observable behavioural output.

Reinforcement, according to Skinner, described the increased probability of a behavioural response without reference to its affective influences or strengthened connections. He may have recognized that the two processes are dissociable but he advocated the position that the study of behavioural output was the only domain of psychology. In contrast, Thorndike accepted and discussed the distinction between affective and strengthening processes: "There is no pleasure with the association. The pleasure does not come until after the association is come and gone. ... the connection thus stamped in is not contemporaneous, but prior to the pleasure" (Thorndike, 1898, p. 104).

In this dissertation, the induction of positive affect and the strengthening of an association will be discussed as separate processes. Following the example of White (1989), they will be referred to as reward and reinforcement respectively. As Skinner pointed out, the problem with defining reward as an affective state is

that it can not be measured directly. Despite this criticism, Young undertook a direct and objective study of hedonic processes (Young, 1936, 1959, 1966; Young & Christensen, 1962). While recognizing that reward implied a state of positive affect, he proposed that the term be operationally defined as a stimulus that induces approach behaviour. Measurements of reward in contemporary psychology are based on this model (White, 1989) and will be adopted in the present studies.

Empirical investigations of this dissertation are primarily concerned with mechanisms by which opiates motivate behaviour. In this context, processes of reward and reinforcement are difficult to distinguish because behaviour may be controlled by its affective consequences (Thorndike, 1898, 1911). That is, stimuli may be both rewarding and reinforcing, the latter by virtue of the former. In the tradition of contemporary theorists (Bechara & van der Kooy, 1989; Bozarth, 1989; Phillips, Pfaus, & Blaha, 1991; Robbins, Cador, Taylor, & Everitt, 1989; White, Messier, & Carr, 1987; Wise & Rompre, 1989), the term reward will be used to discuss the influence of stimuli on motivated behaviours. This practice is based on the premise that the rewarding and motivational properties of stimuli are inherently linked. Processes of reinforcement contribute to the expression of motivated behaviours, but in the present discussion usage of the term will be restricted: reinforcement will refer specifically to a strengthened association. Finally, although reward and reinforcement will be described and discussed as separate phenomena, it is understood that the two processes may be mediated by the same underlying process (Wise, 1989).

Behavioural Measures of Reward

The rewarding effects of opiates, and other abused drugs, are most frequently examined using the self-administration and conditioned place preference (CPP) paradigms. In the former, drug administration is contingent upon the performance of an operant, for example, a lever press. Animals learn to associate the operant and its consequences, that is the lever press and drug administration. If the drug administration induces rewarding effects, it will increase the probability that the operant will be repeated.

The CPP paradigm involves a series of conditioning sessions in which drug administration is paired with distinctive environmental stimuli. On test day, animals are given the choice between spending time in the environment previously paired with drug administration and one paired with neutral stimuli. Preference for the drug-paired compartment reflects the development of a CPP. It also indicates that the drug administration is rewarding because animals approach and maintain contact with drug-paired stimuli.

Evidence from both self-administration and CPP studies suggest that opiates are rewarding: they will be self-administered by a variety of laboratory animals (Deneau, Yanagita, & Seevers, 1969; Schuster & Thompson, 1969; Woods & Schuster, 1968; Weeks, 1962), and produce a preference for an environment paired with their administration (Beach, 1957). Given that the rewarding effect of opiates may be assessed in self-administration and CPP tests, these two paradigms have been used extensively to examine the neural mechanisms underlying opiate-induced reward. The present study is an extension of these investigations.

Opiate-Induced Reward and Endogenous Systems

An understanding of the neural mechanisms mediating the rewarding effect of opiates has been enhanced by pharmacological investigations. The discoveries that opiates bind specifically to receptor sites in the central nervous system (CNS) (Pert, Kuhar, & Snyder, 1976; Simon, Hiller, & Edelman, 1973; Terenius, 1973) and that the brain contains neuropeptides that mimic the action of opiate agonists (Bradbury, Smyth, & Snell, 1976; Hughes, 1975; Simantov & Snyder, 1976) suggest that opiates produce their effects by activating endogenous systems. The rewarding effect of opiates appears to be mediated via this mechanism because animals will develop a CPP to morphine, but not to its inactive isomer (Mucha & Herz, 1986; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), and opioid antagonists block a CPP (Hand, Stinus, & Le Moal, 1989) or self-administration (Britt & Wise, 1983; Corrigall & Vaccarino, 1988; Vaccarino, Bloom, & Koob, 1985) induced by opiates.

Initial investigations into endogenous opioid systems revealed that the pharmacological profiles of morphine and its congeners differ (Gilbert & Martin, 1976; Martin, Eases, Thompson, Huppler, & Gilbert, 1976). For example, administration of opiates with different chemical structures elicit different behavioural responses. Moreover, withdrawal symptoms produced by chronic administration of one opiate substance can not always be alleviated by administration of another. Based on this evidence, it was proposed that different effects produced by opiate administration are mediated through distinct populations of receptors (Gilbert & Martin, 1976; Lord, Waterfield, Hughes, & Kosterlitz, 1977; Martin, Eases, Thompson, Huppler, & Gilbert, 1976). The recent cloning of *u*, *d* and *k* opioid receptors (Chen, Mestek, Lui, Hurley, & Yu, 1993; Evans, Keith,

Morrison, Magendzo, & Edwards, 1992; Keiffer, Befort, Gaveriaux-Ruff, & Hirth, 1992; Meng et al., 1993; Thompson, Mansour, Akil, & Watson, 1993; Wang et al., 1993; Yasuda et al., 1993), confirms the existence of opioid receptor subtypes.

Reward may be mediated through an action at *u* or *d* receptors because intra-cerebral administration of agonists at either receptor produce a CPP (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Bals-Kubik, Shippenberg, & Herz, 1990; Barr & Rossi, 1992; Bozarth, 1987b; Motta & Brandao, 1993; Olds, 1982; Phillips & LePiane, 1980; Shippenberg, Bals-Kubik, & Herz, 1987; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and support self-administration (Bozarth & Wise, 1981b, 1984; Devine & Wise, 1994; Goeders, Lane, & Smith, 1984; Olds, 1982). Given that *u* antagonists do not affect a CPP to *d* agonists and *d* antagonists do not affect a CPP to *u* agonists (Shippenberg, Bals-Kubik, & Herz, 1987), rewarding effects which are mediated via *u* and *d* opioid receptors may be independent. In support of this suggestion, *u* but not *d* receptor antagonists produce extinction-like responding for intravenous heroin (Negus et al., 1993).

Animals will not self-administer opioids with *k* receptor agonist activity (Woods, Smith, Mezihradsky, & Swain, 1979) and antagonists at the *k* receptor do not affect responding for intravenous heroin (Negus et al., 1993). Furthermore, *k* receptor agonists produce conditioned place and taste aversions (Bals-Kubik, Herz, & Shippenberg, 1989; Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Mucha & Herz, 1985; Shippenberg & Herz, 1987; Shippenberg, Emmett-Oglesby, Ayesta, & Herz, 1988) as well as aversive effects in humans (Pfeiffer, Brantl, Herz, & Emrich, 1986; Pfeiffer, 1991). The rewarding effect of opiates, therefore, appear to be produced by an action at *u* and *d* but not *k* receptors.

Because opiate-induced reward is mediated through specific populations of receptors, the rewarding effect of different opiates may be related to their affinities for opioid receptor subtypes. Morphine, which is the prototypical opiate, acts predominantly at *u* receptors (Simantov, Childers, & Snyder, 1978). Heroin (diacetylmorphine) is a semi-synthetic derivative of morphine that is rapidly hydrolysed to 6-acetylmorphine and more slowly to morphine following its administration (Nakamura, Thornton, & Noguchi, 1975; Way, Young, & Kemp, 1965). Heroin does not bind to opioid receptors but acts primarily as a lipid soluble prodrug for the central distribution of morphine (Inturris et al., 1983; Gianutsos et al., 1986). The primary action of heroin, therefore, is produced via an action at *u* opioid receptors. In summary, the evidence outlined in this section suggests that the rewarding effects of opiates are mediated through endogenous systems, specifically through an action at the *u* opioid receptor in the CNS.

Neural Mechanisms Underlying Motivational Processes

If opiates produce their rewarding effect through endogenous systems, they may do so by activating the same neural substrates that mediate the motivational properties of natural rewards (Fibiger, 1978; Wise, 1980, 1982; Wise & Bozarth, 1987). Early investigations suggested that motivated behaviours are mediated through neurons in the lateral hypothalamus (LH) because stimulation of the area induces feeding (Delgado & Anand, 1953; Miller, 1960) and drinking behaviour (Greer, 1955; Mogenson & Stevenson, 1966), and LH lesions produce aphagia (Anand & Brobeck, 1951) and adipsia (Montemurro & Stevenson, 1957). With the development of a neurotoxin that selectively destroys dopamine (DA) neurons (Ungerstedt, 1968), it became apparent that deficits induced by LH lesions were

due to destruction of DA fibers that pass through the LH (Fibiger, Ais, & McGeer, 1973; Marshall & Teitelbaum, 1973; Ungerstedt, 1971) rather than to neurons that are intrinsic to the region. This suggested that processes of motivation are mediated through dopaminergic mechanisms.

Neuroanatomical projections of DA systems were mapped and classified in the mid 1960s (Dahlstrom & Fuxe, 1964). Motivated behaviours were associated with the mesolimbic DA system (Anden, Carlsson, & Haggendal, 1969; Anden et al., 1966; Fuxe & Hokfelt, 1969) which originates in the ventral tegmental area (VTA) and medial substantia nigra (SN) and projects rostrally to the ventral striatum (VS), the prefrontal and cingulate cortices, the amygdala, and the hippocampus (Fallon & Moore, 1978; Lindvall & Bjorklund, 1974; Lindvall, Bjorklund, Moore, & Steveni, 1974; Simon, Le Moal, & Calas, 1979; Swanson, 1982; Ungerstedt, 1971). Although mesolimbic DA fibers terminate in a number of sites, the system that has been the focus of studies examining motivated behaviours is the projection from the VTA to the VS.

The suggestion that mesolimbic DA projections to the VS are important in motivated behaviours is supported by the following evidence: injections of DA agonists into the VS induce feeding (Evans & Vaccarino, 1986; Wise, Fortuhi, & Colle, 1989) and potentiate responding for a stimulus associated with food (Burns, Robbins, & Everitt, 1993), water (Cador, Robbins, & Everitt, 1989; Taylor & Robbins, 1986), or sexual (Everitt, Cador, & Robbins, 1989) rewards; DA release in the VS is stimulated during feeding (Blackburn, Phillips, Jakubovic, & Fibiger, 1986; Church, Justice, & Neill, 1987; Hernandez & Hoebel, 1988a; Hernandez & Hoebel, 1988b; Radhakishum, Van Ree, & Westernik, 1988); finally lesions of the mesolimbic DA system block a CPP for a food-paired environment (Spyraki,

Fibiger, & Phillips, 1982a) and reduce the ability of DA to potentiate responding for a conditioned reinforcer (Taylor & Robbins, 1986).

The mesolimbic DA system may be involved in processes of motivation as a mediator of the rewarding effect of incentive stimuli (Wise, Spindler, de Wit, & Gerber, 1978; Wise, 1982). Known as the anhedonia hypothesis, this proposal suggests that disruption of dopaminergic mechanisms reduces the affective properties of rewarding stimuli. It is based on evidence that DA antagonists produce deficits in responding for food or water reward (Nakajima, 1986; Wise, 1978) and that these changes are similar to the behavioural effects of extinction (Gerber, Sing, & Wise, 1981; Mason, Beninger, Fibiger, & Phillips, 1960; Wise, Spindler, de Wit, & Gerber, 1978; Wise, Spindler, & Legault, 1978). Moreover, when DA antagonists are administered intermittently during training to run an alley for food or water, animals exhibit an increased resistance to extinction that is similar to the effects produced by a partial reinforcement schedule (Ettenberg & Camp, 1986a, 1986b).

Challenges to the anhedonia hypothesis arose when it was reported that mesolimbic DA lesions do not attenuate food or water intake (Kelley & Stinus, 1985; Koob, Riley, Smith, & Robbins, 1978; Le Moal et al., 1977; Robbins & Koob, 1980). In addition, evidence from *in vivo* voltammetry studies demonstrates that mesolimbic DA release occurs in anticipation of reward, but not during its consumption (Blackburn, Phillips, Jakubovic, & Fibiger, 1986; Blackburn, Phillips, Jakubovic, & Fibiger, 1989). It appears, therefore, that mesolimbic DA does not mediate the motivational properties of primary rewards, but may be involved in secondary or conditioned rewarding effects.

In order to account for this evidence, it has been proposed that DA release in the VS gates or facilities behavioural responses to motivationally relevant stimuli (Mogenson, 1987), facilitates motivated behaviours (Kelley & Stinus, 1985), has a permissive role in motivated behaviours (Blackburn, Pfaus, & Phillips, 1992), modulates behavioural arousal associated with incentive motivation (Phillips, Pfaus, & Blaha, 1991), amplifies the transfer of rewarding information from one structure to another (Cador et al., 1991), or mediates the pull of external stimuli, but not the push of internal drives or motives (Wise & Rompre, 1989).

One of the primary targets of mesolimbic DA projections is the nucleus accumbens septi (NAS) region of the VS. Anatomical evidence suggests that this region is an important component in systems mediating motivated behaviours. The NAS receives afferents from limbic and corticolimbic structures including the amygdala, the hippocampus, and parts of the frontal cortex (De France, Marchand, Stanley, Sikes, & Chronister, 1980; Heimer & Wilson, 1975; Kelley & Domesick, 1982; Kelley, Domesick, & Nauta, 1982; Kelley & Stinus, 1984; Krettek & Price, 1978; McGeorge & Faull, 1989). In turn, it projects to structures associated with motor functioning including the ventral pallidum (VP) and SN (Cohen, Kimes, & London, 1991; Swanson & Cowan, 1975). The VP sends fibers to the pedunculopontine tegmental nucleus (PPTg) (Heimer, Switzer, & Van Hoesen, 1982; Swanson, Mogenson, Gerfen, & Robinson, 1984), a region that is associated with the initiation of forward locomotion (Garcia-Rill, 1991; Garcia-Rill, Houser, Skinner, Smith, & Woodward, 1987). Because it receives input from limbic structures associated with emotion and transmits information to locomotor generating sites the NAS may represent a neural region wherein emotional signals

are translated into action (Mogenson, 1987; Mogenson, Jones, & Yim, 1980; Mogenson & Yang, 1991).

Using locomotor activity as a measure of motivated behaviour, Mogenson and his colleagues investigated the role of the NAS-VP-PPTg connections in processes of motivation (Brudzynski, Houghton, Brownlee, & Mogenson, 1986; Brudzynski & Mogenson, 1985; Brudzynski, Wu, & Mogenson, 1988; Jones, Mogenson, & Wu, 1981; Mogenson & Nielsen, 1984; Mogenson, Swanson, & Wu, 1985; Mogenson & Wu, 1986; Mogenson, Wu, & Tsai, 1989; Milner & Mogenson, 1988). Based on evidence from these studies, they suggested that the NAS is a region of limbic-motor interface (Mogenson, 1987; Mogenson, Jones, & Yim, 1980), and that projections from the VP to the PPTg contribute to the expression of motivated behaviours by producing locomotor responses during food procurement or escape (Mogenson, 1984). Recently, both the VP and PPTg have been implicated in motivational processes because lesions of either structure reduce a CPP to food (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992a; McAlonan, Robbins, & Everitt, 1993).

Over the last 50 years, research examining the neural mechanisms underlying motivational processes has demonstrated that motivation and its behavioural expression may be produced through a circuit that includes DA projections from the VTA to the VS, VS projections to the VP, and VP projections to the PPTg.

Neural Mechanisms Underlying the Rewarding Effect of Morphine

Based on the premise that drugs of abuse are rewarding because they activate substrates associated with motivation, Koob and Bloom (1988) suggested

that a neural circuit which includes the mesolimbic DA system, the VS, the VP, and the PPTg mediates the rewarding effect of abused drugs. According to these authors, drugs of abuse may access the system at different points, however, they all elicit their rewarding effects by activating this neural circuit of drug reward. The rewarding effect of stimulants depends critically on structures within the circuit (Pulvirenti, Swerdlow, Hubner, & Koob, 1991), but the role of each site in opiate-induced reward has not been determined.

Three factors suggest that opiates may produce their effects through activation of the mesolimbic system: opioid receptors are located on or near cell bodies of the mesolimbic DA neurons (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987; Moskowitz & Goodman, 1984); morphine increases single unit activity of VTA DA neurons (Gysling & Wang, 1983; Matthews & German, 1984); and systemic or intra-VTA administration of *u* receptor agonists increases DA release in the VS (Di Chiara & Imperato, 1988a, 1988b; Leone, Pocock, & Wise, 1991; Spangel, Herz, & Shippenberg, 1990). The ability of opiates to activate VTA DA neurons may be related to their rewarding effect because intra-VTA injections of *u* receptor agonists produce a CPP (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Barr & Rossi, 1992; Bozarth, 1987b; Phillips & LePiane, 1980) and support self-administration (Bozarth & Wise, 1981b, 1984; Devine & Wise, 1994). More importantly, the boundary of VTA sites in which morphine injections are rewarding corresponds to the distribution of DA cells within the region (Bozarth, 1983).

Although an action of opiates in the VTA appears to be rewarding, it is not clear whether opiate-induced reward depends on the mesolimbic DA system. For example, systemic administration of DA antagonists have been reported both to block (Bozarth & Wise, 1981a; Leone & Di Chiara, 1987; Schwartz & Marchok,

1974; Shippenberg & Herz, 1987, 1988) or not affect (Bechara, Harrington, Nader, & van der Kooy, 1992; Nader, Bechara, Roberts, & van der Kooy, 1994) an opiate-induced CPP. In some studies, mesolimbic DA lesions blocked a CPP to opiates (Spyraki, Fibiger, & Phillips, 1983; Shippenberg, Bals-Kubik, & Herz, 1993), but in another they did not (Mackey & van der Kooy, 1985). Furthermore, self-administration of opiates is not disrupted by DA antagonists (Ettenberg, Pettit, Bloom, & Koob, 1982; Gerrits, Ramsey, Wolterink, & Van Ree, 1994; Van Ree & Ramsey, 1987) or by lesions of the mesolimbic DA system (Pettit, Ettenberg, Bloom, & Koob, 1984).

The role of the VS cell bodies in the rewarding effect of opiates is also the subject of considerable controversy. Lesions of the VS are reported to block a CPP to morphine (Kelsey, Carlezon, & Falls, 1989) but the electrolytic lesions employed in this study would have destroyed fibers passing through the VS. Although lesions of VS cell bodies disrupt responding for heroin self-administration (Zito, Vickers, & Roberts, 1985), the effect is not as pronounced as the effect of the same lesions on cocaine self-administration (Zito, Vickers, & Roberts, 1985). Similarly, microinjection studies examining the role of VS cell bodies in opiate-induced reward have produced inconsistent results. Morphine microinjections into the VS produced a CPP in one study (van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), but the high dose used may have produced behavioural effects through an action at other neural sites (Wise & Hoffman, 1992). Some reports indicate that animals will self-administer opiates into the VS (Goeders, Lane, & Smith, 1984; Olds, 1982) although one study suggested that they will not (Bozarth, 1983). Finally, there is disagreement whether microinjections of opioid antagonists

into the VS disrupt heroin self-administration (Britt & Wise, 1983; Vaccarino, Bloom, & Koob, 1985).

There is preliminary evidence that both the VP and the PPTg are involved in mediating the rewarding effect of opiates. VP lesions reduce responding for heroin self-administration, however lesions in this study may not have been in the VP proper (Hubner & Koob, 1990). PPTg lesions block a CPP to morphine (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1989; Olmstead & Franklin, 1993) or heroin (Nader, Bechara, Roberts, & van der Kooy, 1994), and disrupt the acquisition of responding for intravenous heroin (Olmstead, Munn, & Wise, 1993). Although these findings suggest that the PPTg is involved in the rewarding effect of opiates, lesion-induced deficits have been attributed to disruptions in motivation (Bechara, Harrington, Nader, & van der Kooy, 1992), associative learning (Fujimoto, Ikeguchi, & Yoshida, 1992), or cognitive functioning (Steckler, Inglis, Winn, & Sahgal, 1994).

In addition to these four sites (mesolimbic DA, VS, VP, PPTg), there is evidence that other neural regions may also be involved in the rewarding effect of opiates. For example, animals will self-administer morphine into the LH (Cazala, Darracq, & Saint-Marc, 1987; Cazala, 1990; Olds, 1979) and develop a CPP for an environment paired with intra-LH injections of morphine (van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982). Administration of opiates into the periacqueductal gray (PAG) may also produce a CPP (Motta & Brandao, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and support self-administration (Cazala, 1990). In addition, blockade of opioid receptors in the PAG disrupts heroin self-administration (Corrigall & Vaccarino, 1988), suggesting that opiate-induced reward is mediated in this region. The hippocampus has also been

implicated in the rewarding effect of morphine because morphine injections into the region produce a CPP (Corrigall & Linseman, 1988). In contrast to these reports, there is evidence that the LH, the PAG, and the hippocampus are not involved in the rewarding effect of opiates: LH lesions do not block self-administration of heroin (Britt & Wise, 1981); one report suggests that animals will not self-administer morphine into the PAG (Bozarth & Wise, 1984); and lesions of the hippocampus decrease the threshold dose required to maintain responding for intravenous morphine (Glick & Cox, 1978), suggesting that the rewarding effect of morphine is increased by destruction of the hippocampal region.

The evidence summarized in this section indicates that the neural mechanisms underlying morphine's rewarding effect remain elusive. It is plausible that morphine-induced reward is mediated through the neural circuit of drug reward, but experimental evidence has not confirmed this proposal. Studies examining the role of mesolimbic DA or VS cell bodies in the rewarding effect of opiates have produced inconsistent results. The question of whether the VP mediates opiate-induced reward merits further investigation because the results from studies examining this possibility are inconclusive. Findings from studies examining the role of the PPTg in opiate-induced reward are consistent, but interpretations of them are not. Finally, a number of other neural sites have also been implicated in the rewarding effect of morphine, but their role in mediating the effect has not been thoroughly tested.

The Present Investigation

The present set of experiments were designed to investigate the neural mechanisms underlying the rewarding effect of morphine. The issue was

addressed by considering two questions: Where is morphine's rewarding effect initiated, and what structures are involved in mediating the effect? The evidence outlined above demonstrates that a number of neural sites have been implicated in both the initiation and the mediation of morphine-induced reward. Nonetheless, there is little consensus on which structures contribute to the effect. It is difficult to evaluate conflicting evidence because methodologies, drug doses, and control groups varied across studies. The present study, therefore, re-evaluated the role of several structures in the rewarding effect of morphine using a consistent protocol throughout an extended series of studies.

The CPP paradigm was used to assess the rewarding effect of morphine because it has a number of advantages over other behavioural measurements of reward (Carr, Fibiger, & Phillips, 1989; Swerdlow, Gilbert, & Koob, 1989; van der Kooy, 1987): first, a CPP may be produced at doses lower than those that maintain self-administration -- animals will develop a preference for an environment paired with injections of as little as 0.08 mg/kg morphine (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982), 0.5 mg/kg d-amphetamine (Spyraki, Fibiger, & Phillips, 1982c) or 0.05 mg/kg heroin (Nader, Bechara, Roberts, & van der Kooy, 1994); second, a CPP may be induced with a single morphine injection (Bardo & Neisewander, 1986; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982); third, a CPP may be retained for up to one month (Mucha & Iversen, 1984); fourth, testing is conducted under drug free conditions, thereby eliminating confounds of sensory and/or motor changes that may influence responding; fifth, drug dose is determined and controlled by the experimenter, whereas in the self-administration paradigm the animal's rate of responding determines the dose that is administered; finally, the fact that animals will show a CPP to food (Bechara, Harrington, Nader,

& van der Kooy, 1992; McDonald & White, 1993; Papp, 1988; Spyraki, Fibiger, & Phillips, 1982a; Swerdlow, van der Kooy, Koob, & Wenger, 1983; Tombaugh, Grandmaison, & Zito, 1982), sexual partners (Miller & Baum, 1987), and a number of abused drugs including amphetamine (Gilbert & Cooper, 1983; Hiroi & White, 1991; Reicher & Holman, 1977; Sherman, Roberts, Roskam, & Holman, 1980; Spyraki, Fibiger, & Phillips, 1982c), apomorphine (Papp, 1988; Spyraki, Fibiger, & Phillips, 1982; van der Kooy, Koob, & Swerdlow, 1983), cocaine (Mackey & van der Kooy, 1985; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Phillips, Broekkamp, & Fibiger, 1983; Spyraki, Fibiger, & Phillips, 1982b), heroin (Bozarth & Wise, 1981a; Nader, Bechara, Roberts, & van der Kooy, 1994; Spyraki, Fibiger, & Phillips, 1983; Schenk, Hunt, Colle, & Amit, 1983; Schenk, Ellison, Hunt, & Amit, 1985), and morphine (Beach, 1957; Katz & Gormezano, 1978; Kumar, 1972; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Rossi & Reid, 1976; Sherman, Roberts, Roskam, & Holman, 1980), suggests that it is a valid measure of the rewarding effect of appetitive stimuli.

The first set of experiments addressed the question of where the rewarding effect of morphine is initiated. The literature cited above indicates that it is produced through endogenous circuits, specifically via an action at *u* opioid receptors in the CNS. Because *u* receptors are located in many neural sites (Pert, Kuhar, & Snyder, 1976; Mansour, Khachaturian, Lewis, Akil, & Watson, 1987), the populations which mediate opiate-induced reward must be identified experimentally. The possibility that morphine acts at one or more of thirteen specific neural sites to produce its rewarding effect was examined by testing whether microinjections of nanomolar concentrations of morphine into each region produced a CPP. To determine that morphine microinjections produced their

rewarding effect via an action at opioid receptors, an opioid antagonist was injected into positively identified sites prior to CPP conditioning with systemic morphine. If morphine-induced reward is produced through an action at opioid receptors in a specific brain locus, blocking receptors in the region should disrupt the development of a CPP to morphine.

The CPP paradigm is particularly advantageous as a measure of reward in microinjection studies because injection dose and volume are controlled by the experimenter; variations in drug diffusion and the possibility that the drug is acting at multiple sites is thereby minimized. Furthermore, few conditioning trials (intracerebral injections) are required to produce a CPP; the use of multiple intracerebral injections introduces problems such as infection and necrosis.

The second set of experiments examined which neural sites are necessary for the mediation of morphine-induced reward. The role of eight different neural sites was examined by testing the effect of lesions of each region on the development of a CPP to morphine. If morphine's rewarding effect is mediated through a particular structure, lesions of the site should disrupt the development of a CPP to morphine. The effects of mesolimbic DA, VS, VP, and PPTg lesions were tested because processes of motivation appear to be mediated through a circuit composed of these structures, and the rewarding effects of drugs may be mediated through the same system. The effects of PAG, hippocampus, lateral nucleus of the amygdala (LA), and caudate putamen (CPu) lesions were also tested because these regions have been implicated in opiate-induced reward, or in processes that may be involved in the expression of a CPP.

GENERAL METHODS

Subjects

All experiments were carried out in accordance with the guidelines for the ethical use of animals in biomedical research outlined by the Canadian Council on Animal Care and the McGill University Animal Care Committee.

Subjects were four hundred and seventy male Long Evans rats (Charles River, St. Constance, Quebec), weighing 275-325 g at the beginning of the experiment. Animals were housed individually on a 12 hours on and 12 hours off light/dark schedule with *ad libitum* food and water except during experimental procedures. Testing was conducted during the light phase.

Surgical Procedures

All surgical procedures were conducted under xylazine (0.5 mg/kg Romprin i.m.) and sodium pentobarbital (40-45 mg/kg i.p.) anaesthesia. Animals were secured in a stereotaxic apparatus in the flat skull position according to the protocol described by Paxinos and Watson (1986). All animals were monitored for at least 6 hours following surgery.

Microinjection Studies

In Experiments 1 and 2 animals received intra-cerebral injections of drug or vehicle during conditioning sessions. These animals were surgically prepared by implanting stainless-steel, 23G guide cannulae (bilaterally) 1.5 mm above the intended site of injection. Stainless steel stylettes (00G insect pins), 1 mm longer than the guide cannulae, were inserted into the guide cannulae to keep them free of debris. Animals were left to recover at least 7 days before behavioural testing began.

Lesion Studies

In Experiments 3 and 4 animals received bilateral lesions of different brain sites prior to behavioural testing. Neurotoxic lesions were induced by infusing the neurotoxin through a 30G stainless-steel injector connected to a section of polyethylene tubing (PE-10). The tubing was connected to a 10 *ul* Hamilton syringe mounted on an infusion pump (Harvard Apparatus, Mass.). Injectors were left in place for an additional 5 minutes following neurotoxic infusions. With the exception of 6-hydroxydopamine (6-OHDA) (Experiment 3), neurotoxins were dissolved in phosphate buffer vehicle, pH 7.0. 6-OHDA was dissolved in a solution of 0.3 mg/ml ascorbic acid in 0.9% saline and was stored in melting ice during use. For each lesion, control subjects (shams) were lesioned by infusing vehicle into the intended site.

Electrolytic or radio-frequency lesions were induced by passing current through a nichrome electrode (0.25 mm in diameter) with enamel insulation. The tips of the electrodes were exposed by removing the insulation with Strip X (GC Electronics). Control subjects (shams) were lesioned by lowering electrodes without turning on the current. Animals were left to recover for two weeks before behavioural testing began.

Apparatus

Conditioned Place Preference

The three compartment CPP apparatus, based on the design of Carr and White (1983), was made of wood with a plexiglass front wall. Two of the compartments were identical in size (45 X 45 X 30 cm), but differed in shading (white or black), texture (smooth floor or wire mesh floor), and olfactory cues

(wood chips on smooth floor or vinegar on wire mesh floor). The third compartment was an unpainted tunnel (36 X 18 X 20 cm). It protruded from the rear of the two large compartments and connected the entrances to them. In this apparatus, rats show no consistent preference for either compartment (Carr & White, 1983; Clarke, White, & Franklin, 1990).

Drugs

Morphine sulphate (Sabex International, Quebec) was dissolved in 0.9% physiological saline. Dosages and method of administration (intra-cerebral or systemic) varied across experiments. Vehicle injections were 0.9% physiological saline.

Intra-cerebral Injections

Intra-cerebral injections were made through 30 G stainless-steel injectors which fitted into the guide cannulae and extended 1.5 mm beyond the cannulae tips. Injectors were connected to a length of polyethylene tubing (PE-10) filled with drug or vehicle. All injections were made in a volume of 0.5 *ul* over 60 seconds by means of a syringe pump (Harvard Apparatus, Mass.). The progress of drug infusion was monitored by the movement of a small air bubble in the tubing. Inner cannulae were left in place for an additional 60 seconds to reduce the possibility of reflux.

Behavioural Procedures

The CPP procedure required habituation, conditioning, and test sessions. On day 1, subjects were habituated to the apparatus and testing environment. Entrances to the tunnels remained open, and rats were allowed to explore the three compartments for 30 minutes. Animals were then conditioned for 2, 4, or

6 days depending on the experiment. On each conditioning day, animals received injections of morphine or saline and were immediately confined to one of the large compartments. The order of injection and the compartment paired with the drug injection were counterbalanced within groups. This conditioning protocol ensures that animals have equal exposure to both large compartments. Conditioning sessions in all experiments were 30 minutes. Although the size of a morphine-induced CPP is not affected by changes in length of conditioning sessions, 30 minutes is used most frequently and yields the most reliable CPP (Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982).

On the test day, no drug was injected. Entrances to the connecting tunnel were opened, each subject was placed in the tunnel and allowed to explore the three compartments for 20 minutes. Data were collected by recording the time at which animals entered and exited each compartment. Three variables were recorded: which compartment the subjects entered first, the amount of time they spent in each compartment, and the number of entries they made to each compartment.

Histology

After the completion of behavioural testing, subjects were deeply anaesthetized with chloral hydrate, and perfused through the heart with 0.9% saline followed by 10% formalin. Brains were removed, stored in 10% formalin for at least 24 hours, frozen, and sliced in 20 *um* sections.

Slices were stained with thionin in Experiments 1 and 2 to verify the location of cannulae and microinjector tips. Data from individual subjects were discarded

if the bilateral injections were not symmetrical (less than 0.5 mm apart) or fell beyond the boundary of the target site.

Sections were stained with cresyl violet in Experiments 3 and 4 to determine the location and extent of lesion-induced damage.

Statistical Analysis

Data were analyzed using ANOVAs with planned comparisons. Independent variables were group and compartment (drug-paired and saline-paired). Dependent variables were time spent in, or entries to, each compartment. In the CPP apparatus, a tunnel connects the drug- and saline-paired compartments. Given that subjects spend part of the time in the connecting tunnel, time spent in one large compartment is independent of the time spent in the other large compartment. Because subjects spend time in both large compartments, the compartment factor was considered to be a repeated measure.

Two types of planned comparisons were applied to the data. In the first, the time spent in the drug-paired compartment was compared to the time spent in the saline-paired compartment. This test determined whether individual groups displayed a CPP. The number of entries to drug- and saline-paired compartments were compared in the same way.

The second comparison was between difference scores (time in drug-paired minus time in saline-paired compartments) across groups. This analysis determined whether the CPP (or lack thereof) exhibited by a given group differed from its control group. The same analysis was used to determine whether the total number of entries (entries to drug-paired plus entries to saline-paired compartments) differed across groups.

Incidental observations suggested that the CPP expressed by individual groups of animals was exhibited at different times during the test. To examine this possible trend statistically, the 20 minute test session was divided into two 10 minute periods. Data from individual groups were analyzed during the first and second half of the 20 minute test if the experimental manipulation was effective. This included groups that displayed a CPP following morphine microinjections in Experiment 1 and groups that exhibited a reduced CPP to morphine following CNS lesions in Experiments 3 and 4. Similar to the analyses of the 20 minute test, planned comparisons were applied to the time spent in, or entries to, drug- and saline-paired compartments. The size of the difference score (time in drug-paired minus time in saline-paired compartments) between the first and second half of the test was compared for each group. Because difference scores were measured in the same animals (during the first and second 10 minutes of testing), group was considered to be a repeated measure.

In Experiments 3 and 4, some lesions blocked a morphine-induced CPP. Animals in these groups were re-tested for a CPP in a drugged state (2 mg/kg morphine s.c.). The state dependent test always occurred the day after the drug free test. To determine whether drug administration affected time spent in drug- and saline-paired compartments, the size of the CPP (difference scores) in drug free and state dependent tests were compared for each group. For this analysis, group was treated as a repeated measure because the size of the CPP in drug free and state dependent tests was compared in the same group of animals.

EXPERIMENT 1

Effects of Intra-cerebral Morphine Injections on the Development of a CPP

Experiment 1 was designed to address the question of where morphine's rewarding effect is initiated. The possibility that the drug acts at one or more of thirteen different CNS sites was assessed by testing whether microinjections of morphine into each site produced a CPP.

The effects of morphine injections into the VTA, the VS, the VP, and the PPTg were tested because these structures constitute the neural circuit of drug reward, and it has been proposed that drugs may produce their rewarding effect through an action at one or more of these sites (Koob & Bloom, 1988). Previously, the VTA has been identified as a site wherein opiate-induced reward is initiated in both CPP (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Bozarth, 1987b; Phillips & LePiane, 1980) and self administration (Bozarth, 1983; Bozarth & Wise, 1981b, 1984; Devine & Wise, 1994) studies. Nonetheless, the role of VTA opioid receptors has not been systematically compared to that of other neural sites which may also be involved in mediating the effect.

Some reports suggest that an action at *u* opioid receptors in the VS is rewarding (Olds, 1982; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), although other findings contradict these results (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Bozarth, 1983; Stewart & Vezina, 1988). Given the heterogenous makeup of the VS (Brog, Salyapongse, Deutch, & Zahm, 1993; Deutch & Cameron, 1992; Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Zahm & Brog, 1992; Zahm & Heimer, 1993), discrepancies across studies may be due to different injection sites within the structure. In the present study, separate

groups of subjects received injections into core and shell regions of the NAS, the latter of which has a higher concentration of *u* opioid receptors (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987). The rewarding effect of opiate injections into the VP or PPTg has not previously been tested.

The effect of morphine microinjections into the hippocampus, the LH, and the PAG were also tested because there is evidence that opiates may produce their rewarding effect through an action at these sites. For example, microinjections of *u* receptor agonists into the hippocampus, the LH, or the PAG produce a CPP (Corrigall & Linseman, 1988; Motta & Brandao, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and support self-administration (Cazala, 1990; Cazala, Darracq, & Saint-Marc, 1987; Olds, 1979; Stevens, Shiotsu, & Stein, 1991). Results from these studies have been challenged, however, because doses producing the effect were much higher than those required to produce an effect with intra-ventricular or intra-VTA injections (Wise & Hoffman, 1992). The present study addressed this issue by comparing behavioural effects of all microinjections to those produced by injections into the lateral ventricles (LV).

Anatomical and behavioural evidence indicates that two other CNS sites may also be involved in the mediation of opiate-induced reward. The medial frontal cortex (MFC) projects topographically to the VS (Berendse, Galis-deGraaf, & Groenewegen, 1992; Brog, Salyapongse, Deutch, & Zahm, 1993; McGeorge & Faull, 1989) suggesting that the two sites may be involved in similar processes. Functions of the CPu may also be closely linked to those of the VS: the VS and CPu share neurochemical and cytoarchitectural features, and have similar afferent and efferent connections (Heimer & Wilson, 1975). It appears that both the MFC and CPu are involved in the rewarding effect of opiates because lesions of either

site disrupt responding for intravenous morphine (Glick & Cox, 1978; Glick, Cox, & Crane, 1975). The possibility that morphine acts at the MFC or CPu to produce its rewarding effect was therefore tested in the present study.

The amygdala has not been directly implicated in processes that mediate the rewarding effect of opiates, however, the region is involved in the formation of stimulus-reward associations (Gaffan & Harrison, 1987; Jones & Mishkin, 1972; Spiegler & Mishkin, 1981; Weiskrantz, 1956). This effect appears to be mediated in the region of the LA (Hiroi & White, 1991; McDonald & White, 1993) and transmitted via direct projections to the VS (Cador, Robbins, & Everitt, 1989; Everitt, Cador, & Robbins, 1989; Everitt, Morris, O'Brien, & Robbins, 1991). The amygdala has a high concentration of opioid receptors (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987, 1988), suggesting that opioid-induced activation may contribute to amygdala-mediated processes. It is possible, therefore, that morphine microinjections into the LA will produce a CPP.

Neural substrates mediating the rewarding and analgesic effects of opiates appear to overlap in the VTA and VS (Manning, Morgan, & Franklin, 1994; Morgan & Franklin, 1990). Based on this evidence, it has been suggested that the two effects depend on a common mechanism (Franklin, 1989; Le Magnen, Margaing-Jallat, Miceli, & Devos, 1980). Because the posterior hypothalamus (PH) is also a site where morphine microinjections are analgesic (Manning, Morgan, & Franklin, 1994), it is possible that opiate-induced activation of this region is rewarding. For this reason, the ability of intra-PH morphine injections to induce a CPP was tested.

Methods

Subjects

One hundred and twenty subjects (8 per group) were used in this experiment.

Surgical Procedures

The stereotaxic coordinates of the 13 targeted sites are shown in Table 1. Injected substances may reach dorsally located structures via efflux up the outside of cannulae shafts (Johnson & Epstein, 1975). Thus, in order to minimize the possibility that morphine injections spread to the ventricles of overlying structures, cannulae aimed at the NAS (shell) were angled laterally 5 degrees, and those aimed at the NAS (core), VTA, and PAG were angled 10 degrees laterally. In addition, two groups served as anatomical controls for sites where where microinjections produced a CPP (VTA and PAG). Animals in these groups were implanted with cannulae 1 mm dorsal to either the VTA or PAG.

Behavioural Procedures

There were four conditioning days. On two days, 1 *u*g (2.63 nmol) of morphine sulphate in 0.5 *u*l of vehicle was injected into the target site. On the two intervening days, animals received vehicle microinjections into the same site. A control group of subjects with LV cannulae received vehicle injections on all four conditioning days.

Table 1

Stereotaxic coordinates for intra-cerebral morphine injections. Values represent distances in millimetres, anterior (+) or posterior (-) to bregma, lateral to the midline, and ventral to the skull surface at bregma according to the atlas of Paxinos and Watson (1986).

	Ant/Post	Lateral	Ventral
MFC	+3.5	0.5	3.0
NAS (core)[a]	+1.6	1.4	7.0
NAS (shell)[b]	+1.6	2.3	7.9
CPu	+1.2	2.5	5.0
VP	-0.3	2.0	7.5
LV	-1.0	1.4	4.0
CA1	-3.0	1.5	3.0
LA	-3.5	5.5	8.0
LH	-4.2	1.4	8.0
PH	-4.2	0.3	8.6
VTA[a]	-5.0	0.9	8.6
PAG[a]	-6.0	0.5	5.3
PPTg	-7.8	1.6	7.0

[a] Cannulae angled laterally 10 degrees

[b] Cannulae angled laterally 5 degrees

Results

Histology

The results of histological analyses are shown in Figures 1 and 2. The symbols plotted indicate whether or not each animal displayed a CPP. The size of the CPP produced by LV morphine was used as a standard (mean = 262; standard deviation = 63). It was assumed that animals expressed a CPP if their difference score (time in drug-paired minus time in saline-paired compartment) exceeded a score of one standard deviation below the mean of the LV group. Therefore, for purposes of histological representation, an injection was classified as producing a CPP if the difference score produced by that injection was greater than 200 seconds over the 20 minute test period.

Behavioural Results

Time in Compartments

Animals receiving LV vehicle injections did not show a preference for either the black or white compartment [$F(1,7) = 0.89$; $p > 0.05$] confirming that the CPP apparatus used in this experiment is balanced.

Results of the planned comparisons are presented in Table 2. The first column presents the F value for the comparison between time spent in drug-paired and saline-paired compartments for each group. In the second column, the F value for the comparison of mean difference scores (time in drug-paired minus time in saline-paired compartments) is presented. Difference scores for each group are compared to difference scores of animals conditioned with intra-LV morphine.

Figure 1

Distribution of sites at which microinjections of morphine into the VTA or PAG produced a CPP (filled symbols), or failed to produce a CPP (open symbols). The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the intended site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

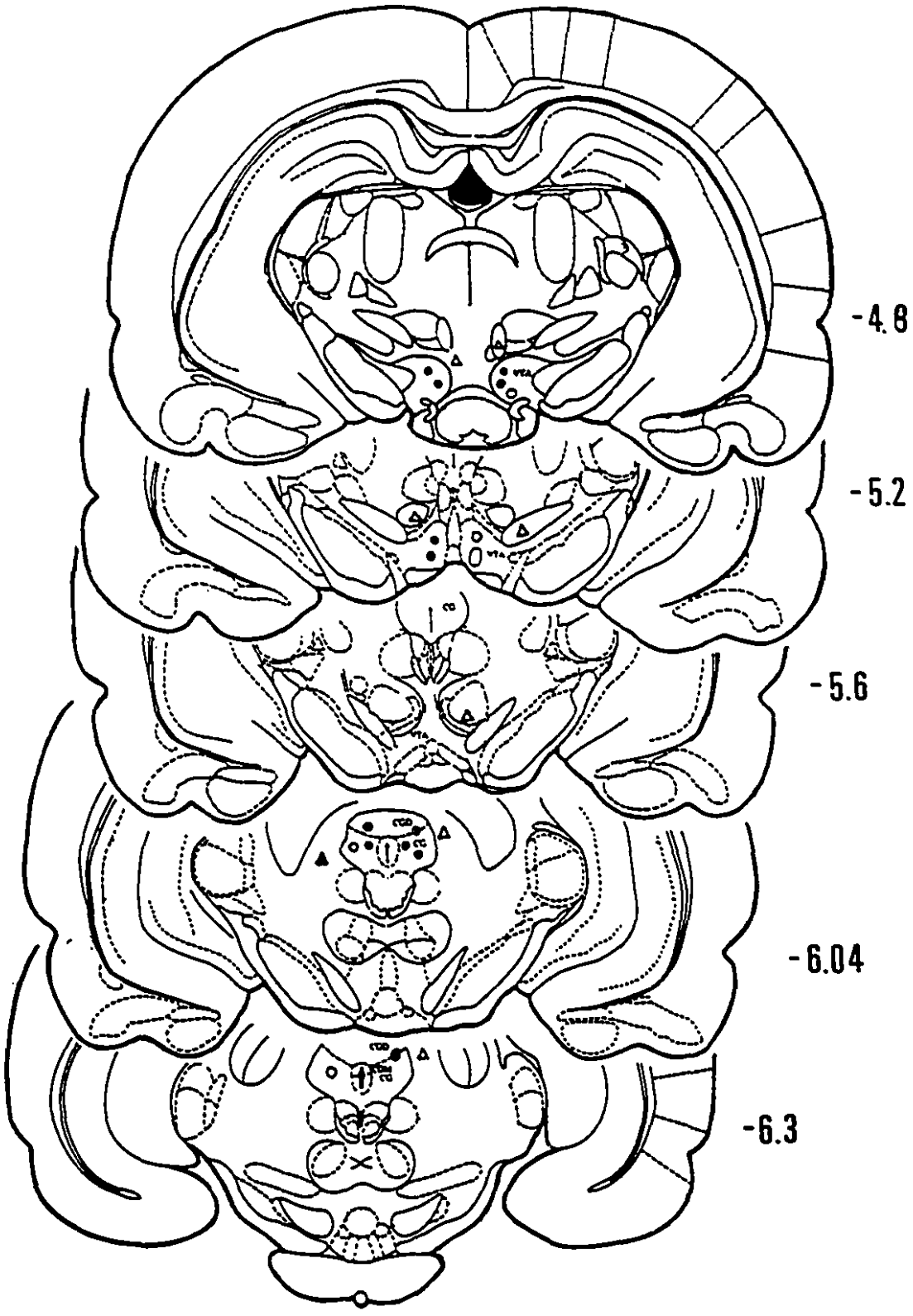

-4.8
-5.2
-5.6
-6.04
-6.3

Figure 2

Distribution of sites at which microinjections of morphine into the MFC, NAS, CPu, VP, CA1, LA, LH, PH, and PPTg produced a CPP (filled symbols), or failed to produce a CPP (open symbols). The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the designated site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

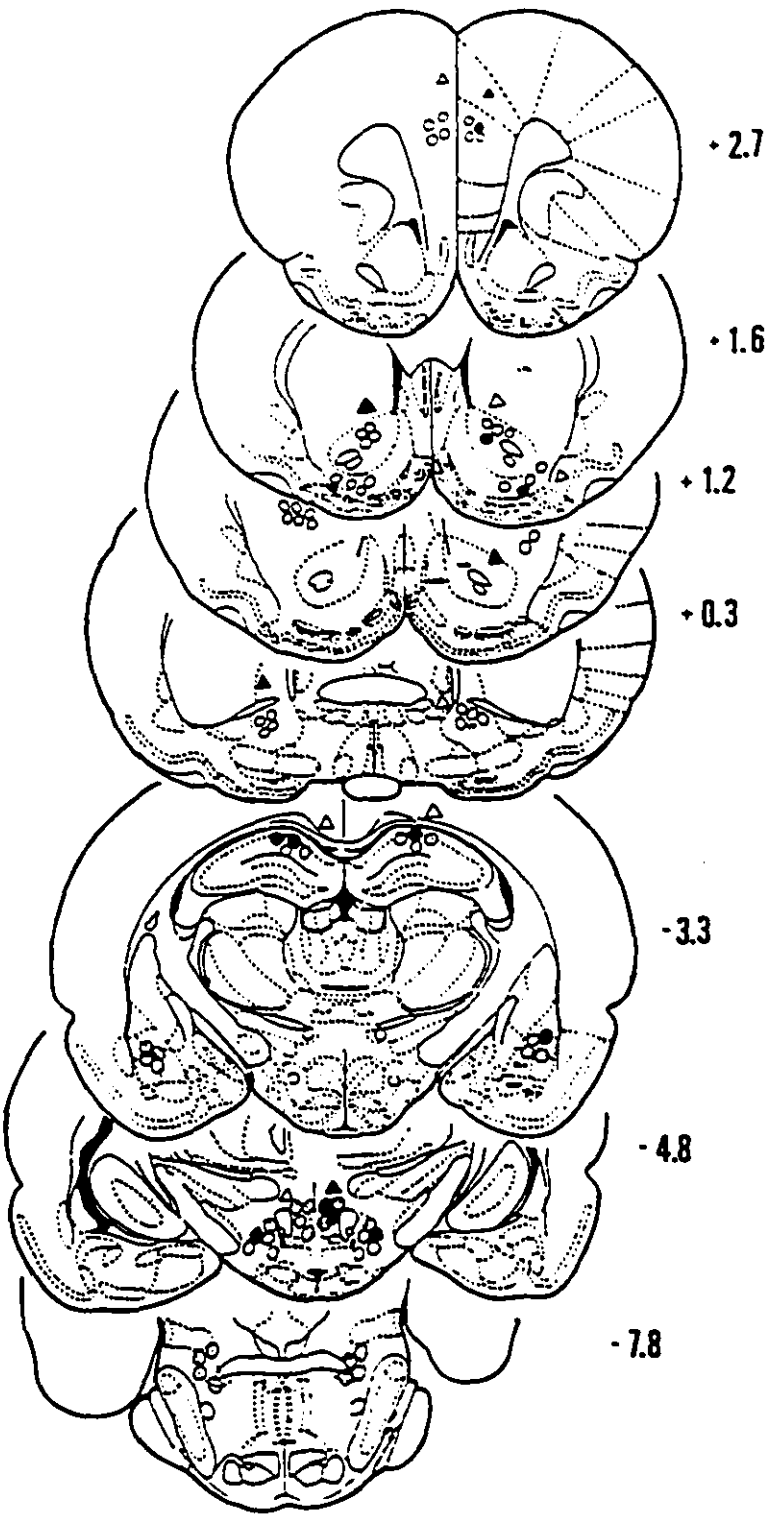

+ 2.7
+ 1.6
+ 1.2
+ 0.3
- 3.3
- 4.8
- 7.8

Table 2

Results of planned comparisons for groups conditioned with morphine injections into different CNS sites. Drug vs Saline: F value for the comparison between time spent in drug-paired and saline-paired compartments for each group. Difference score: F value for the comparison of the mean difference score of each group to the mean difference score of animals conditioned with intra-LV morphine.

Site	Drug vs Saline *df* = 1,105	Difference Score *df* = 1,210
LV	4.60*	
VTA	7.90**	0.56
VTA (1 mm dorsal)	0.01	4.19*
PAG	3.92*	0.02
PAG (1 mm dorsal)	0.35	4.10*
MFC	0.01	4.80*
NAS (core)	0.66	7.81*
NAS (shell)	0.36	6.33*
CPu	0.01	4.30*
VP	0.69	5.89*
CA1	1.72	4.10*
LA	0.78	7.62*
LH	0.10	4.01*
PH	0.01	4.60*
PPTg	2.30	11.01[a]

* Significant at $p < 0.05$ level

** Significant at $p < 0.01$ level

[a] Conditioned Place Aversion

As shown in Table 2, and in Figure 3, that animals conditioned with intra-LV, intra-VTA, and intra-PAG morphine developed a significant preference for the drug-paired compartment. The size of the CPP was not significantly different for these three groups. Injections 1 mm dorsal to the VTA or PAG did not produce a CPP. Figure 4 illustrates that injections into all other sites tested were also ineffective.

Entries to Compartments

The mean (± SEM) number of entries that animals in each group made to the drug- and saline-paired compartments are shown in Table 3. For statistical purposes, the total number of entries (entries to drug-paired plus entries to saline-paired compartments) for each group were compared to total number of entries made by animals receiving LV saline during conditioning.

Animals receiving intra-VTA morphine during conditioning made significantly more entries to the drug-paired than to the saline-paired compartment [$F(1,105) = 11.30$; $p < 0.01$]. Microinjections into the LV or PAG did not produce this effect (LV [$F(1,105) = 3.1$; $p > 0.05$] and PAG [$F(1,105) = 1.9$; $p > 0.05$]). There was no significant difference in the number of entries to drug- and saline-paired compartments for any other group.

For each group, the total number of entries (entries to drug-paired plus entries to saline-paired compartment) were compared to the total number of entries made by animals receiving LV injections of vehicle. Based on these criterion, morphine injections into seven sites produced a significant increase in total entries on test day: LV [$F(1,224) = 6.2$; $p < 0.05$], VTA [$F(1,224) = 10.8$; $p < 0.01$], PAG [$F(1,224) = 4.0$; $p < 0.05$], PAG (control) [$F(1,224) = 4.05$; $p < 0.05$], NAS (core) [$F(1,224) = 8.9$; $p < 0.01$], LA [$F(1,224) = 4.15$; $p < 0.05$], and PH [$F(1,224) = 4.97$; $p < 0.05$].

Figure 3

Effects of morphine injections into the LV, VTA, and PAG on the development of a CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for each group. Control injections are 1 mm dorsal to the intended site.

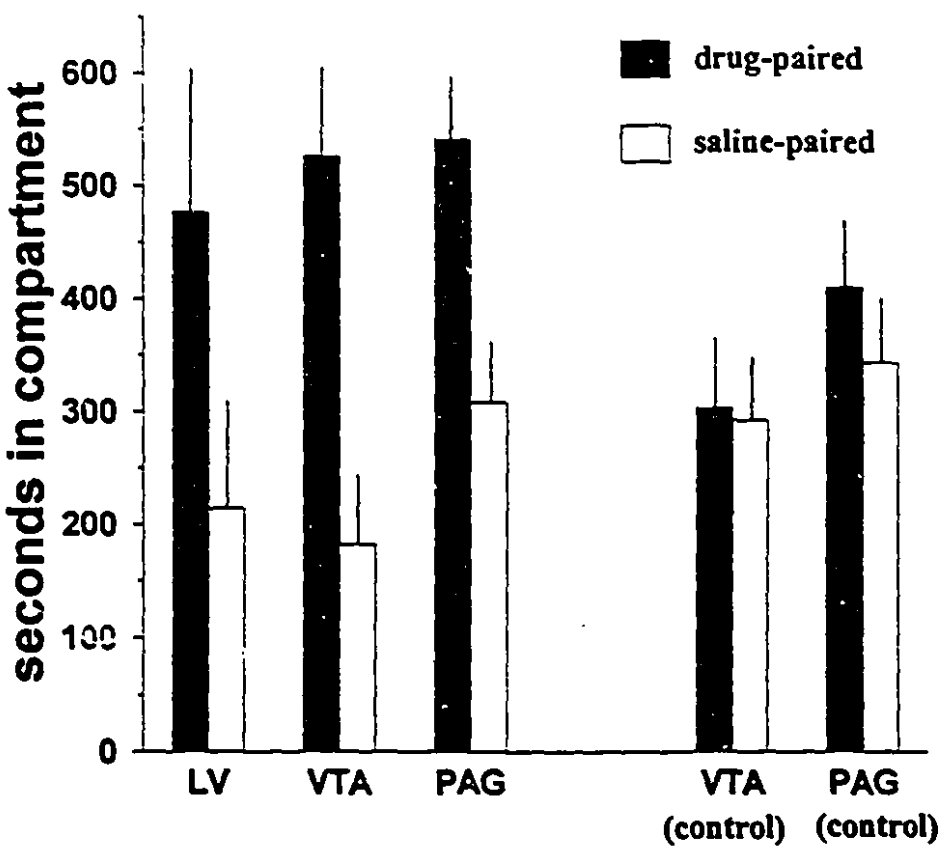

seconds in compartment
600
500
400
300
200
100
0
drug-paired
saline-paired
LV
VTA
PAG
VTA
(control)
PAG
(control)

Figure 4

Effects of morphine injections into the MFC, NAS (core and shell regions), CPu, VP, CA1, LA, LH, PH, and PPTg on the development of a CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for each group.

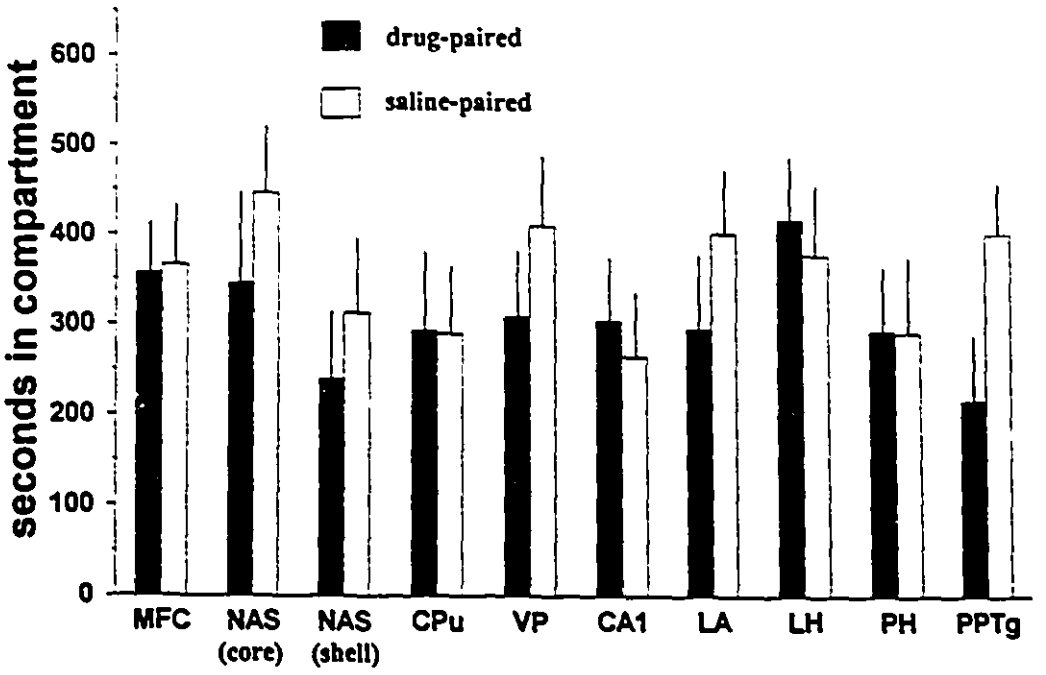

drug-paired
saline-paired
seconds in compartment
600
500
400
300
200
100
0
MFC
NAS (core)
NAS (shell)
CPu
VP
CA1
LA
LH
PH
PPTg

Table 3

Mean number of entries (± SEM) to drug-paired and saline-paired compartments for groups conditioned with morphine injections into different CNS sites.

	Drug-Paired	Saline-Paired	Total
LV (saline)	6.3 ± 1.1	4.6 ± 1.3	10.9 ± 1.7
LV	20.8 ± 3.6	16.0 ± 1.4	36.8 ± 4.2*
VTA[a]	26.9 ± 3.6	18.1 ± 3.5	45.0 ± 6.8**
VTA (control)	6.8 ± 0.8	7.8 ± 0.6	14.6 ± 3.8**
PAG	17.6 ± 2.2	14.0 ± 1.7	31.6 ± 3.0*
PAG (control)	15.1 ± 1.5	16.6 ± 1.9	31.8 ± 2.4*
MFC	10.8 ± 0.8	12.5 ± 1.2	22.0 ± 1.6
NAS (core)	20.1 ± 2.7	22.6 ± 1.4	42.8 ± 3.8*
NAS (shell)	10.6 ± 1.3	11.0 ± 1.3	20.1 ± 2.0
CPu	9.3 ± 1.0	9.1 ± 0.6	18.4 ± 0.9
VP	8.1 ± 1.0	9.6 ± 0.8	17.8 ± 1.5
CA1	6.8 ± 0.8	6.0 ± 0.8	13.8 ± 1.4
LA	17.0 ± 2.3	16.3 ± 1.9	33.3 ± 2.3*
LH	12.8 ± 2.3	12.8 ± 3.3	25.5 ± 5.6
PH	17.1 ± 1.4	16.9 ± 1.9	34.0 ± 3.5*
PPTg	11.5 ± 1.2	15.0 ± 1.5	25.4 ± 2.0

[a] Entries to drug-paired compartment significantly greater than entries to saline-paired compartment

* Significant at $p < 0.05$

** Significant at $p < 0.01$

CPP Induced by intra-VTA and intra-PAG Morphine

As outlined in the General Methods, behavioural data were re-analyzed for groups that displayed a CPP because initial observations indicated that individual groups expressed a CPP at different times during the test. Figure 5 presents the data from the groups conditioned with intra-VTA and intra-PAG morphine as time spent in, and entries to, drug- and saline-paired compartments across testing.

Animals conditioned with intra-VTA morphine exhibited a CPP during both the first [$F(1,7) = 46.9$; $p < 0.05$] and second [$F(1,7) = 7.8$; $p < 0.05$] ten minutes of testing. The size of the CPP was not significantly different during these two portions of the test [$F(1,7) = 0.008$; $p > 0.05$].

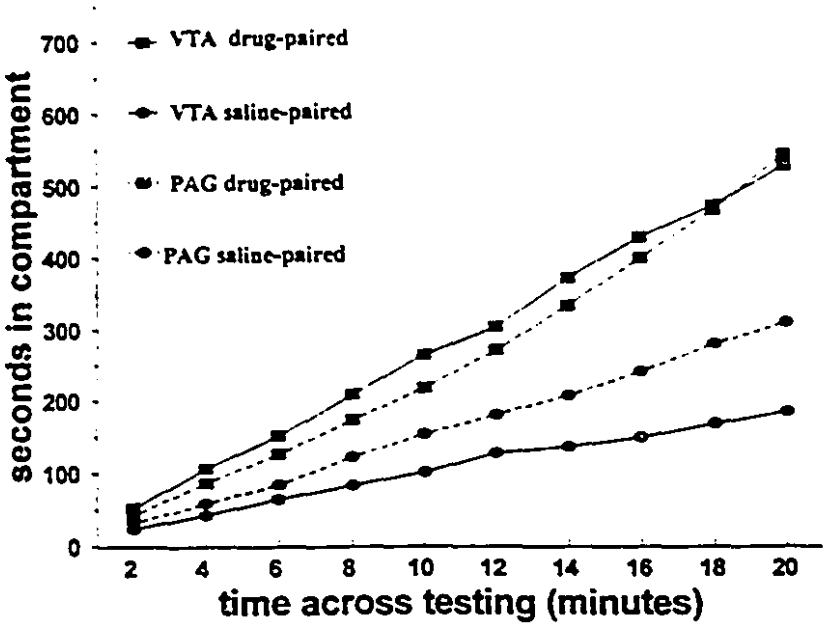
In contrast, animals conditioned with intra-PAG morphine did not show a CPP during the first half of the test [$F(1,7) = 1.7$; $p > 0.05$], but they did during the second half of the test [$F(1,7) = 15.5$; $p < 0.05$]. The difference score for these animals was significantly larger during the second half of the test [$F(1,7) = 0.008$; $p < 0.05$].

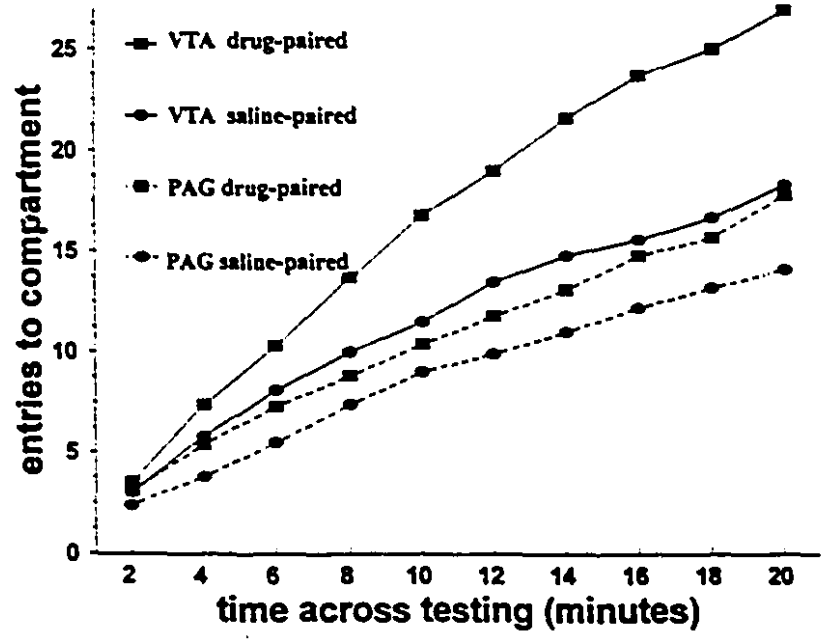
Intra-VTA injections of morphine produced more entries to the drug-paired compartment during the first [$F(1,7) = 18.0$; $p < 0.05$] as well as the second [$F(1,7) = 7.2$; $p < 0.05$] ten minutes of testing.

Animals conditioned with intra-PAG morphine did not make more entries to the drug-paired compartments during either portion of the test [$F(1,7) = 2.1$; $p > 0.05$] and [$F(1,7) = 1.3$; $p > 0.05$].

Figure 5

Time course of the CPP exhibited by animals conditioned with intra-VTA (solid lines) and intra-PAG (dotted lines) morphine. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals for each group. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals for each group. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.


VTA drug-paired
VTA saline-paired
PAG drug-paired
PAG saline-paired
seconds in compartment
0
100
200
300
400
500
600
700
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


VTA drug-paired
VTA saline-paired
PAG drug-paired
PAG saline-paired
entries to compartment
0
5
10
15
20
25
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

Discussion

Consistent with previous reports (Shippenberg, Bals-Kubik, & Herz, 1987; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), morphine microinjections into the LV produced a significant preference for an environment paired with the injection. Given that intra-ventricular drug injections are diluted as they diffuse, an injection at the true site of action should be equal, or superior, in efficacy to a comparable LV injection. On the basis of this criterion, only the VTA and PAG were identified as sites wherein morphine microinjections are rewarding. Neither effect can be attributed to diffusion up the cannula tract because injections 1 mm dorsal to either structure did not produce a CPP. Although thirteen different sites were tested, the mapping of putative sites was not exhaustive, and it is possible that there are CNS sites not tested in the present study which are capable of supporting a CPP.

Effects of Intra-cerebral Morphine Injections

Findings from the present study support evidence that injections of *u* agonists into the VTA produce a CPP (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; Bozarth, 1987b; Phillips & LePiane, 1980). Opiate injections into the VTA also support self-administration (Bozarth & Wise, 1981b, 1984; Devine & Wise, 1994). The rewarding effect of intra-VTA opiates is thought to be mediated through activation of DA neurons (Bozarth & Wise, 1981b; Wise & Bozarth, 1987; Wise & Rompre, 1989). In support of this hypothesis, a CPP induced by *u* agonist injections into the VTA is blocked by administration of DA antagonists, and by lesions of the mesolimbic DA system (Shippenberg, Bals-Kubik, & Herz, 1993).

The present results also confirm reports that intra-PAG morphine injections produce a CPP (Motta & Brandao, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) but appear to contradict the report that PAG microinjections do not support self-administration (Bozarth & Wise, 1984). Although the CPP and self-administration paradigms occasionally produce discrepant findings, the different results are probably related to one of two factors. First, the dose of morphine employed by Bozarth and Wise (1984) may have been too high. Animals will self-administer low, but not high, doses of morphine into the PAG (Cazala, 1990; David & Cazala, 1994), and intra-PAG injections of morphine are rewarding at low doses and aversive at high doses (Motta & Brandao, 1993). Second, injection sites in the present study may not be in the same region of the PAG as those in Bozarth and Wise's study. Morphine injections into the rostral PAG induce signs of physical dependence that are more pronounced than injections into the caudal PAG (Bozarth, 1994). Given that intra-PAG injections in the Bozarth and Wise (1984) study induced signs of physical dependence, they were probably located in the rostral regions of the nucleus, whereas injections in the present study were concentrated in the caudal PAG. Morphine injections into discrete regions within the PAG may have different behavioural effects because the neurochemical constitution of the PAG varies along its longitudinal axis (Onstott, Mayar, & Beitz, 1993).

Results of the present study are in agreement with reports that injections of *u* agonists into the amygdala, LH, MFC, or CPu do not produce a CPP (Bals-Kubik, Ableitner, Herz, & Shippenberg, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982). On the other hand, the findings that morphine microinjections into the hippocampus, NAS, or LH are ineffective contrast

with those of previous studies (Corrigall & Linseman, 1988; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982). The different results are probably related to doses which were 10 (van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and 20 (Corrigall & Linseman, 1988) times higher than those tested in the present study, and much greater than the dose required to produce a CPP through ventricular injections. It is likely that the identification of the hippocampus, the NAS, or the LH as sites where morphine's rewarding effect is initiated is the result of drug diffusion into the ventricles.

The suggestion that intra-NAS injections of morphine are rewarding (Olds, 1982; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) may also be related to the high doses employed in these studies. At high doses, morphine may produce an action at *d* opioid receptors (Simantov, Childers, & Snyder, 1978) and specific *d* receptor agonists have been shown to be rewarding in the NAS (Goeders, Lane, & Smith, 1984). Indeed, it has been suggested that reward is mediated by *u* receptors in the VTA (Bozarth, 1987b) and by *d* receptors in the NAS (Goeders, Lane, & Smith, 1984).

In the present study, morphine was injected in both the core and shell regions of the NAS. The core and shell represent distinct subregions of the NAS which have been associated with the motor and limbic systems respectively (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Zahm & Brog, 1992). The present results provide further evidence for a functional distinction between the two sites because morphine infusions into the core produced higher activity levels (total entries to large compartments) than did infusions into the shell. The finding that morphine injections into the core produce an increase in activity supports evidence

that NAS mediated locomotion depends on neuronal processes in this region (Maldonado-Irizarry & Kelley, 1994).

It is interesting to note that morphine injections into the PPTg appeared to produce a conditioned place aversion. Animals conditioned with intra-PPTg morphine spent less time in the drug-paired than in the saline-paired compartment. Although the time spent in drug- and saline-paired compartments did not differ for these animals, their difference scores varied significantly from the difference scores of animals conditioned with LV morphine injections. As outlined previously, activation of the PPTg is associated with reward related processes; morphine injections into this site may produce aversive effects because the drug inhibits PPTg activity (Serafin, Khateb, & Muhlethaler, 1990).

The total number of entries that animals make to both large compartments is an estimate of their locomotor activity during testing. Although injections which produced a CPP (LV, PAG, and VTA) also caused increased activity during testing, injections into other sites (PAG control, NAS core, LA, PH) produced an increase in activity, but no CPP. The absence of a clear relationship between the two measures supports evidence that the effect of opiates on locomotor activity and reward are dissociable (Cunningham & Kelley, 1992; Stinus et al., 1989).

In some cases, intra-cerebral morphine injections may have produced secondary effects such as disrupting memory processes (Gallagher & Kapp, 1978), which could interfere with CPP conditioning. The absence of a CPP may indicate that the drug injection was not rewarding, or that the animal can not remember the association between rewarding effects and environmental stimuli. The possibility that microinjections induce a state of positive affect, but block the ability to

associate this condition with salient cues, needs to be examined using tests other than the CPP which independently assess reward and memory processes.

A Comparison of the CPP Induced by Intra-VTA and Intra-PAG Morphine

In the present study, animals conditioned with intra-VTA or intra-PAG morphine injections exhibited a preference for the morphine-paired compartment. Although the magnitude of the CPP was the same for these two groups, the time at which they displayed it was not. Animals conditioned with intra-VTA morphine exhibited a significant preference for the drug-paired compartment during the first half of the test, and the size of the effect did not change across testing. In contrast, animals conditioned with PAG microinjections exhibited a CPP during the second, but not the first, ten minutes of testing. The two groups also differed in their pattern of entries: animals conditioned with intra-VTA morphine made more entries to the drug-paired compartment during both halves of the test, whereas animals conditioned with intra-PAG morphine did not. These findings suggest that the mechanisms which contribute to a CPP induced by intra-VTA or intra-PAG morphine injections may differ.

This raises the question of which factors control behavioural responding during CPP testing. Although the CPP is a relatively simple and effective method of investigating reward-related processes (Carr, Fibiger, & Phillips, 1989; van der Kooy, 1987), underlying psychological mechanisms that contribute to a CPP are difficult to ascertain. That is, why do animals choose to return to the environment where they experienced drug effects? Presumably they approach salient environmental cues because the cues, through their association with primary reward, have acquired conditioned rewarding properties. If this is true, animals

express a CPP by approaching and maintaining contact with previously neutral stimuli that have become capable of producing a state of positive affect.

The affective state influencing responding during CPP testing, however, probably reflects more than simple conditioned reward. Morphine administration produces a variety of physiological effects, any number of which may be conditioned to neutral cues (Stewart & Eikelboom, 1987). If intra-VTA and intra-PAG morphine injections produce different physiological effects, responses elicited by drug-paired cues may differ following conditioning to these two injections. Conditioned effects elicited on test day may be perceived by the animal as positive or negative, and this perception may subsequently affect preferences for the drug-paired stimuli. The issue is particularly complex because morphine is a paradoxical drug with both rewarding and aversive properties (Koob, Wall, & Bloom, 1989; Stefurak, Martin, & van der Kooy, 1990; White, Sklar, & Amit, 1977).

The present results, along with those cited above (Cazala, 1990; David & Cazala, 1994; Motta & Brandao, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982), provide evidence that morphine injections into the PAG are rewarding. Reports that intra-PAG morphine produces fearful hyperactivity indicates that the injections can also be aversive (Jacquet, Saedrup, & Squires, 1987; Jacquet & Squires, 1988; Motta & Brandao, 1993). In some cases, a single injection may produce both effects. For instance, although animals will self-administer morphine into the PAG, they demonstrate an increased latency to initiate the response and exhibit signs of aversion following the injection (Cazala, 1990; David & Cazala, 1994). Electrical stimulation of the PAG also produces behavioural responses which suggest that animals find the stimulation aversive as well as rewarding (Cazala, 1986; Cazala, Bendani, & Zielinski, 1985). Given these

findings, it is possible that intra-PAG morphine injections in the present study produced both rewarding and aversive effects. The fact that animals conditioned with these injections exhibited a CPP during the latter portion of the test may reflect an ambivalence to the drug-paired cues. The suggestion that intra-PAG morphine elicits aversive effects does not discount the possibility that morphine injections into the VTA or PAG produce their rewarding effects via the same underlying mechanism. It suggests, however, that behavioural responding in the CPP paradigm may also be influenced by effects that are not related to reward.

The development of a CPP depends not only on the affective properties of the unconditioned stimulus, but also on animals' ability to learn an association between conditioned and unconditioned stimuli, and to remember and express this learning on test day. Because drugs may enhance or inhibit these processes, intra-VTA and intra-PAG morphine injections may influence the expression of a CPP by interacting with more than one mechanism. For example, the two injections may differentially strengthen the association between conditioned and unconditioned stimuli, enhance retention of this association, increase the salience of conditioned stimuli, or increase the ability to discriminate between drug- and saline-paired cues. Modulation of one or more of these processes by intra-VTA or intra-PAG injections may affect the time spent in drug- and saline-paired compartments on test day. It is possible that intra-VTA morphine enhances discriminative abilities because it was the only injection that produced more entries to the drug-paired than to the saline-paired compartment.

In summary, the present results suggest that neural mechanisms underlying the rewarding effect of morphine are initiated by an action at the VTA or the PAG.

These results also suggest that the expression of a CPP may be influenced by processes that are not necessarily related to the rewarding properties of the drug.

EXPERIMENT 2

The Role of VTA and PAG Opioid Receptors in the Development of a CPP to Morphine

Results from Experiment 1 demonstrate that morphine injections into the VTA or PAG produce a CPP. Experiment 2 assessed the relative sensitivity of the VTA and the PAG in mediating the effect by testing whether a higher (5.0 ug) and a lower (0.2 ug) dose of morphine produced a CPP when injected into either site. In order to confirm that morphine reward is mediated via opioid receptors in these two regions, an opioid receptor antagonist was injected into the VTA or the PAG prior to CPP conditioning with systemic morphine. If morphine reward is produced through an action at opioid receptors in these sites, blocking the receptors should disrupt a CPP induced by systemic morphine administration.

Methods

Subjects

Ninety six subjects (12 groups with eight subjects per group) were used. Cannulae, aimed at the VTA or the PAG, were implanted following the protocol described in the General Methods and Experiment 1.

Behavioural Procedures

Four separate groups of animals were tested for the development of a CPP following intra-VTA or intra-PAG microinjections of a 0.2 ug (0.53 nmol) or a 5 ug (13.15 nmol) dose of morphine sulphate. As in Experiment 1, all groups underwent four conditioning sessions, two with morphine and two with saline.

In eight other groups, the role of VTA and PAG opioid receptors in the development of a CPP to morphine was tested by injecting the opioid antagonist naloxone methiodide (NM) (RBI 0-002) into one of these sites prior to CPP conditioning with systemic morphine (4 mg/kg morphine sulphate). NM is a hydrophillic quaternary salt of naloxone which exhibits peripherally acting opioid antagonist properties similar to naloxone, but does not cross the blood brain barrier (Iorio & Frigeni, 1984; Milne, Coddington, & Gamble, 1990). NM, therefore, should diffuse more slowly from the injection site than the highly lipophilic antagonist naloxone.

The effect of opioid antagonist injections into the VTA and the PAG was tested using three separate groups of animals who underwent different conditioning protocols. In the first, animals received intra-cerebral vehicle injections prior to a systemic injection of morphine or saline. In the second, animals received intra-cerebral NM (2 nmol) followed by systemic morphine or intra-cerebral vehicle followed by systemic saline. In the third, animals received intra-cerebral NM on one conditioning day and intra-cerebral vehicle on the other; both intra-cerebral injections were followed by a systemic injection of saline. The latter group was used to determine whether intra-cerebral injections of NM are aversive.

Because intra-PAG injections of NM (2 nmol) did not effectively block the CPP to systemic morphine, two other groups of animals were tested. One group received injections of intra-PAG NM (5 nmol) followed by systemic morphine and intra-PAG vehicle followed by systemic saline. The last group received injections of intra-PAG NM (5 nmol) and intra-PAG vehicle, both followed by systemic saline.

Results

Sensitivity

The results of histological analyses are shown in Figures 6 and 7. The symbols plotted indicate whether or not each animal displayed a CPP according to the criterion outlined in Experiment 1. That is, for purposes of histological representation, an injection was classified as producing a CPP if the difference score produced by that injection was greater than 200 seconds over the 20 minute test period.

It can be seen in Figure 8 that 0.2 ug of morphine produced a CPP when injected into the VTA [$F(1,14) = 8.3$; $p < 0.05$], but not when injected into the PAG [$F(1,14) = 2.1$; $p > 0.05$]. Injections of 5.0 ug of morphine into either site produced a CPP (VTA [$F(1,14) = 21.7$; $p < 0.01$] and PAG [$F(1,14) = 32.7$; $p < 0.01$]).

Data from Experiment 1 (1 ug) were compared with data from the present experiment (0.2 ug and 5.0 ug) to determine whether different doses of morphine produced different behavioural effects. Planned comparisons applied to the difference score for each group revealed that the size of the preference for the drug-paired compartment was not significantly different for VTA groups conditioned with 0.2 ug and 1 ug of morphine [$F(1,42) = 1.4$; $p > 0.05$] or for groups conditioned with 1 ug and 5 ug of morphine [$F(1,42) = 0.003$; $p > 0.05$]. Similarly, the difference scores of animals conditioned with intra-PAG morphine did not vary across doses (0.2 ug and 1 ug [$F(1,42) = 0.49$; $p > 0.05$], and 1 ug and 5 ug [$F(1,42) = 0.8$; $p > 0.05$]).

The difference scores of animals conditioned with intra-VTA and intra-PAG morphine were compared at each dose. Although an injection of 0.2 ug of morphine produced a CPP when injected into the VTA, but not when injected into

Figure 6

Distribution of sites at which microinjections of 0.2 ug of morphine into the VTA or PAG produced a CPP (filled symbols), or failed to produce a CPP (open symbols). The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the intended site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

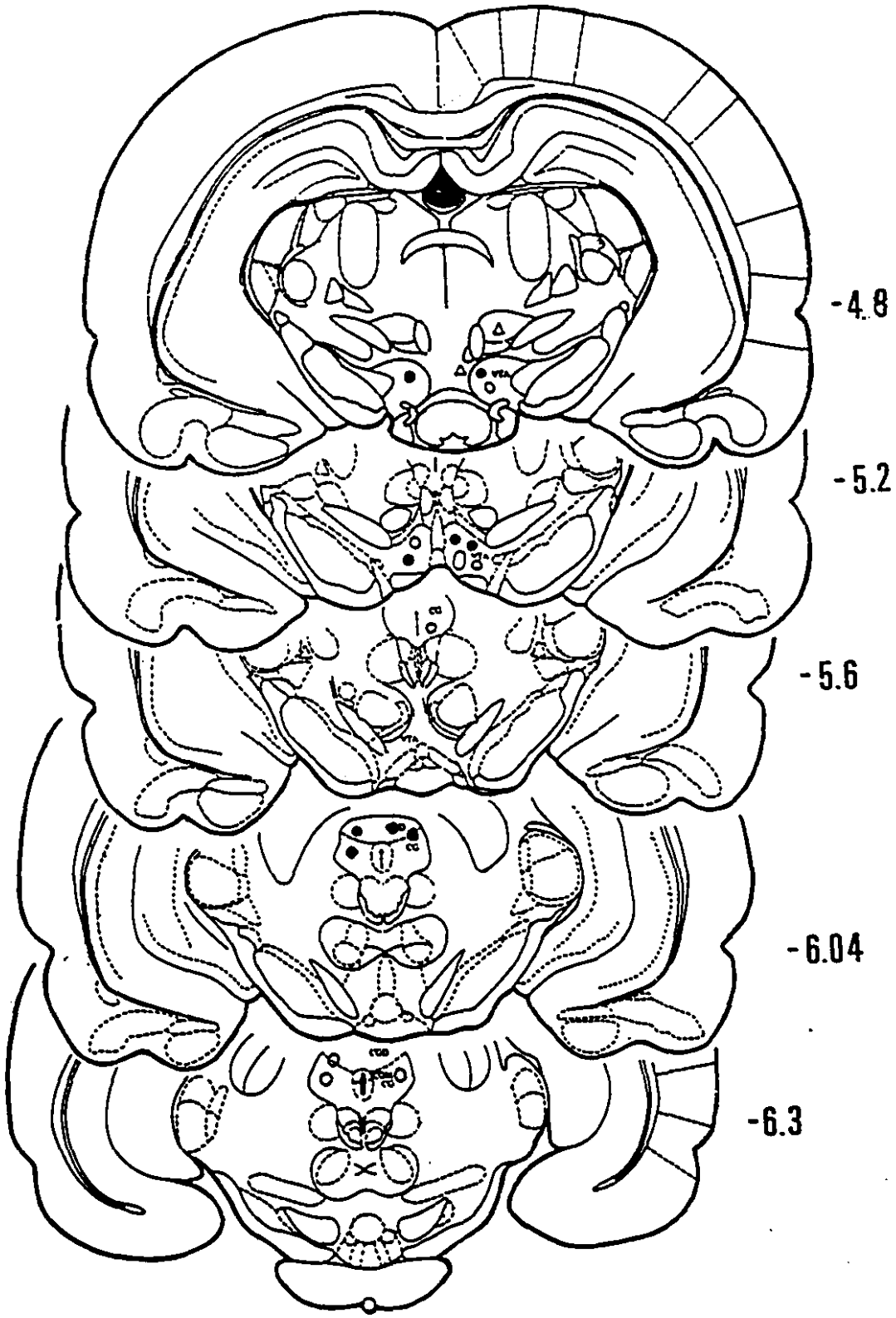

-4.8
-5.2
-5.6
-6.04
-6.3

Figure 7

Distribution of sites at which microinjections of 5.0 *u*g of morphine into the VTA or PAG produced a CPP (filled symbols), or failed to produce a CPP (open symbols). The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the intended site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

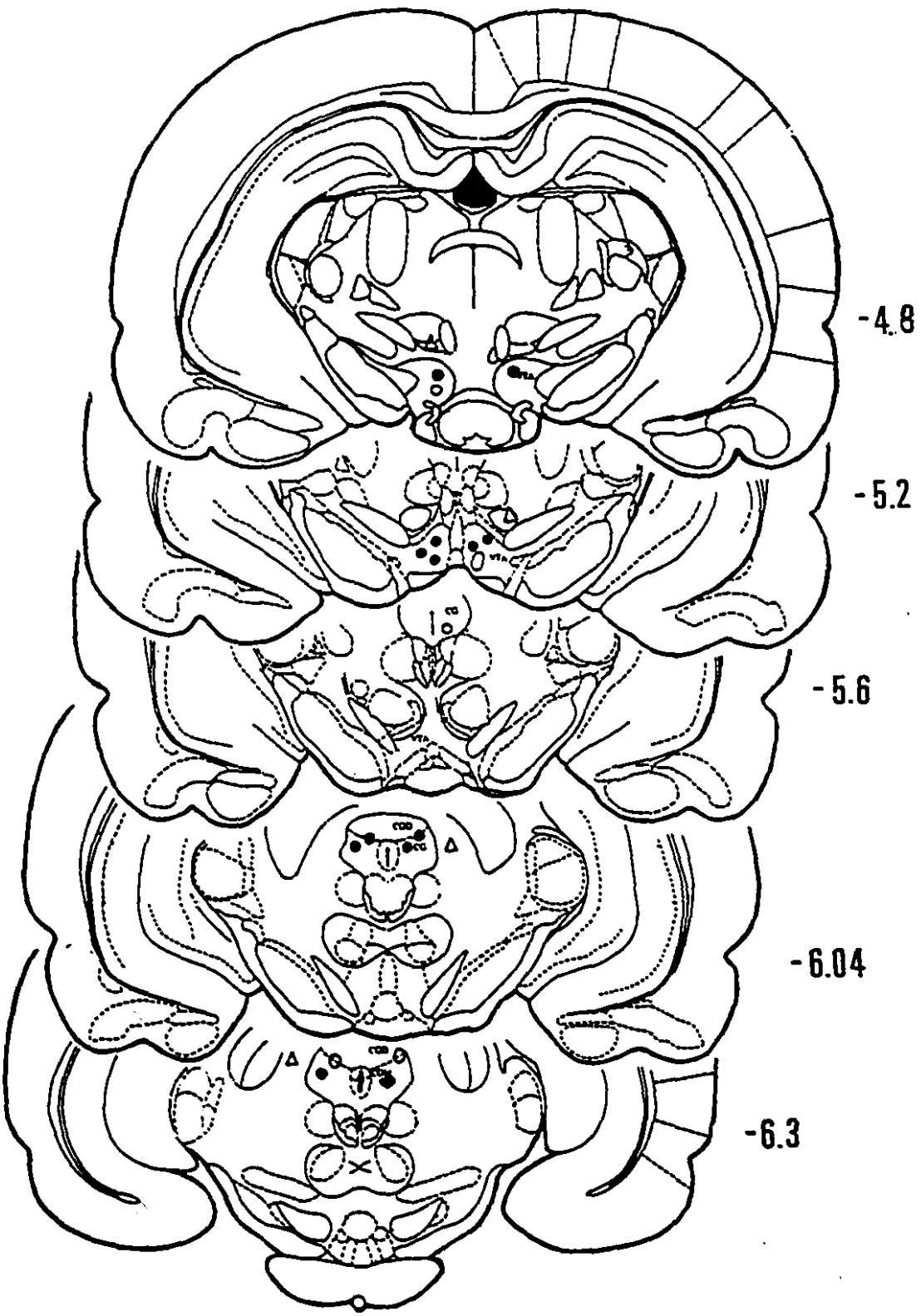
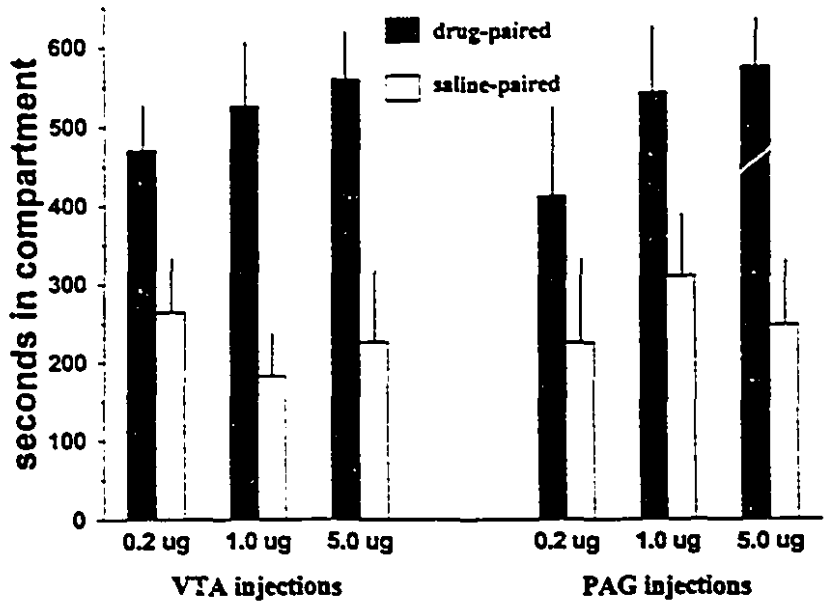
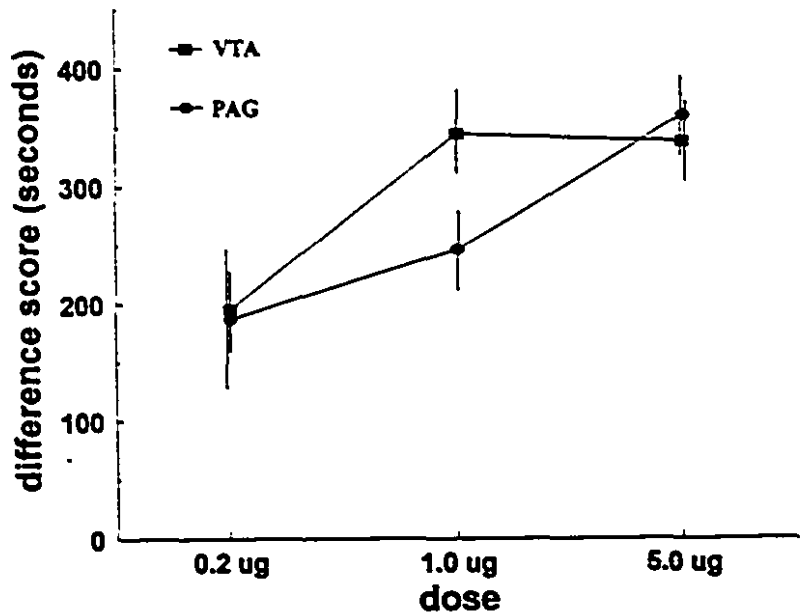

-4.8
-5.2
-5.6
-6.04
-6.3

Figure 8

Effects of intra-VTA or intra-PAG morphine (0.2 *u*g or 5.0 *u*g) on the development of a CPP. Top panel: Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for each grcup. Bottom panel: Difference score (time in drug-paired minus time in saline-paired compartment) across doses of morphine for each group. Results from Experiment 1 (1 *u*g) are included in order to show a larger dose-effect range.


drug-paired
saline-paired
seconds in compartment
600
500
400
300
200
100
0
0.2 ug
1.0 ug
5.0 ug
VTA injections
0.2 ug
1.0 ug
5.0 ug
PAG injections


VTA
PAG
difference score (seconds)
400
300
200
100
0
0.2 ug
1.0 ug
5.0 ug
dose

the PAG (see above), there was no difference in the size of the preference for the morphine-paired compartment following these two injections [$F(1,14) = 0.09$; $p > 0.05$]. Similarly, the size of the CPP did not differ following infusions into the VTA or the PAG of 1 ug [$F(1,14) = 0.6$; $p > 0.05$] or 5 ug [$F(1,14) = 0.03$; $p > 0.05$] of morphine.

Opioid Antagonism

The results of histological analyses are shown in Figures 9 and 10. Figure 11 illustrates that blockade of opioid receptors in the VTA or the PAG attenuate a morphine-induced CPP. NM injections into either site did not produce a preference or an aversion for the NM-paired compartment (VTA (2nmol NM) [$F(1,21) = 0.002$; $p > 0.05$], PAG (2nmol NM) [$F(1,21) = 0.02$; $p > 0.05$], and PAG (5 nmol NM) [$F(1,21) = 0.4$; $p > 0.05$]).

As shown in Figure 11, animals conditioned with systemic morphine and intra-VTA vehicle infusions developed a CPP [$F(1,14) = 25.6$; $p < 0.01$]. Intra-VTA infusions of NM (2 nmol) blocked the effect [$F(1,14) = 0.12$; $p > 0.05$]. The size of the preference for the drug-paired compartment was significantly reduced following intra-VTA injections of NM [$F(1,28) = 4.7$; $p < 0.05$]. The higher dose of NM (5 nmol) was not tested in the VTA because the lower dose (2 nmol) eliminated a CPP induced by systemic morphine.

Systemic morphine administration produced a CPP in animals who received intra-PAG infusions of vehicle during conditioning [$F(1,21) = 30.1$; $p < 0.01$]. Animals conditioned with intra-PAG injections of NM (2 nmol) and systemic morphine also showed a significant preference for the drug-paired compartment [$F(1,21) = 8.4$; $p < 0.05$]. There was no difference in the size of the CPP between

Figure 9

Distribution of sites at which microinjections of 2 nmol of NM into the VTA or PAG blocked (open symbols), or failed to block (filled symbols), a CPP induced by systemic morphine. The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the intended site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

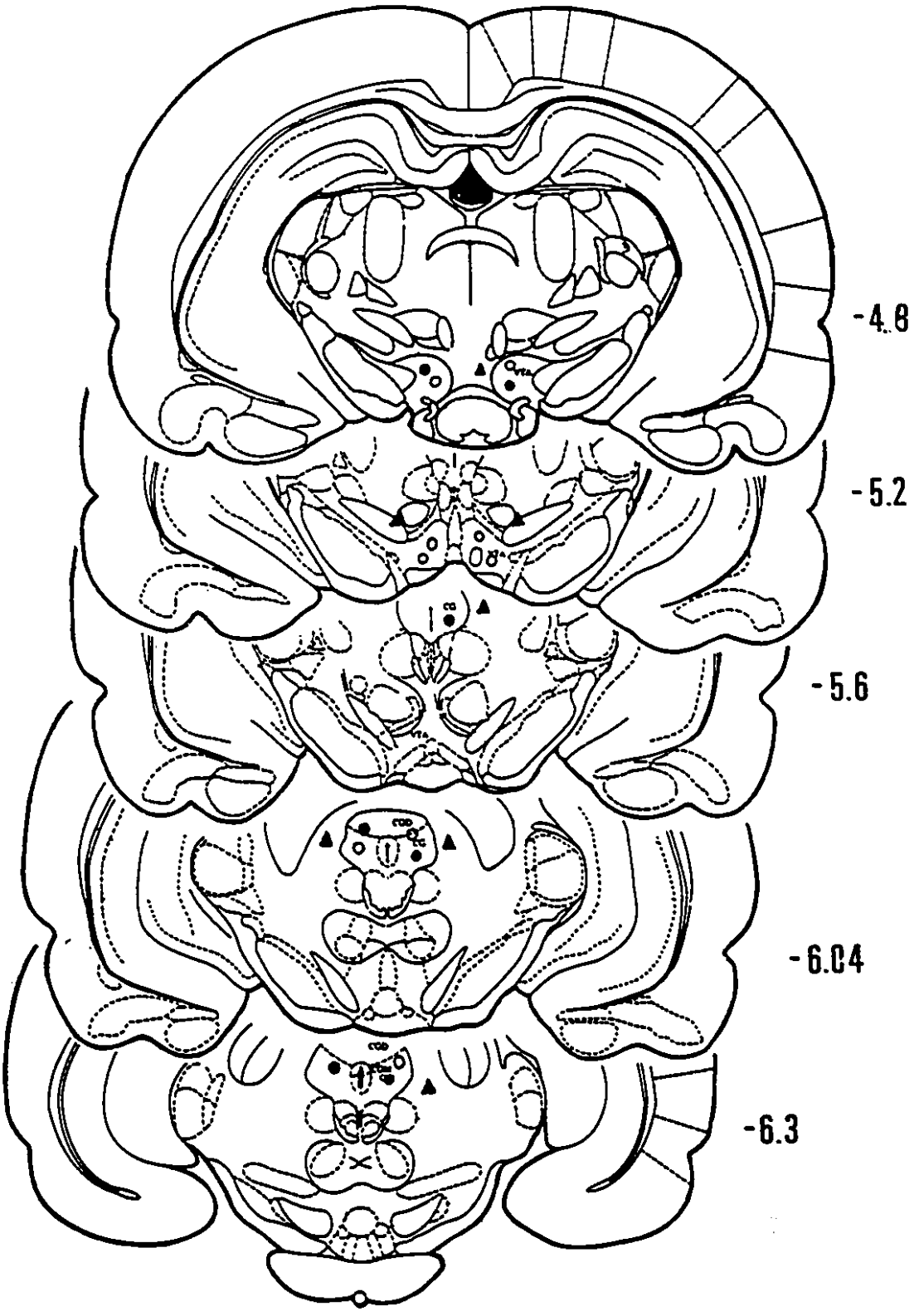

-4.8
-5.2
-5.6
-6.04
-6.3

Figure 10

Distribution of sites at which microinjections of 5 nmol of NM into the PAG blocked (open symbols), or failed to block (filled symbols), a CPP induced by systemic morphine. The left or right cannula from each animal was selected at random to be plotted so that the bilateral distribution is unbiased. Circles represent animals included in statistical analyses. Triangles represent animals discarded from the study because injections fell beyond the anatomical boundaries of the intended site. Numbers to the right are distances, in mm, anterior (+) or posterior (-) to bregma.

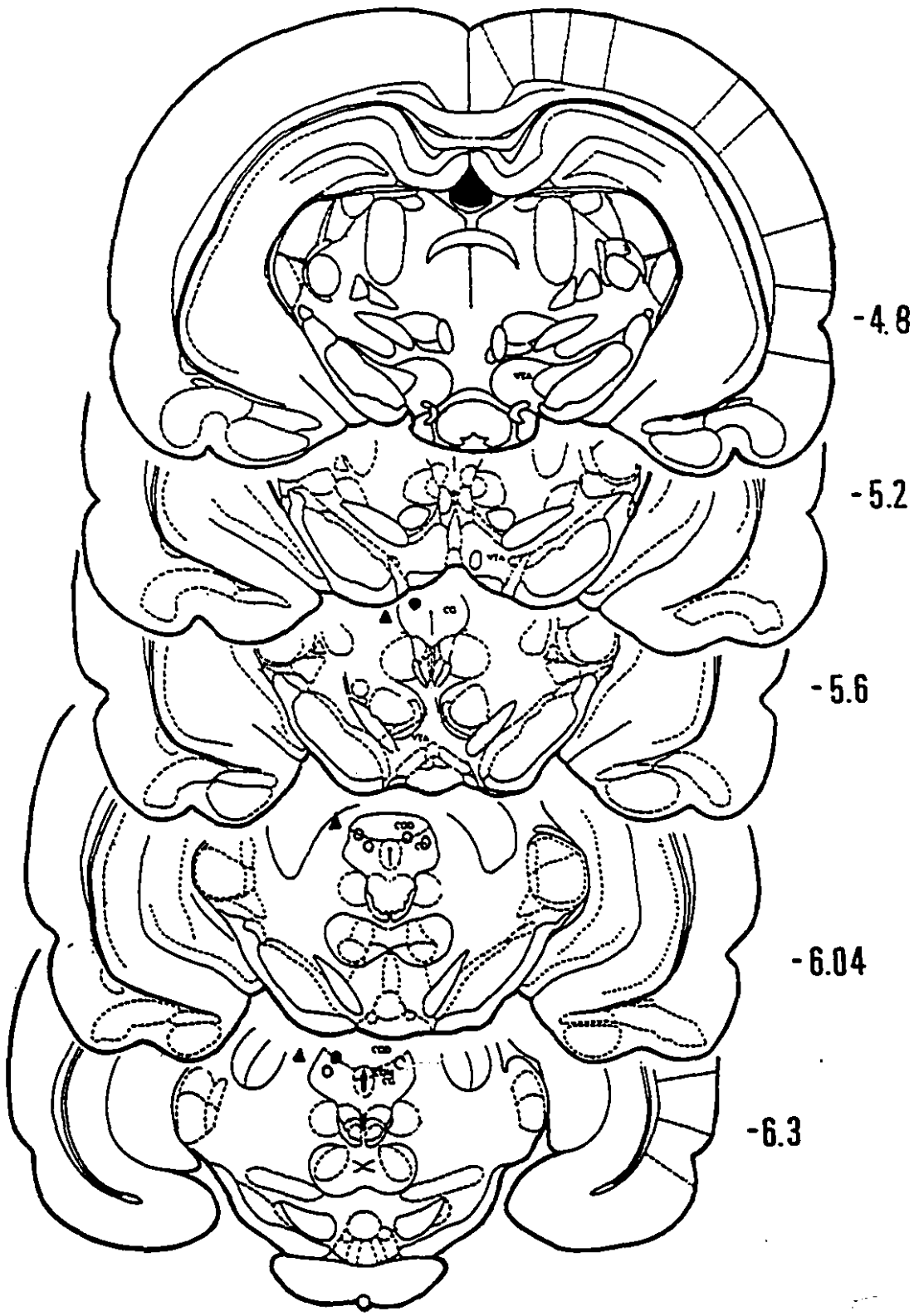
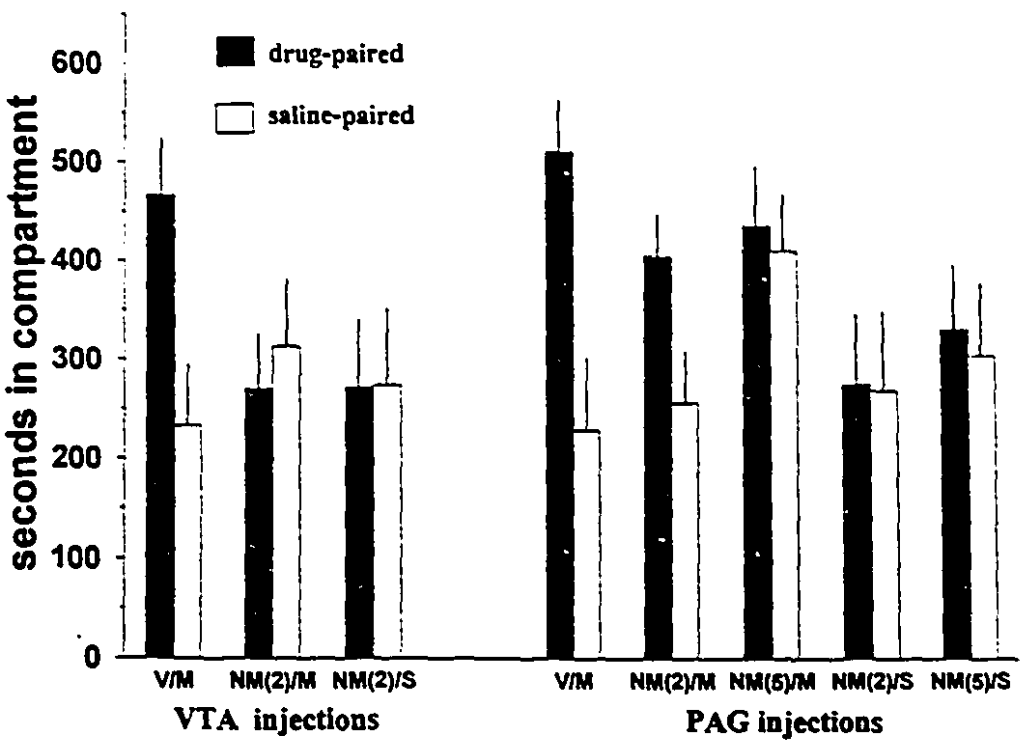

-4.8
-5.2
-5.6
-6.04
-6.3

Figure 11

Effects of opioid antagonist injections into the VTA or PAG on the CPP induced by systemic morphine. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments under different combinations of intracerebral and systemic injections (for description, see methods). Abbreviations: NM(2) = 2 nmol intra-cerebral NM; NM(5) = 5 nmol intra-cerebral NM; V = intra-cerebral vehicle; M = systemic morphine; S = systemic saline.


drug-paired
saline-paired
seconds in compartment
600
500
400
300
200
100
0
V/M
NM(2)/M
NM(2)/S
VTA injections
V/M
NM(2)/M
NM(6)/M
NM(2)/S
NM(5)/S
PAG injections

Discussion

In the present study, increasing doses of intra-VTA or intra-PAG morphine did not produce increasing preferences for the drug-paired compartment. This is in contrast to findings in previous studies that morphine's rewarding effect in the CPP paradigm is dose-dependent (Barr, Paredes, & Gridger, 1985; Bozarth, 1987a; Motta & Brandao, 1993; Mucha & Iversen, 1984; Mariaelvina, Braida, Calcaterra, Leone, & Gori, 1992). It is possible that the dose increments employed in the present study were too large to reveal a dose-response relationship because threshold and maximum effective doses that produce a CPP are very close (Bozarth, 1987a).

VTA Versus PAG Sensitivity

The present results indicate that the VTA is more sensitive than the PAG to both opioid-induced agonist and antagonist activity. The lowest dose of morphine tested (0.2 *u*g) produced a significant CPP following intra-VTA, but not intra-PAG, injections. Furthermore, 2 nmol of NM eliminated a morphine-induced CPP when injected into the VTA, but only reduced the effect when injected into the PAG. It is possible that intra-VTA injections of morphine and NM were effective at lower doses than intra-PAG injections because there is a higher concentration of *u* opioid receptors in the VTA than in the targeted site within the PAG (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987).

It is unlikely that the behavioural effects produced by intra-PAG injections of morphine or NM are due an action at the VTA. First, diffusion from a 0.5 *u*l microinjection of morphine is approximately 0.75 mm in 30 minutes (Yaksh & Rudy, 1978): PAG injections were 1 mm posterior, 0.4 mm medial, and 3.3 mm

dorsal to the VTA injections. Second, morphine microinjections that were closer to the VTA than to the PAG (LH or PH) failed to produce a CPP (see Figures 1 and 2). Third, NM injections (5 nmol) into the PAG blocked a CPP, whereas those that fell outside the boundaries of the structure, some closer to the VTA, did not (see Figure 9).

VTA and Morphine-induced CPP

Findings in both Experiments 1 and 2 support previous evidence that intra-VTA injections produce a CPP (Barr & Rossi, 1992; Bozarth, 1987b; Phillips & LePiane, 1980). Experiment 2 is the first report that blockade of opioid receptors in the VTA reduces a CPP induced by systemic morphine administration. Taken together, these findings indicate that an action at opioid receptors in the VTA is rewarding. This suggestion is further supported by evidence that animals will self-administer morphine into the VTA (Bozarth & Wise, 1981b, 1984; Devine & Wise, 1994). Because opioid receptors are located on GABA interneurons in the VTA (Dilts & Kalivas, 1989; Johnson & North, 1992), it has been suggested that opiates produce their rewarding effects by reducing GABAergic influences on VTA DA neurons (Devine, Leone, & Wise, 1993).

PAG and Morphine-induced CPP

The present results also support the findings that morphine injections into the PAG produce a CPP (Cazala, 1990; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982) and are consistent with the report that intra-PAG injections of morphine support self-administration (Motta & Brandao, 1993). In addition, the finding that intra-PAG injections of NM blocked a CPP induced by systemic

morphine supports evidence that blockade of opioid receptors in the region reduces responding for intravenous opiates (Corrigall & Vaccarino, 1988). It appears, therefore, that morphine's rewarding effect may be initiated by an action at opioid receptors in the PAG.

Despite the evidence outlined above indicating that the PAG is involved in the rewarding effect of opiates, the region is not generally associated with reward related processes. Behavioural investigations into PAG function have demonstrated that PAG stimulation elicits defensive reactions (Abrahams, Hilton, & Zbrozyna, 1960; Bandler & Carrive, 1988; Depaulis, Keay, & Bandler, 1992) and antinociception (Lewis & Gebhart, 1977; Pert & Yaksh, 1974; Yaksh, 1979; Yaksh, Yeung, & Rudy, 1976), and that PAG lesions abolish defensive responses to threatening stimuli (Fernandez De Molina & Hunsperger, 1962). These findings are taken as evidence that the PAG is part of a system that controls behavioural responses to aversive stimuli (Holstege, 1990, 1992).

If this is true, morphine injections into the PAG may produce a CPP because they reduce the aversive effects of the behavioural testing. This hypothesis is feasible because morphine injections into PAG suppress nociceptive sensory input (Jenck, Schmidtt, & Karli, 1983), attenuate escape responses to painful environmental stimuli (Mayer & Price, 1976; Pert & Yaksh, 1974; Yaksh, Yeung, & Rudy, 1976), and produce analgesia in the formalin test (Manning, Morgan, & Franklin, 1994). The most aversive aspects of CPP conditioning and testing are probably stress related, particularly in microinjection studies in which animals are restrained while the injectors are inserted and removed. The possibility that intra-PAG injections of morphine produce a CPP via negative as opposed to positive reinforcement could be tested by examining whether changes

in levels of stress (or other aversive effects) alters the behavioural responding of animals conditioned with intra-PAG morphine.

Relationship Between the VTA and the PAG

The findings in Experiment 2 appear to indicate that morphine's rewarding effect is mediated through a serially connected system that includes both the VTA and PAG because blockade of opioid receptors in either site eliminated a CPP to morphine. The analysis of behavioural responding in Experiment 1, however, suggests that the CPPs induced by intra-VTA and intra-PAG morphine may not be produced by the same underlying mechanism. This may only be an apparent conflict because opiate injections into one neural region are capable of influencing opioid activity in other sites. For example, injections of morphine into either the amygdala, the NAS, or the PAG induce opioid release in the other two sites (Ma & Han, 1991b) and the effect is blocked by administration of an opioid antagonist (Ma & Han, 1991a, 1992; Ma, Shi, & Han, 1992). Furthermore, naloxone administration into the NAS or the PAG blocks antinociception induced by morphine injections into the other site (Ma, Shi, & Han, 1992).

Based on the findings from their studies, Ma and colleagues have proposed that the amygdala, the NAS, and the PAG are part of a circuit that mediates opiate-induced antinociception. They further suggest that the three structures are connected in a positive feedback system such that they contribute to an all-or-none effect. If the rewarding effect of morphine is produced through a similar mechanism, blockade of opioid receptors in either the VTA or the PAG could disrupt activity in the other site. That is, opioid-induced activity in both the VTA and the PAG may be critically involved in the mediation of morphine-induced

reward even if the two structures are not serially connected. If this is true, the VTA and the PAG may make different, albeit necessary contributions to the development of a CPP to morphine. As previously suggested, morphine injections into the VTA and the PAG may produce a CPP through processes of positive and negative reinforcement respectively.

EXPERIMENT 3

Effects of Lesions of the Mesolimbic DA System, the VS, the VP, or the PPTg on the Development of a CPP to Morphine

Results from Experiments 1 and 2 suggest that neural mechanisms underlying morphine-induced reward are initiated by an action at opioid receptors in the VTA and the PAG. Activity at these sites is transmitted directly and via synaptic connections to other neural structures which participate in mediating the rewarding effect of opiates. One proposal is that the rewarding effect of morphine (and other abused drugs) is mediated through a neural circuit of drug reward that includes the mesolimbic DA system, the VS, the VP, and the PPTg (Koob & Bloom, 1988).

The mesolimbic DA system appears to mediate the rewarding effect of psychostimulants because lesions of this system block a CPP to amphetamine (Spyraki, Fibiger, & Phillips, 1982c) and disrupt self-administration of cocaine or amphetamine (Lyness, Friedle, & Moore, 1979; Roberts, Corcoran, & Fibiger, 1977; Roberts, Koob, Klonoff, & Fibiger, 1980; Roberts & Koob, 1982). Studies examining the role of mesolimbic DA in the rewarding effect of opiates, however, have produced inconsistent results. Mesolimbic DA lesions have been reported to block an opiate-induced CPP (Spyraki, Fibiger, & Phillips, 1983; Shippenberg, Bals-Kubik, & Herz, 1993), but not responding for intravenous opiates (Dworkin, Guerin, Co, Goeders, & Smith, 1988; Pettit, Ettenberg, Bloom, & Koob, 1984). Similarly, some studies suggest that administration of DA antagonists blocks the rewarding effect of opiates (Bozarth & Wise, 1981a; Leone & Di Chiara, 1987; Schwartz & Marchok, 1974; Shippenberg & Herz, 1987, 1988), whereas others suggest that it does not (Bechara, Harrington, Nader, & van der Kooy, 1992;

Ettenberg, Pettit, Bloom, & Koob, 1982; Gerrits, Ramsey, Wolterink, & Van Ree, 1994; Mackey & van der Kooy, 1985; Nader, Bechara, Roberts, & van der Kooy, 1994; Van Ree & Ramsey, 1987). There is little or no disagreement that opiates increase mesolimbic DA activity when they are administered systemically (Di Chiara & Imperato, 1988a; Gysling & Wang, 1983; Matthews & German, 1984) or directly into the VTA (Leone, Pocock, & Wise, 1991; Matthews & German, 1984). Given the contradictory findings cited above, however, it is not clear whether this effect is critical for morphine-induced reward.

One of the primary targets of mesolimbic DA fibers is the VS (Dahlstrom & Fuxe, 1964; Fallon & Moore, 1978; Ungerstedt, 1971), a region which includes the NAS, the medium sized cells of the olfactory tubercle (OT) and the ventromedial portions of the CPu (Heimer & Wilson, 1975). The region appears to be involved in psychostimulant reward because lesions of VS cell bodies disrupt responding for intravenous cocaine (Zito, Vickers, & Roberts, 1985). In the same study, similar lesions reduced heroin self-administration, although the effect was less pronounced. VS lesions have been reported to block (Dworkin, Guerin, Goeders, & Smith, 1988) or not affect (Glick & Cox, 1978) self-administration of morphine. One report suggests that VS lesions block a CPP to morphine (Kelsey, Carlezon, & Falls, 1989) but this study employed electrolytic lesions which would have destroyed fibers passing through the VS. Thus, the role of VS cell bodies in opiate-induced reward remains unclear.

Efferents from the VS terminate in the VP (Heimer & Wilson, 1975; Mogenson, Swanson, & Wu, 1983; Nauta, Smith, Faull, & Domesick, 1978; Swanson, 1967; Swanson & Cowan, 1975), suggesting that the VP may be involved in mediating the rewarding effect of natural rewards (Mogenson, 1987)

and of drugs of abuse (Koob & Bloom, 1988). The VP is also part of the basal forebrain (Alheid & Heimer, 1988), a region where lesions produce profound deficits in a variety of memory tasks (Dunnett, Everitt, & Robbins, 1991). Given its relationship with these two systems, the VP may be involved in reward-related learning (Richardson & DeLong, 1988). Findings that VP lesions reduce the rewarding effect of food in the CPP paradigm (McAlonan, Robbins, & Everitt, 1993) and of heroin or cocaine in the self-administration paradigm (Hubner & Koob, 1990; Robledo & Koob, 1993) support this suggestion. Although it has not been tested directly, it is possible that the VP is also involved in the rewarding effect of morphine in the CPP paradigm.

The PPTg, as a target of VP projections (Heimer, Switzer, & Van Hoesen, 1982; Swanson, Mogenson, Gerfen, & Robinson, 1984), is the final component in the neural circuit of drug reward (Koob & Bloom, 1988). The PPTg appears to be critically involved in opiate-induced reward because PPTg lesions block the development of a CPP to opiates (Bechara & van der Kooy, 1989; Nader, Bechara, Roberts, & van der Kooy, 1994; Olmstead & Franklin, 1993) and reduce responding for intravenous heroin (Olmstead, Munn, & Wise, 1993). The effect of PPTg lesions on a morphine-induced CPP was re-examined in the present study in order to assess changes in behavioural responding during CPP testing in greater detail than has been done in previous studies.

The evidence outline above indicates that the role of the structures which constitute the neural circuit of drug reward in the rewarding effect of opiates is controversial. Experiment 3 was designed to assess the role of the mesolimbic DA system, the VS, the VP, and the PPTg in opiate-induced reward by testing whether lesions of each site block a CPP to morphine. If a particular neural structure

mediates the rewarding effect of morphine, lesioning the region should disrupt a CPP to morphine. Moreover, if the rewarding effect of abused drugs depends on an intact neural circuit, destruction of any part of the system should block a morphine-induced CPP.

One advantage of the CPP paradigm is that animals are tested in a drug free state thereby eliminating the possibility that drug-induced motor effects are influencing responding. Although opiates produce state dependent learning (Overton, 1973), morphine administration prior to testing does not change the size of the CPP (Mucha & Iversen, 1984). Nonetheless, lesion-induced deficits may be altered during state dependent testing and differences between drug free and state dependent tests may reveal how lesions produce their effect. To test these possibilities, lesioned animals were re-tested for a CPP in a drugged state.

Methods

Subjects

One hundred and thirty subjects were used in this experiment.

Surgical Procedures

Neurotoxic-induced lesions of the mesolimbic DA system, the VS, the VP, or the PPTg were made in different groups of subjects. In the forthcoming description, coordinates for each lesion are given as mm anterior (+) or posterior (-) to bregma, mm lateral to bregma, and mm ventral to the skull surface, according to the atlas of Paxinos and Watson (1986).

Mesolimbic DA Lesions

Thirty minutes prior to surgery, subjects were treated with pargyline HCl 50 mg/kg and desmethylimipramine (DMI) 25 mg/kg i.p. to protect noradrenaline (NA) fibers. Subjects received infusions of 2 *u*l of a solution containing 8 *u*g free base 6-OHDA HBr over 20 minutes: sham (n = 10) and lesion (n = 10). 6-OHDA was dissolved in a solution of 0.3 mg/ml ascorbic acid in 0.9% saline. The solution was kept on ice and shielded from light during surgery. Coordinates, aimed at the NAS, were +1.7, 1.5, 7.0.

VS Lesions

Four separate groups of subjects received lesions of the VS. In the first groups, lesions of the medial VS were induced by infusing N-methyl-D-aspartate (NMDA) (0.5 *u*l of 0.1 M solution): sham (n = 9) and lesion (n = 7). In the second group, lesions of the same area of the VS were induced by infusing kainic acid (0.5 *u*g in 1 *u*l of solution) over 10 minutes: sham (n = 10) and lesion (n = 10). Coordinates for both of these lesions were +2.0, 1.8, 7.5. Kainic acid was used in the second group because VS lesions induced by kainic acid disrupt responding for intravenous heroin (Zito, Vickers, & Roberts, 1985).

The third group of animals received lesions of the entire VS induced by infusing kainic acid at two sites: 0.7 *u*g in 0.7 *u*l of solution was injected over 10 minutes at coordinates +1.2, 1.8, 7.3, and 0.5 *u*g in 0.5 *u*l of solution was injected over five minutes at coordinates +2.5, 1.5, 7.0: sham (n = 12) and lesion (n = 10). In the fourth group, lesions restricted to the anterior VS were induced by infusing 0.5 *u*g in 0.5 *u*l kainic acid solution over five minutes at coordinates 2.5, 1.5, 7.0: sham (n = 8) and lesion (n = 8).

VP Lesions

Lesions of the VP were induced by infusing 0.5 *u*l of 0.2 M NMDA over five minutes at coordinates -0.3, 2.2, 8.0: sham (n = 10) and lesion (n = 10).

PPTg Lesions

Lesions of the PPTg were produced by infusing 0.5 *u*l of 0.1 M NMDA over 10 minutes at coordinates -7.8, 1.6, 7.0: sham (n = 8) and lesion (n = 8). Animals in this group were treated post-surgically with diazepam (1 mg/kg) to reduce seizure activity.

Behavioural Procedures

Subjects underwent six alternating conditioning sessions, three with morphine (2 mg/kg) and three with saline, using the protocol described in the General Methods.

Histology

Brain amine levels were determined for animals receiving 6-OHDA lesions. Following cervical dislocation, brains were removed, cut at midthalamic level, and three coronal sections of the forebrain were taken with a freezing microtome. The rostral section was 1.5 mm thick and the two caudal sections were 1.0 mm thick. Tissue samples were hand dissected from the three sections which spanned the following anterior-posterior coordinates: MFC, +2.2 - +3.7; NAS, +0.9 - +1.9; CPu, -0.1 - +0.9; Samples of OT were taken from the middle and posterior sections.

Tissue samples from the left and right sides of the brain were combined, weighed and homogenized in a volume of solvent consisting of 135 *u*l of 0.1M perchloric acid containing 50 mg/l EDTA and 15 *u*l of dihydroxybenzylamine

hydrochloride (DHBA). Homogenates were spun for 15 minutes at 15,000 RPM at 0C. 20 or 25 *ul* of the supernatant was injected by an automatic sample injector (Shimadsu, Sil-6A Autoinjector) into the column (Waters Microbond Pack C18). Electrochemical detection was by a Amperometric detector LC-4B (Bioanalytic Systems) with voltage of 0.680. The mobile phase was 0.1M sodium acetate and 0.02M citric acid with pH adjusted to 4 with glacial acetic acid. 0.02 M sodium octyl sulphate, 50 mg/l EDTA, and 2% of methanol were added. Flow rate was 2 ml/min. DA, NA, and DHBA were identified by comparing retention times of their peaks to those of the standards. Standard curves were calculated from six interna' standards which consisted of DHBA, six concentrations of DA, and six concentrations of NA. Concentrations of DA and NA were estimated from the curves and expressed as ng/g wet tissue weight.

Results

6-OHDA Lesions

Histology

Table 4 presents the results of brain amine level analyses following infusions of 6-OHDA (lesion) or vehicle (sham) into the NAS. These data show that 6-OHDA lesions produced a significant depletion of DA in the NAS [$F(1,18) = 5.67$; $p < 0.05$], but not in the CPu [$F(1,18) = 1.76$; $p > 0.05$], OT [$F(1,18) = 0.04$; $p > 0.05$], or MFC [$F(1,18) = 1.1$; $p > 0.05$]. NA levels were modestly, but not significantly, reduced in all four regions.

Table 4

Regional concentrations of DA and NA in the NAS, CPu, OT, and MFC following infusions of 6-OHDA (lesion) or vehicle (sham) into the NAS. Absolute concentrations are given as mean ± SEM in ng/g wet tissue. Percent refers to the concentration of DA and NA in lesioned animals as a percentage of that found in sham lesioned animals.

	Site	Sham N=10	Lesion N=10	Percent
DA	NAS	12.5 ± 0.05	5.0 ± 0.01	40*
	CPu	11.69 ± 0.08	10.17 ± 0.05	87
	OT	1.87 ± 0.02	2.20 ± 0.08	120
	MFC	0.34 ± 0.04	0.34 ± 0.02	100
NA	NAS	0.11 ± 0.001	0.09 ± 0.004	82
	CPu	0.57 ± 0.01	0.51 ± 0.01	90
	OT	0.53 ± 0.04	0.5 ± 0.05	82
	MFC	0.11 ± 0.006	0.09 ± 0.002	82

Behavioural Results

Figure 12 shows that 6-OHDA lesions of the NAS had no effect on a morphine-induced CPP. Both sham and lesioned animals developed a CPP for the drug-paired compartment, sham [$F(1,18) = 39.5$; $p < 0.01$] and lesion [$F(1,14) = 54.9$; $p < 0.01$]. There was no difference between the groups in the size of preference for the drug-paired compartment [$F(1,36) = 0.76$; $p > 0.05$].

VS Lesions

Histology

The location and extent of NMDA- or kainic acid-induced lesions of the medial VS are presented in Figure 13. Lesions were concentrated in the caudal end of the VS, typically damaging most of the NAS. Both NMDA and kainic acid infusions produced neuronal damage throughout the core of the NAS. Portions of the surrounding shell were also destroyed, but in general the rostral pole was spared. There was little or no damage in the rostral one-third of the VS or in the ventromedial portion of the CPu. The OT was also frequently spared. Consistent with previous reports (Dunnett, Whishaw, Jones, & Bunch, 1987; Peterson & Moore, 1980), kainic acid infusions produced a larger area of cell loss and/or destruction than did NMDA, destroying cells in more lateral and ventral portions of the VS.

The location and extent of kainic acid-induced lesions of the entire VS are shown in Figure 14. These lesions destroyed the same regions that were damaged in the first two VS lesioned groups as well as more rostral and ventral areas including the OT and some dorsal VP neurons. The ventromedial CPu was damaged in two animals.

Figure 12

Effects of 6-OHDA lesions of the NAS on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.

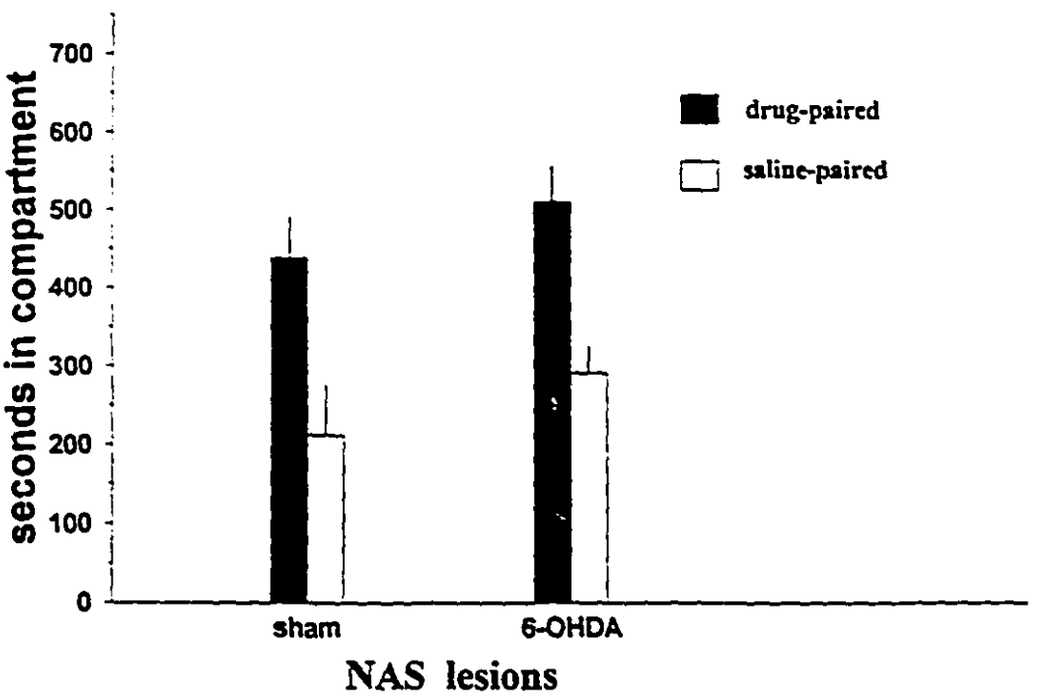

700
600
500
400
300
200
100
0
seconds in compartment
drug-paired
saline-paired
sham
6-OHDA
NAS lesions

Figure 13

Schematic representation of NMDA- or kainic acid-induced lesions of the medial VS. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

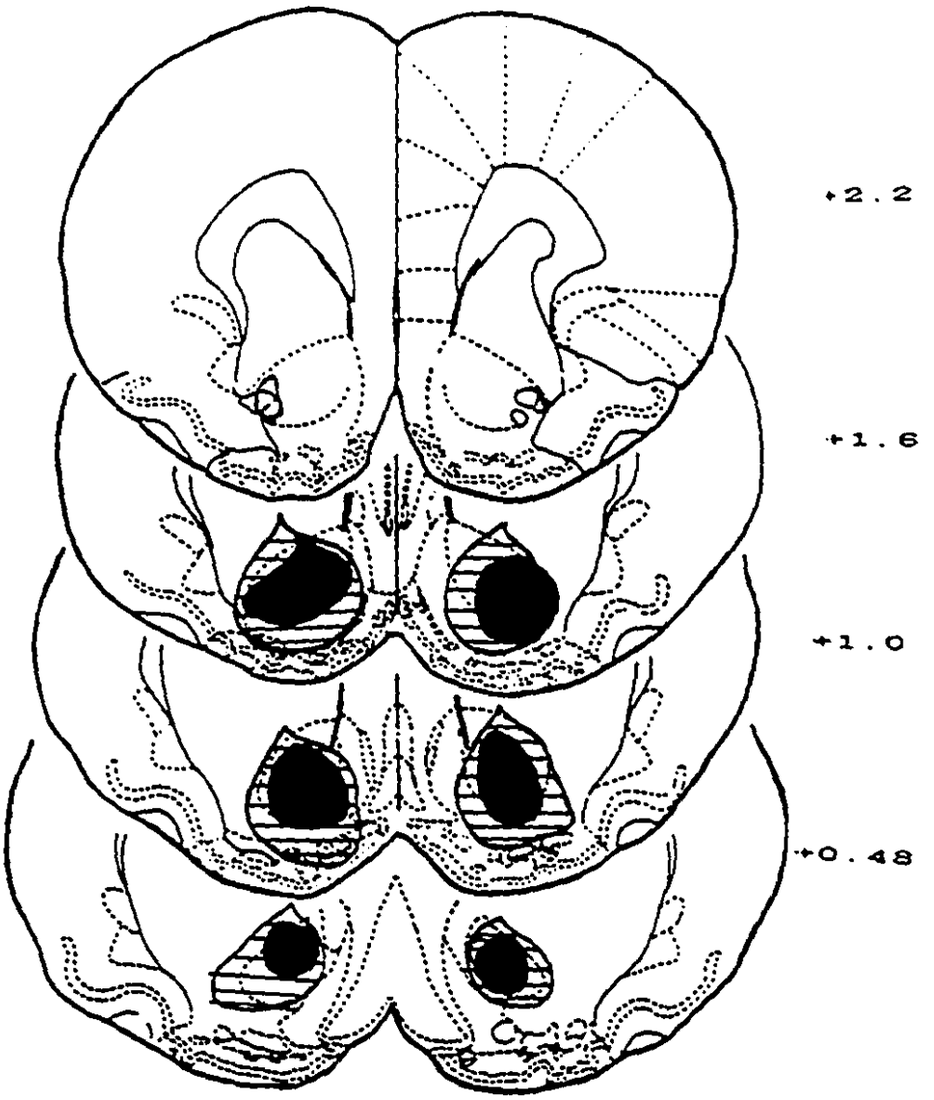
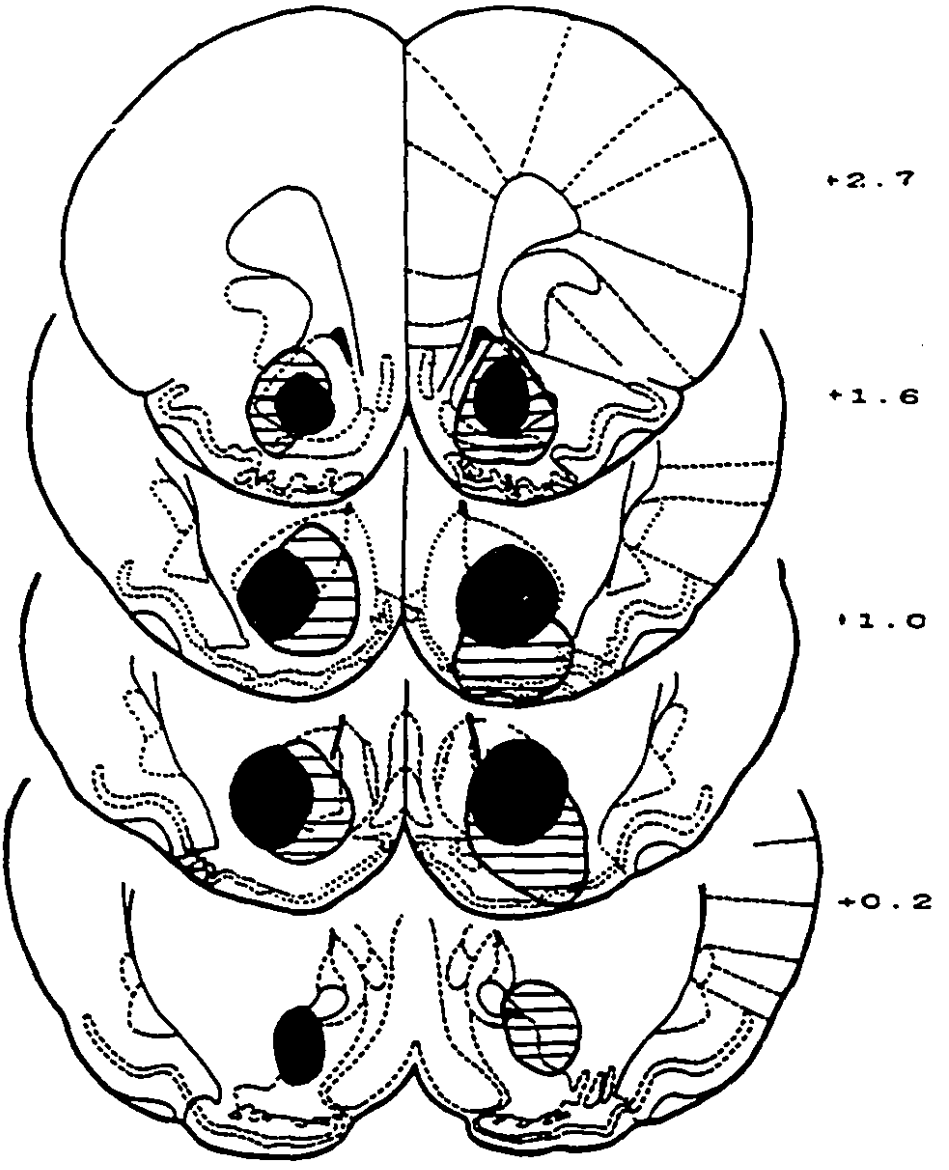

+2.2
+1.6
+1.0
+0.48

Figure 14

Schematic representation of kainic acid-induced lesions of the entire VS. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).


+2.7
+1.6
+1.0
+0.2

Kainic acid-induced lesions of the anterior VS are represented in Figure 15. Lesions destroyed approximately the rostral half of the VS. In all animals, OT and VP neurons were spared. Neither large nor anterior VS lesion destroyed septal nuclei.

Behavioural Results

As shown in Figure 16, lesions of the medial VS had no effect on the development of a CPP to morphine. Following NMDA-induced lesions, animals with sham and NMDA-induced lesions showed a CPP for the morphine-paired compartment, sham [$F(1,16) = 19.7$; $p < 0.01$] and lesion [$F(1,16) = 35.2$; $p < 0.01$]. There was no difference in the size of the CPP between these two groups [$F(1,32) = 0.95$; $p > 0.05$]. Similarly, kainic acid-induced lesions had no effect on a morphine-induced CPP. Sham and lesioned groups showed a preference for the drug-paired compartment (sham [$F(1,18) = 13.9$; $p < 0.01$] and lesion [$F(1,18) = 16.4$; $p < 0.01$]), and the size of preference did not differ between the two groups [$F(1,36) = 0.36$; $p > 0.05$].

As shown in Figure 17, kainic acid-induced lesions which destroyed the entire VS reduced a CPP to morphine. Both groups of animals developed a CPP (sham [$F(1,22) = 36.8$; $p < 0.01$] and lesion [$F(1,22) = 7.8$; $p < 0.05$], but the size of the preference for the drug-paired compartment was significantly smaller in the lesioned group [$F(1,44) = 3.9$; $p < 0.05$].

Lesions confined to the anterior portion of the VS had no effect on the development of a CPP to morphine. Sham [$F(1,14) = 10.9$; $p < 0.01$] and lesioned [$F(1,14) = 13.8$; $p < 0.01$] groups developed a CPP to the drug-paired compartment, and there was no difference in the size of the preference between groups [$F(1,28) = 0.08$; $p > 0.05$].

Figure 15

Schematic representation of kainic acid-induced lesions of the anterior VS. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

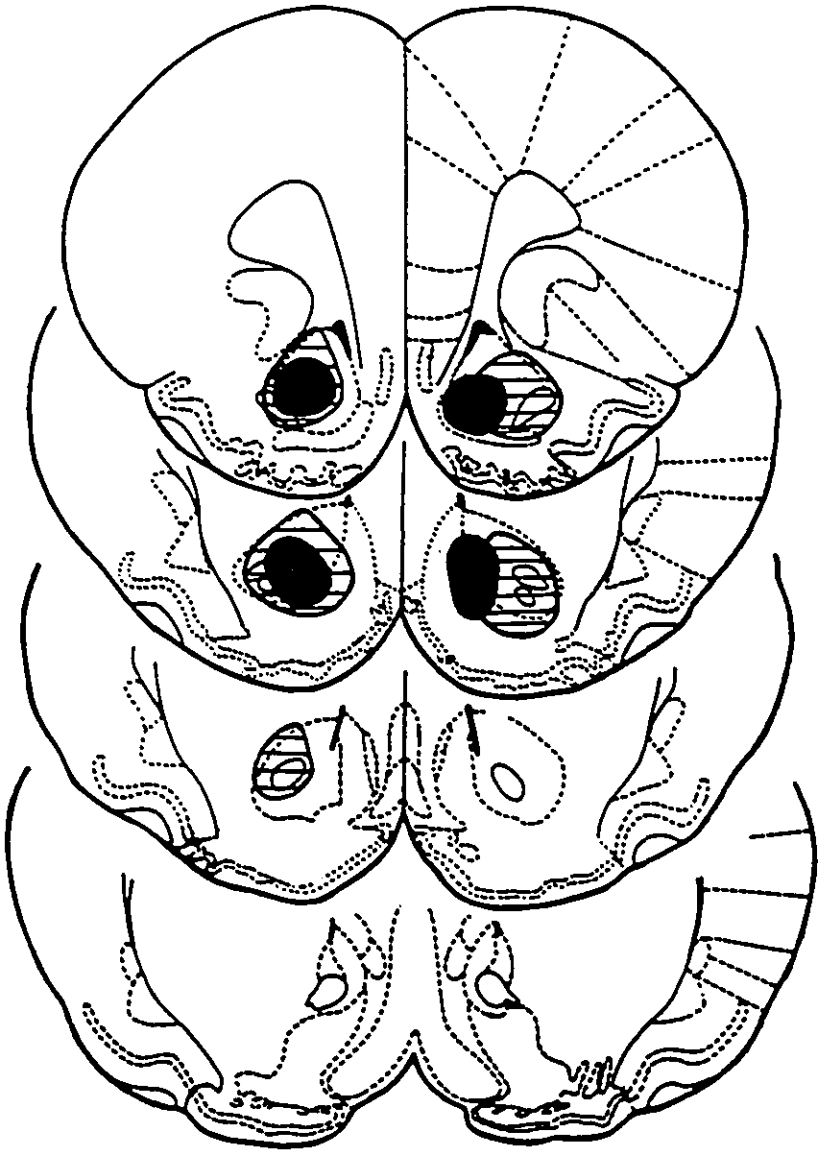

+2.7
+1.6
+1.0
+0.2

Figure 16

Effects of kainic acid- and NMDA-induced lesions of the medial VS on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.

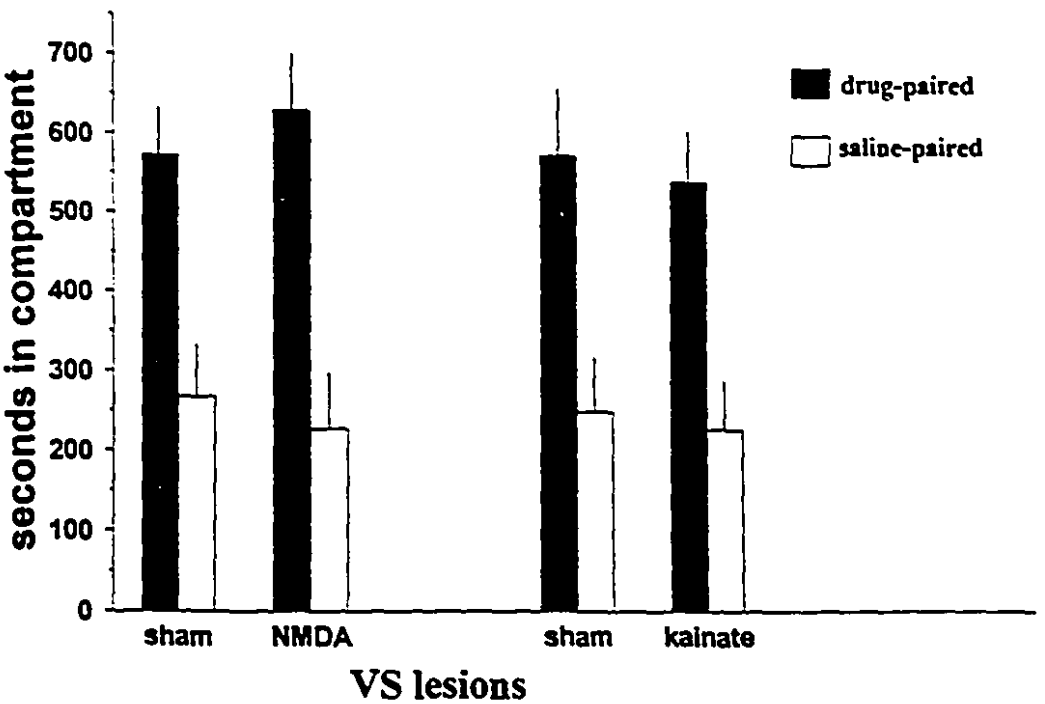
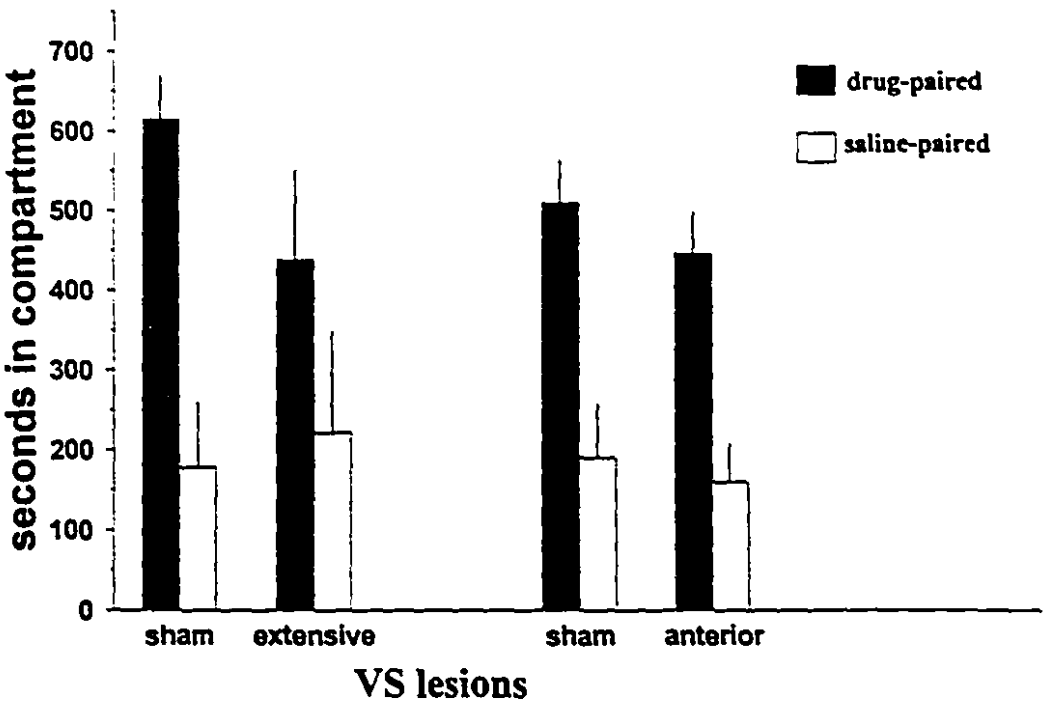

seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NMDA
sham
kainate
VS lesions

Figure 17

Effects of kainic acid-incuded lesions of the entire or anterior VS on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.


seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
extensive
sham
anterior
VS lesions

Data from the animals with extensive VS lesions were re-analyzed and is presented in Figure 18 as time spent in, and entries to, drug- and saline-paired compartments at 2 minutes intervals. Animals with large VS lesions exhibited a CPP during the first [$F(1,9) = 10.6$; $p < 0.05$] and second [$F(1,9) = 17.1$; $p < 0.05$] 10 minutes of testing. The size of the CPP did not differ across the two 10 minute sessions [$F(1,9)$ 4.9; $p > 0.05$].

The number of entries that animals made to the drug- and saline-paired compartments did not differ during the first [$(F1,9) = 0.89$; $p > 0.05$] or second [$F(1,9) = 4.1$; $p > 0.05$] half of the test. Compared to their sham lesioned controls, animals with large VS lesions made more total entries over the 20 minute test period [$F(1,44) = 18.6$; $p < 0.01$].

VP Lesions

Histology

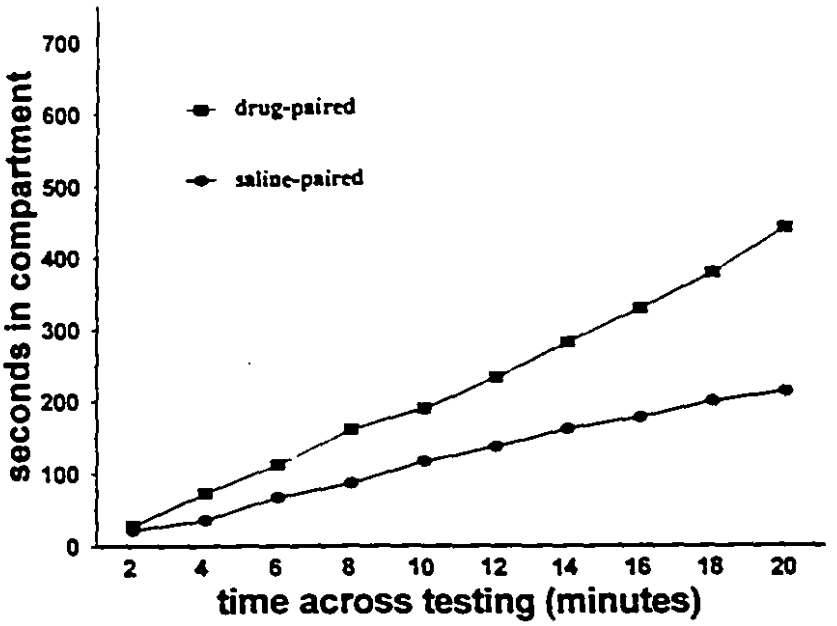
The location and extent of NMDA-induced lesions of the VP are shown in Figure 19. Lesions produced damage in the anterior portion of the VP below the decussation of the anterior commissure. The posterior VP was partially damaged in half of the animals and completely spared in the other half. Lesions also destroyed, to a varying degree, portions of the olfactory tubercle and nucleus basalis of Meynert. This damage was variable and never destroyed a significant portion of either structure. No gliosis was visible in the NAS, CPu, dorsal pallidum, or structures medial to the VP.

Behavioural Results

There was no effect of VP lesions on the CPP induced by morphine (Figure 20). Animals with sham and neurotoxin-induced lesions developed a significant

Figure 18

Time course of the CPP exhibited by animals with large VS lesions. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.


seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

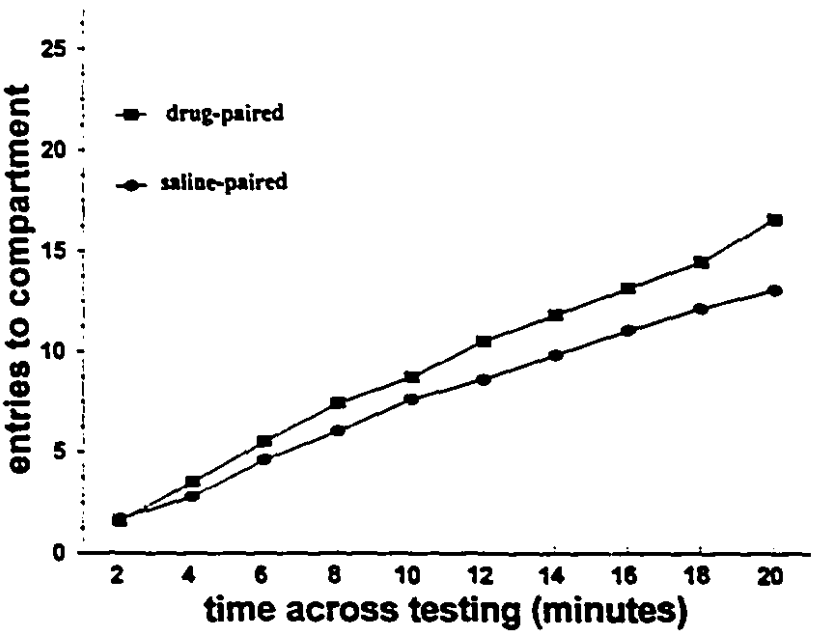

entries to compartment
25
20
15
10
5
0
drug-paired
saline-paired
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

Figure 19

Schematic representation of NMDA-induced lesions of the VP. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

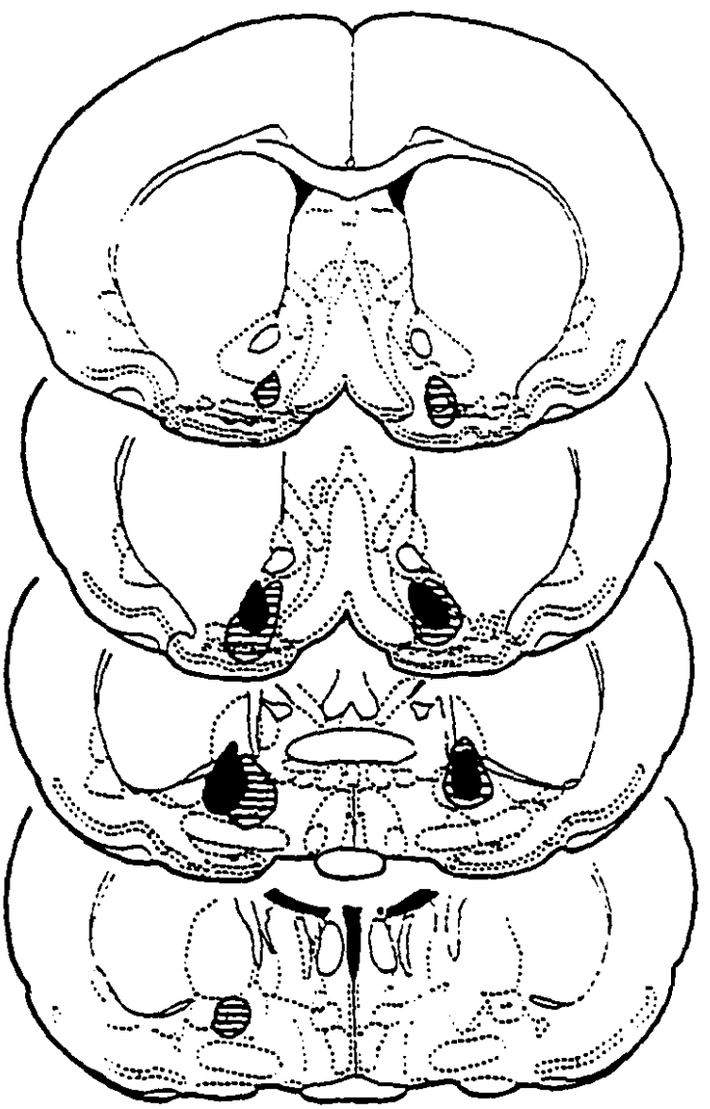
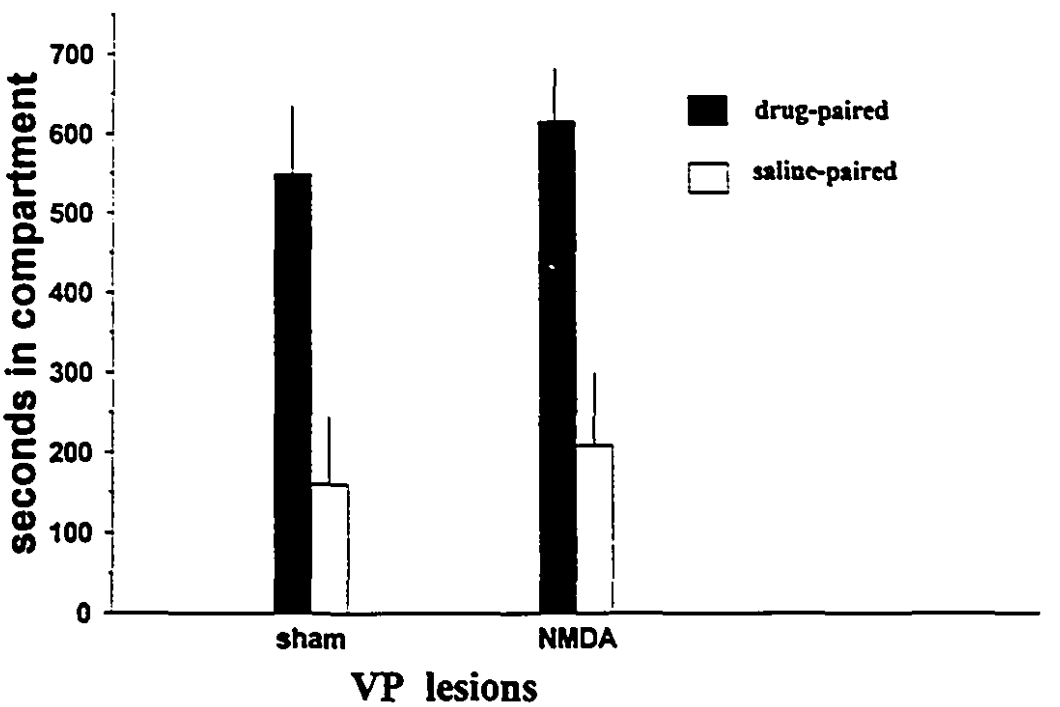

+0.4 8
+0.2
-0.4
-0.8

Figure 20

Effects of NMDA-induced lesions of the VP on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.


seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NMDA
VP lesions

preference for the drug-paired compartment, sham [$F(1,18) = 44.5$; $p < 0.01$] and lesion [$F(1,18) = 40.7$; $p < 0.01$]. There was no difference in the size of the CPP exhibited by these two groups [$F(1,36) = 0.05$; $p > 0.05$].

PPTg Lesions

Histology

The location and extent of NMDA-induced lesions of the PPTg are shown in Figure 21. Lesions produced cell loss lateral and ventral to the decussation of the superior cerebral peduncle. Damage extended from the anterior tip to the posterior one third of the PPTg. Half of the animals sustained damage in the retrorubral nucleus. Surrounding regions, including the PAG, cuneiform nucleus, substantia nigra, and VTA were all spared.

Behavioural Results

Figure 22 shows that PPTg lesions blocked a morphine-induced CPP. Sham lesioned animals displayed a CPP [$F(1,14) = 55.1$; $p < 0.01$] but animals with NMDA-induced lesions did not [$F(1,14) = 3.8$; $p > 0.05$]. The size of the CPP was significantly reduced following PPTg lesions [$F(1,28) = 15.7$; $p < 0.05$].

A further analysis of the data revealed that PPTg lesions blocked a morphine-induced CPP during the first [$F(1,7) = 1.7$; $p > 0.05$] and second half of the test [$F(1,7) = 2.04$; $p > 0.05$]. Difference scores did not vary between the two halves of the test [$F(1,7) = 0.36$; $p > 0.05$].

Lesioned animals did not make more entries to the drug-paired compartment in the first [$F(1,7) = 1.46$; $p > 0.05$] or second [$F(1,7) = 2.01$; $p > 0.05$] 10 minutes of testing. There was no difference in number of total entries

Figure 21

Schematic representation of NMDA-induced lesions of the PPTg. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

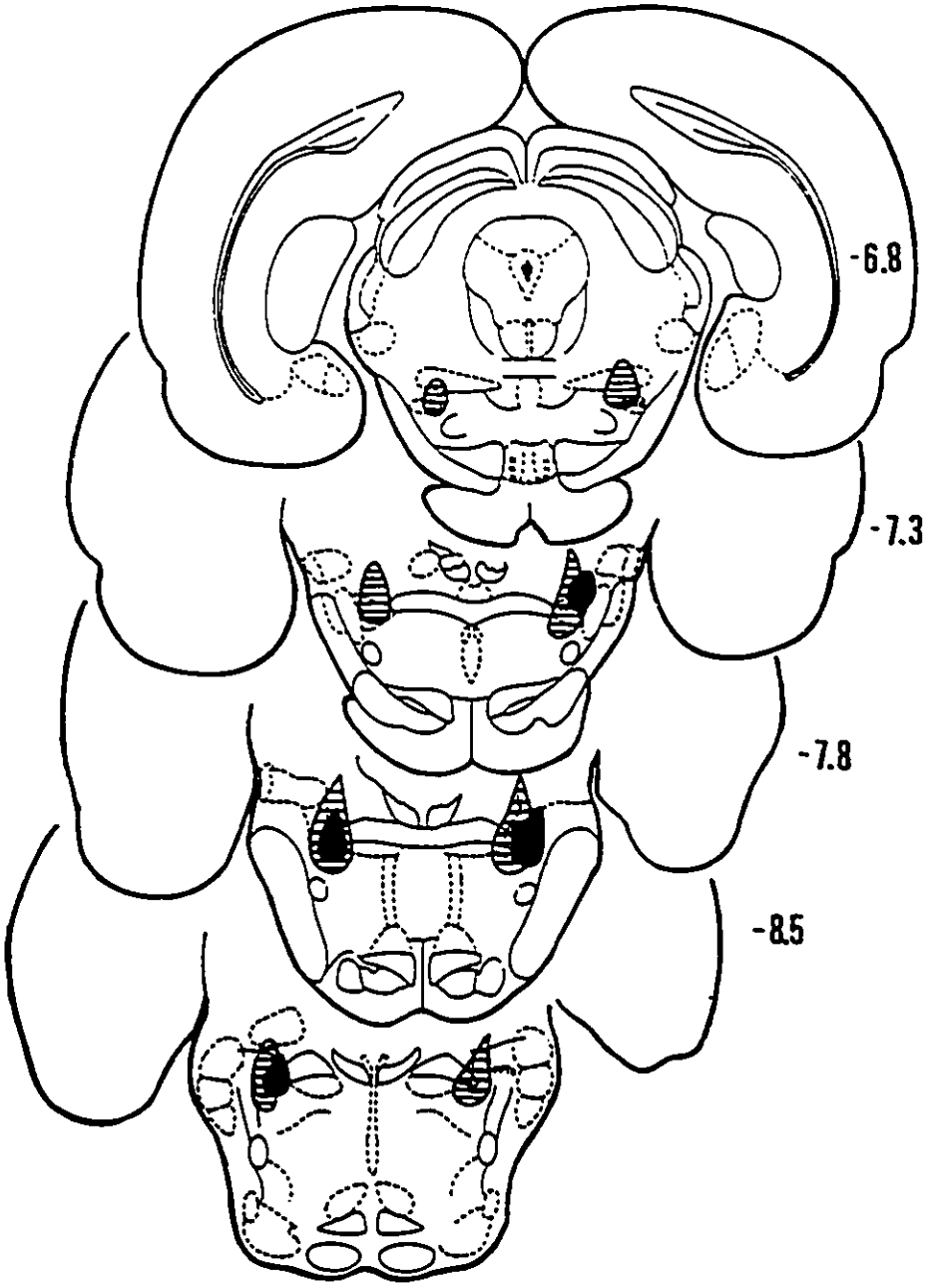
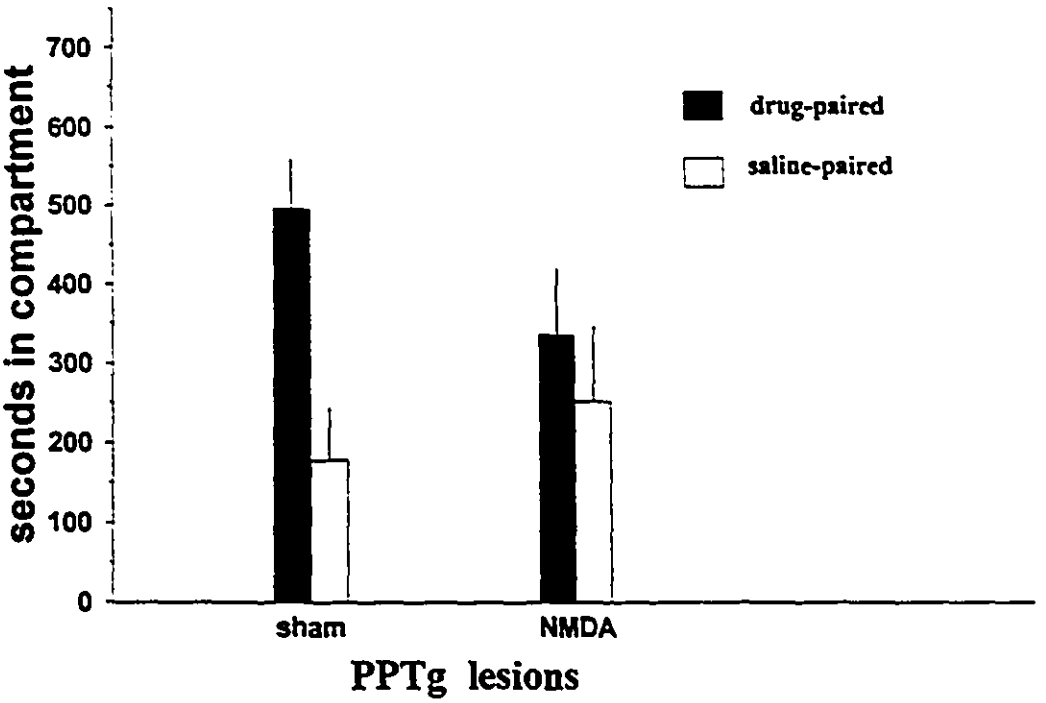

-6.8
-7.3
-7.8
-8.5

Figure 22

Effects of NMDA-induced lesions of the PPTg on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.


seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NMDA
PPTg lesions

over the 20 minute test for PPTg sham and lesioned animals [$F(1,28) = 0.95$; $p > 0.05$].

When re-tested in a drugged state, PPTg lesioned animals did not display a CPP [$F(1,7) = 2.6$; $p > 0.05$]. Data from the drug free and state dependent tests is presented in Figure 23 as time spent in, and entries to, drug- and saline-paired compartments at two minute intervals across each test. It appears that drug administration induced a CPP in PPTg lesioned animals because the time they spend in the drug-paired compartment is larger in the state dependent than in the drug free test. Nonetheless, the size of the CPP was not significantly different in the two tests [$F(1,7) = 2.8$; $p > 0.05$]. The apparent preference that PPTg lesioned animals exhibit for the drug-paired compartment during state dependent testing (see Figure 23) is probably not statistically significant because the group variance is large (SEM = 176.9).

Discussion

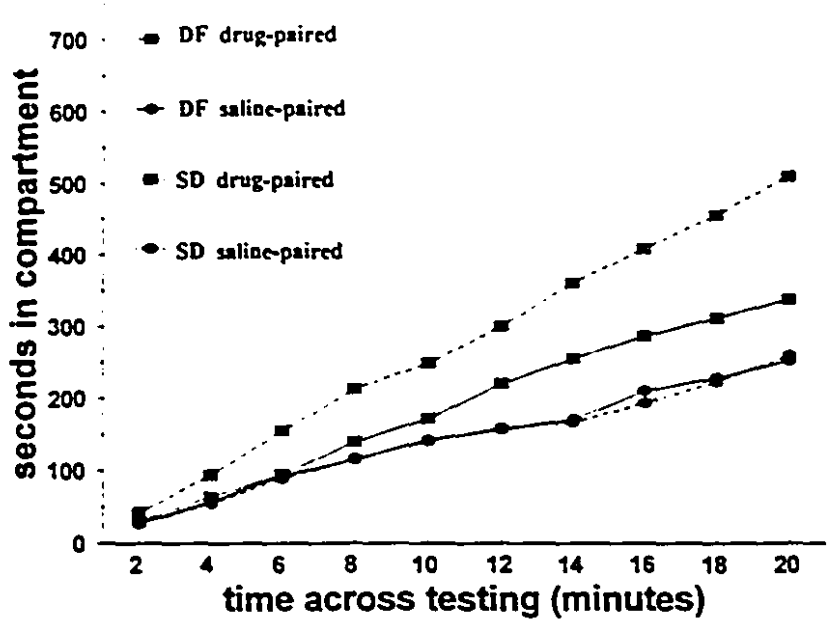
In the present set of experiments lesions of the mesolimbic DA system or the VP had no affect on the development of a CPP to morphine. Lesions that destroyed the entire VS potentiated a morphine-induced CPP, whereas lesions that were confined to rostral or caudal ports of the nucleus were ineffective. Finally, PPTg lesions completely eliminated the preference for a morphine-paired compartment in both drug free and state dependent tests.

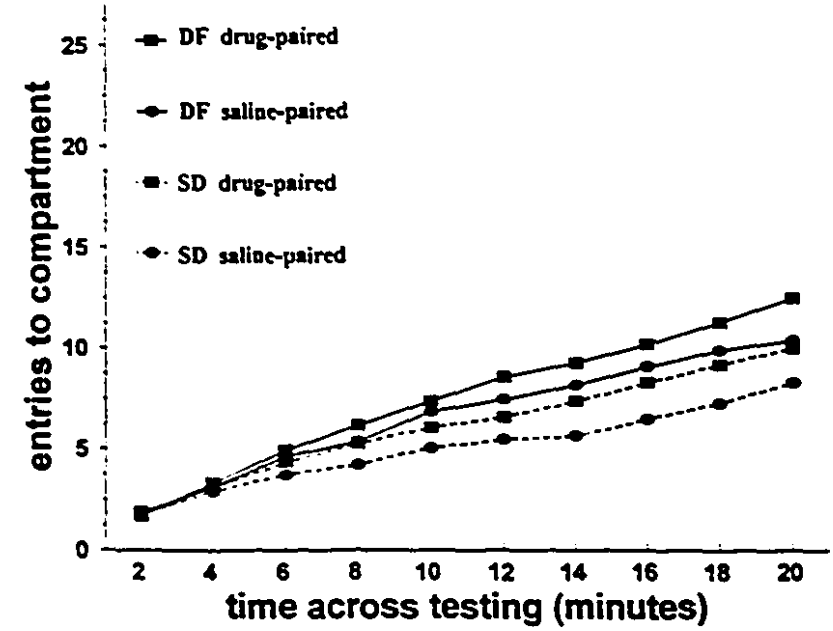
6-OHDA Lesions and Morphine-induced CPP

The finding that 6-OHDA lesions of the NAS do not affect a CPP to morphine appear to contradict a previous report that similar lesions block a

Figure 23

Time course of the CPP exhibited by animals with PPTg lesions during drug free (DF) (solid lines) and state dependent (SD) (dotted lines) tests. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals in each test. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals in each test. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
seconds in compartment
0
100
200
300
400
500
600
700
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
entries to compartment
0
5
10
15
20
25
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

heroin-induced CPP (Spyraki, Fibiger, & Phillips, 1983). Although heroin does not act on endogenous opioid receptors (Inturris et al., 1983; Gianutsos et al., 1986), it crosses the blood brain barrier more quickly and has fewer aversive side effects than morphine. Its increased potency may be related to an action of 6-acetylmorphine, which is the first derivative of heroin (Hubner & Kornetsky, 1992). Because the use of morphine and heroin does not generally produce different effects in CPP studies, it is unlikely that contradictory results are related to the use of morphine in the present study and heroin in the Spyraki et al. (1983) study. Rather, procedural differences between the two studies probably account for the conflicting findings. Spyraki et al. (1983) used a biased CPP procedure in which reward is assessed as an increase in time spent in the drug-paired compartment between pre- and post-conditioning test sessions. Reward in the present study reflects a preference for the drug-paired over the saline-paired compartment on the post-conditioning test day. Given that non-specific factors including stress and anxiety may confound interpretations of data using a biased CPP procedure (Carr, Fibiger, & Phillips, 1989; van der Kooy, 1987), the present results may more accurately assess the role of mesolimbic DA in opiate-induced reward.

It is also possible that 6-OHDA lesions in the present study did not reduce a CPP to morphine because the resulting DA depletion (only 60%) was not sufficient to induce a motivational deficit. When depletion is less than 90%, DA release remains normal and behavioural deficits are seldom apparent (Robinson & Whishaw, 1988; Zhang et al., 1988). It is therefore surprising that lesions which produced less than 50% DA depletion in the NAS were reported to block a morphine-induced CPP (Shippenberg, Bals-Kubik, & Herz, 1993). Discrepancies between results of the present study and those of Shippenberg et al. (1993) are

difficult to reconcile because procedures were similar in both studies. It should be noted, however, that present results agree with findings that 6-OHDA lesions of the VS do not affect self-administration of opiates (Dworkin, Guerin, Co, Goeders, & Smith, 1988; Pettit, Ettenberg, Bloom, & Koob, 1984).

The question of what role mesolimbic DA has in the rewarding effect of opiates is further complicated by the fact that studies examining the effect of DA antagonists on opiate-induced reward have produced conflicting results. In some reports blockade of DA receptors reduced the rewarding effect of opiates (Bozarth & Wise, 1981a; Leone & Di Chiara, 1987; Schwartz & Marchok, 1974; Shippenberg & Herz, 1987, 1988), and in others it did not (Bechara, Harrington, Nader, & van der Kooy, 1992; Ettenberg, Pettit, Bloom, & Koob, 1982; Gerrits, Ramsey, Wolterink, & Van Ree, 1994; Mackey & van der Kooy, 1985; Nader, Bechara, Roberts, & van der Kooy, 1994; Van Ree & Ramsey, 1987).

One explanation of these apparent contradictions is that DA mediates the negative, but not the positive, reinforcing effect of opiates (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992b). First, opiates may serve as positive reinforcers because they are rewarding; that is, they increase responding in the self-administration paradigm or produce a CPP because they induce a state of positive affect. Second, opiates may serve as negative reinforcers because they alleviate a state of aversion. Following repeated administration of opiates, animals become drug dependent and experience withdrawal effects when the drug is eliminated from the body. Opiate administration relieves this state. Animals therefore may self-administer opiates or develop a preference to an opiate-paired environment because the drug administration relieves the aversive effects of withdrawal.

Using a modified version of the CPP paradigm, Bechara and colleagues have demonstrated that disruption of DA transmission reduces a CPP to opiates when animals are drug dependent but not when they are drug naive (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992a, 1992b; Nader, Bechara, Roberts, & van der Kooy, 1994). These findings suggest that DA mediates the negative, but not the positive, reinforcing effect of opiates. In support of this suggestion, 6-OHDA lesions reduce the conditioned place aversion induced by *k* opioid agonists (Shippenberg, Bals-Kubik, & Herz, 1993) and increase responding for heroin when animals are opiate dependent (Smith, Guerin, Co, Barr, & Lane, 1985). The question of whether mesolimbic DA mediates positive or negative reinforcement was not directly addressed in the present study. Nonetheless, findings support Bechara's hypothesis because 6-OHDA lesions did not block a morphine-induced CPP in these animals that were not opiate dependent.

It is also possible that manipulations of DA systems produce inconsistent findings in different studies because DA has a dual function in motivational processes (Di Chiara, Acquas, & Carboni, 1989). According to Di Chiara et al. (1989), the first role of DA activity is to determine the affective valence of a stimulus; they propose that reward is associated with stimulation, and aversion with inhibition, of DA transmission. Di Chiara and colleagues further suggest that the second function of DA is to mediate conditioned incentive learning or the transfer of affective properties to stimuli associated with primary reward.

To examine the possibility that DA contributes to affective valence and incentive learning, Acquas and Di Chiara (1994) tested that effect of increasing doses of a DA antagonist on the development of a CPP to a variety of drugs.

Based on the finding that a CPP induced by amphetamine is seven to eight times more sensitive to DA blockade than is a CPP induced by morphine, they conclude that the rewarding effect of opiates are independent of DA mechanisms, although the conditioned incentive learning to opiates is not (Acquas & Di Chiara, 1994). These authors argue that DA lesions may reduce an opiate-induced CPP because they disrupt the process whereby neutral stimuli acquire the ability to elicit conditioned rewarding effects. The CPP is particularly resistant to antagonism of DA systems because the rewarding effect of opiates is mediated through a non-dopaminergic system. If this is true, it may explain why lesions in the present study which significantly reduced DA levels in the NAS, had no effect on a morphine-induced CPP.

As outlined previously, opiate administration activates the mesolimbic DA system and induces DA release in the VS. Although this may be a sufficient condition to produce reward, the present results (along with evidence cited in this section) suggest that it is not a necessary one.

VS Lesions and Morphine-induced CPP

Four separate groups of animals were tested for the development of a CPP to morphine following different lesions of the VS. In the first two groups, excitotoxin-induced lesions of the caudal two thirds of the VS had no effect on a morphine-induced CPP. These results do not prove that the VS is not involved in opiate-induced reward because both NMDA- and kainic acid-induced lesions spared anterior portions of the nucleus. It is possible that this region mediates morphine's rewarding effect and that sufficient VS cells remained intact to produce a CPP. To test this hypothesis, the third and fourth groups of animals received

neurotoxin infusions that lesioned either neuronal elements throughout the entire VS, or only those in its anterior portions. Animals with extensive VS lesions exhibited a preference for the drug-paired compartment, but compared to their sham lesioned controls, the size of the effect was reduced. Selective lesions of the anterior VS were ineffective.

The fact that a reduction in a morphine-induced CPP is only evident following destruction of the entire VS could indicate that mesolimbic DA lesions were ineffective in the present study because they were incomplete. On the other hand, lesions that destroy either cell bodies or DA fibers in the VS may have different effects on a CPP to morphine because they disrupt different functions. According to Cools et al. (1991), the functional output of the VS is determined by its neurochemical state, specifically by the interaction between DA and NA systems in this region. If lesions of cell bodies and DA fibers in the VS differentially alter DA-NA interactions, they may produce different behavioural outputs. This suggestion is supported by evidence that the two classes of lesions have different effects on a number of behavioural tasks. For example, kainic acid-induced lesions of the VS disrupt opiate self-administration (Dworkin, Guerin, Goeders, & Smith, 1988; Zito, Vickers, & Roberts, 1985), whereas 6-OHDA lesions do not (Dworkin, Guerin, Co, Goeders, & Smith, 1988; Pettit, Ettenberg, Bloom, & Koob, 1984). Exploratory behaviour and spontaneous locomotion are enhanced by NMDA-induced lesions of the VS, but not by 6-OHDA lesions of the same region (Weissenborn & Winn, 1992). Finally, lesions of VS cell bodies impair spatial learning in the Morris water maze (Annett, McGregor, & Robbins, 1989) but 6-OHDA lesions do not (Hagan, Alpert, Morris, & Iversen, 1983).

There may be another reason that kainic acid, but not 6-OHDA, lesions of the VS block a morphine-induced CPP: VS afferents that contribute to opiate-induced reward may be non-dopaminergic. Serotoninergic (5-HT) systems appear to be involved in the rewarding effect of opiates because 5-HT antagonists block a CPP to morphine (Carboni, Acquas, Leone, & Di Chiara, 1989; Nomikos & Spyraki, 1988). Furthermore, lesions of the 5-HT system in the NAS disrupt the development of a CPP to morphine (Nomikos, Spyraki, Galanopoulou, & Papadopoulou, 1986) and responding for intravenous opiates (Smith, Shultz, Co, Goeders, & Dworkin, 1987).

The findings in this experiment suggest that VS lesions reduce, but do not eliminate, a CPP to morphine appear to contradict a previous report (Kelsey, Carlezon, & Falls, 1989). It is difficult to evaluate whether VS lesions in the Kelsey et al. (1989) study attenuated or completely blocked preferences for the morphine-paired compartment because the experiment used a biased CPP procedure. Interpretations of the results are also problematic because electrolytic lesions employed by Kelsey et al. (1989) would have destroyed fibres passing though the VS. In contrast, excitotoxin-induced lesions used in the present study destroy VS cells while leaving fibers of passage intact (Stewart, Price, Olney, Hartman, & Cozzari, 1986; Zito, Vickers, & Roberts, 1985). It is likely, therefore, that the present study more accurately assesses the role of VS cell bodies in a morphine-induced CPP. Moreover, the present results indicating that the VS is not critically involved in opiate-induced reward are supported by evidence that VS lesions reduce, but do not eliminate, self-administration of morphine (Dworkin, Guerin, Goeders, & Smith, 1988; Glick & Cox, 1978).

The behavioural results from the present study must be considered in light of anatomical characteristics of the VS. In particular, the NAS is now viewed as at least three distinct territories, the core, shell, and the rostral pole. The core, located in the caudal three-fourths of the NAS, ensheathes the anterior limb of the anterior commissure and was originally separated from the surrounding shell on the basis of differential distribution of CCK immunoreactivity (Zaborszky et al., 1985). Subsequently, the core and shell were also distinguished by the presence or absence of neuroactive substances, receptors, cytoarchitecture, synaptic organization, DA metabolism, electrophysiological properties, and different vulnerability to neurotoxic or environmental challenges (Deutch & Cameron, 1992; Pennartz, Dolleman-Van der Weel, & Lopes da Silva, 1992; Zahm & Brog, 1992; Zahm & Heimer, 1993).

The NAS may also be partitioned on the basis of anatomical connections: afferents from different neural sites preferentially (but apparently not selectively) innervate discrete regions of the nucleus (Brog, Salyapongse, Deutch, & Zahm, 1993; Phillipson & Griffiths, 1985; Zahm & Heimer, 1993). This topographical relationship is maintained in efferent targets (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991; Kelley, Domesick, & Nauta, 1982; Kelley & Domesick, 1982; Cornwall & Coen, 1991; Zahm & Heimer, 1993). Characteristics of the two regions merge in the rostral one-fourth of the NAS and cannot be distinguished; this third subregion is referred to as the rostral pole (Zahm & Brog, 1992).

Results from the present study appear to indicate that extent, not location, of neuronal damage within the VS determines whether lesions will disrupt a CPP to morphine. VS lesions were effective in reducing preferences for the morphine-paired compartment when they encompassed the entire nucleus, but not when

they were confined to either its anterior or posterior regions. Nonetheless, this interpretation should be accepted with caution because none of the subjects had neuronal destruction confined to either the core, shell, or rostral pole regions of the NAS. Because the shell is more closely associated with the limbic system and the core is associated with the motor system (Zahm & Brog, 1992), VS lesions that destroy the shell may have more pronounced effects on reward related behaviour than lesions confined to the core.

Results from the present study indicating that large VS lesions reduce a morphine-induced CPP do not specify how or why the effect is produced. This question may be addressed by examining hypotheses of VS function and the role that it may play in the development of a CPP to morphine. The VS receives input from numerous limbic and corticolimbic structures including the hippocampus, the amygdala, and the prelimbic area of the MFC (Groenewegen, Vermeulen-Van der Zee, Te Kortschot, & Witter, 1987; Heimer & Wilson, 1975; Kelley & Domesick, 1982; Kelley, Domesick, & Nauta, 1982; Kelley & Stinus, 1984; Krettek & Price, 1978; McGeorge & Faull, 1989). Particularly striking is the overlap within the VS of afferents from the amygdala, hippocampus, and dopaminergic neurons of the VTA (De France, Marchand, Stanley, Sikes, & Chronister, 1980; Groenewegen, Becker, & Lohman, 1980; Heimer & Wilson, 1975; Kelley & Stinus, 1984). These anatomical connections suggest that the VS may integrate information about the emotional significance of stimuli and events. The proposal is supported by evidence that VS neurons respond to stimuli which predict the presentation of reward (Apicella, Ljungberg, Scarnati, & Schultz, 1991; Schultz, Apicella, & Ljungberg, 1993; Schultz, Apicella, Scarnati, & Ljungberg, 1992). Given that the expression of a CPP depends on conditioned reward, VS lesions may affect a

morphine-induced CPP by attenuating the rewarding effect of the drug and/or drug-paired stimuli. If this is true, morphine's rewarding effect must also be mediated through neural sites that are independent of the VS because animals with large VS lesions exhibit a preference, albeit reduced, for the morphine-paired compartment.

It is also possible that the VS does not mediate the rewarding effect of morphine, but that its destruction interferes with processes that contribute to the expression of a CPP. That is, if lesions produce memory deficits or disrupt the ability to discriminate salient stimuli, the size of the CPP may be reduced even if the drug administration is rewarding. These possibilities are difficult, if not impossible, to distinguish in the CPP paradigm. They must be addressed by examining converging evidence from a variety of behavioural paradigms.

VP Lesions and Morphine-induced CPP

The present findings that VP lesions do not block a morphine-induced CPP were surprising because VP lesions disrupt self-administration of heroin and cocaine (Hubner & Koob, 1990; Robledo & Koob, 1993). It should be noted, however, that the VP is exceptionally difficult to lesion. Originally defined as the ventral extension of the globus pallidus (Heimer & Wilson, 1975), the VP lies ventral to the temporal limb of the anterior commissure and stretches from the region of the olfactory tubercle to the extended amygdala (Alheid & Heimer, 1988). The region includes the rostral subcommissural portion of the substantia innominata (Heimer, Switzer, & Van Hoesen, 1982) as well as a portion of acetylcholine (ACh) neurons which constitute the nucleus basalis of Meynert (Rye, Wainer, Mesulam, Mufson, & Saper, 1984; Saper, 1984).

Given the heterogeneity of the VP, different functions may be associated with discrete areas within the nucleus. It appears that distinct regions of the VP have different roles in reward-related processes because a CPP to food is differentially affected by lesions of the anterior and posterior VP (McAlonan, Robbins, & Everitt, 1993). Differences in lesion site, therefore, may account for the inconsistent effects of VP lesions on opiate-induced reward. VP lesions that did not affect a morphine-induced CPP in the present study were confined to caudal portions of the nucleus, whereas VP lesions which reduced heroin self-administration (Hubner & Koob, 1990) destroyed more dorsal and rostral regions of the nucleus.

The role of the VP in the development of a CPP to morphine, and in processes of reward in general, merits further examination. VP efferents from the VS are part of a series of parallel fiber connections which lead from the cerebral cortex through the basal ganglia to the thalamus and back to the cortex (Alexander, DeLong, & Strick, 1986; Alexander & Crutcher, 1990). VP functions, therefore, may best be investigated by examining the role of segregated pathways which incorporate specific elements of thalamo-cortical circuitry. Such experiments are difficult to conduct, however, because neurotoxic infusions do not respect anatomical boundaries. Electrolytic lesions which are more discrete in size and location, destroy fibers of passage, thereby influencing processes that are under the control of regions distant to the site of damage.

PPTg Lesions and Morphine-Induced CPP

Consistent with previous reports (Bechara & van der Kooy, 1992b, 1989; Olmstead & Franklin, 1993, 1994a), PPTg lesions in the present study completely

eliminated a CPP to morphine; PPTg lesioned animals did not exhibit a preference for the drug-paired compartment during any portion of the drug free or state dependent test. PPTg lesions also block the development of a CPP to amphetamine (Bechara & van der Kooy, 1989; Olmstead & Franklin, 1994a), food (Bechara & van der Kooy, 1992a), or heroin (Nader, Bechara, Roberts, & van der Kooy, 1994), and disrupt the acquisition of responding for intravenous heroin (Olmstead, Munn, & Wise, 1993). These findings suggest that the PPTg is a critical component in systems mediating the rewarding effect of appetitive stimuli.

It has also been proposed that the PPTg mediates reward when animals are in a non-deprived state (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992a). This hypothesis is based on evidence that PPTg lesions block a CPP to opiates if animals are drug naive and to food if animals are sated, but have no effect on the behaviour when animals are drug dependent or food deprived (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992a, 1992b; Nader, Bechara, Roberts, & van der Kooy, 1994). Although the present study was not designed to test this proposal, the results are consistent with the hypothesis presented by Bechara and colleagues because PPTg lesioned animals were drug naive prior to CPP conditioning.

Anatomical evidence supports the suggestion that the PPTg is involved in reward-related processes. Projections from limbic forebrain sites associated with reward terminate in the PPTg (Moon Edley & Graybiel, 1983; Nauta, Smith, Faull, & Domesick, 1978; Saper & Loewy, 1982; Swanson, Mogenson, Gerfen, & Robinson, 1984; Swanson, Mogenson, Simerly, & Wu, 1987), and activation of PPTg neurons monosynaptically excites midbrain DA neurons (Bolam, Francis, &

Henderson, 1991; Lacey, Calabresi, & North, 1990) and increases DA release in the NAS (Klitenick & Kalivas, 1994). Despite its connections with neural systems of reward, it is not known whether PPTg activation induces rewarding effects, modulates rewarding signals, or is necessary for the transmission of rewarding effects which are produced elsewhere.

It is also possible that PPTg lesions attenuate a morphine-induced CPP because they affect processes other than reward that are necessary for the development of a CPP. The PPTg has been implicated in both motor and cognitive processes; disruption of either could interfere with the acquisition of a CPP without necessarily reducing the rewarding properties of the stimulus.

An association between the PPTg and structures of the motor system is well established: the PPTg has strong reciprocal connections with the substantia nigra, globus pallidus, and subthalamic nucleus (Beninato & Spencer, 1987; Clarke, Hommer, Pert, & Skirboll, 1987; Goldsmith & van der Kooy, 1988; Hammond, Rouzaire-Dubois, Feger, Jackson, & Crossman, 1983; Jackson & Crossman, 1983; Lee, Rye, Hallanger, Levey, & Wainer, 1988; Moon Edley & Graybiel, 1983; Scarnati, Proia, Campana, & Pacitti, 1986), and PPTg efferents innervate the pontomedullary reticular nuclei (Jackson & Crossman, 1983; Rye, Lee, Saper, & Wainer, 1988), ventral medulla and spinal cord (Goldsmith & van der Kooy, 1988; Rye, Lee, Saper, & Wainer, 1988; Spann & Grofova, 1989).

Behavioural studies also suggest that the PPTg has a role in motor functioning. As part of the mesencephalic locomotor region (MLR) (Garcia-Rill, 1991; Garcia-Rill, Houser, Skinner, Smith, & Woodward, 1987), the PPTg is associated with the initiation of forward locomotion. PPTg efferents project to a region of the medioventral medulla in which stimulation evokes locomotion in

decerebrates (Kinjo et al., 1990). Connections from the VS and VP to the PPTg mediate locomotor stimulation (Bechara & van der Kooy, 1992c; Brudzynski & Mogenson, 1985; Mogenson & Wu, 1986) suggesting that the PPTg may serve as a pathway mediating basal ganglia access to descending motor systems of the brain stem (Kelland & Asdourian, 1989; Scarnati, DiLoreto, & Florio, 1989). The PPTg is involved in the regulation of muscle tone (Kelland & Asdourian, 1989; Lai & Seigel, 1990), postural functions during locomotion (Graybiel & Ragsdale, 1978), and morphine's cataleptogenic effects (Olmstead & Franklin, 1994b). Finally, the PPTg may modulate rhythmic activities (Garcia-Rill, 1991) and the transmission of sensory information from forebrain structures to motor output systems (Swerdlow & Geyer, 1993).

Given the close relationship between the PPTg and the motor system, it is not surprising that PPTg lesions produce abnormalities in motor functioning (Olmstead & Franklin, 1994b; Dunbar et al., 1992). These deficits, however, are subtle, and in most cases they are not observed under superficial examination. Furthermore, PPTg lesions do not alter spontaneous or drug-induced locomotor activity (Dellu, Mayo, Cherkaoui, LeMoal, & Simon, 1991; Fujimoto, Yoshida, Ikeguchi, & Niijima, 1989; Inglis et al., 1994; Olmstead & Franklin, 1994a). It is unlikely, therefore, that disruptions in motor functioning following PPTg lesions account for behavioural deficits in tests that do not depend on fine motor control such as the CPP. The finding in the present study that the total number of entries made by PPTg sham and lesioned animals did not differ further indicates that the effect of PPTg lesions on a morphine-induced CPP is not related to disruptions in locomotor abilities.

An alternative explanation is that PPTg lesions produce deficits in reward paradigms because the region is involved in cognitive processes (Steckler, Inglis, Winn, & Sahgal, 1994). The PPTg may influence cortical systems associated with learning and memory through its projections to the thalamus (Hallanger, Price, Steininger, & Wainer, 1990; Semba, Reiner, & Fibiger, 1990; Steriade & Llinas, 1988) and cholinergic neurons of the basal forebrain (Hallanger & Wainer, 1988; Jones & Cuello, 1989; Semba, Reincer, McGeer, & Fibiger, 1988; Woolf & Butcher, 1986). Furthermore, because it is part of the reticular activating and thalamo-cortical systems (Steriade, Datta, Pare, Oakson, & Curro, 1990; Fitzpatrick, Diamond, & Raczkowski, 1989), the PPTg may contribute to attentional processes. In support of this suggestion, forebrain ACh systems associated with attention (Dunnett, Everitt, & Robbins, 1991) may be regulated by PPTg activity (Bertorelli, Forloni, & Consolo, 1991).

The PPTg appears to be involved in cognitive functions because PPTg lesions produce attentional deficits in operant tasks which are not related to either motor or motivational deficits (Steckler, Inglis, Winn, & Sahgal, 1994). PPTg lesions also impair the acquisition of active and passive avoidance (Fujimoto, Yoshida, Ikeguchi, & Niijima, 1989; Fujimoto, Ikeguchi, & Yoshida, 1992; Kessler, Markowitsch, & Sigg, 1986), and produce deficits in radial arm and water maze learning (Dellu, Mayo, Cherkauoui, LeMoal, & Simon, 1991; Lepore, 1993). Finally, PPTg lesions produce delay dependent deficits in a spatial memory task (Kessler, Markowitsch, & Sigg, 1986).

Despite the evidence that PPTg lesions produce learning deficits, it is unlikely that this accounts for the elimination of a CPP following PPTg lesions because animals with PPTg lesions develop a CPP to methylnaltrexone (Bechara

& van der Kooy, 1989), to morphine if they are drug dependent, or to food if they are food deprived (Bechara & van der Kooy, 1992a; Bechara, Harrington, Nader, & van der Kooy, 1992). Furthermore, PPTg lesions do not disrupt the ability of morphine to act as a discriminative cue (Martin, Bechara, & van der Kooy, 1991).

The evidence outlined above appears to indicate that the PPTg is involved in motivational, motor, and cognitive processes. It also suggests that manipulations of the nucleus may affect one function, but not the others. If this is true, the three functions may be mediated through discrete regions within the PPTg. This suggestion is feasible because the PPTg is a heterogenous structure containing subpopulations of cells with distinct ascending and descending projections (Garcia-Rill, 1991; Spann & Grofova, 1989). An ACh cell group is concentrated in the pars compacta of the PPTg and probably includes the ACh cells of the latero-dorsal tegmental nucleus (Semba & Fibiger, 1992; Sugimoto & Hattori, 1984; Woolf, 1991). At the caudal end of the PPTg, the ACh group is located dorsolaterally to the superior cerebellar peduncle. A second non-ACh, possibly glutamatergic (Clements & Grant, 1990; Scarnati, Proia, Campana, & Pacitti, 1986), component is situated medioventral to the superior cerebellar peduncle at the caudal end of the nucleus (Goldsmith & van der Kooy, 1988; Rye, Saper, Lee, & Wainer, 1987; Semba & Fibiger, 1992). ACh and non-ACh portions of the PPTg also have distinct ascending and descending projections (Goldsmith & van der Kooy, 1988; Scarnati, Proia, Campana, & Pacitti, 1986). Some authors have argued that these differences constitute a distinction between the ACh PPTg and the non-ACh midbrain extrapyramidal region (Lee, Rye, Hallanger, Levey, & Wainer, 1988; Rye, Lee, Saper, & Wainer, 1988; Steininger, Rye, & Wainer, 1992); however, both groups of cells are interdigitated throughout the region (Mesulam,

Geula, Bothwell, & Hersh, 1989), suggesting that the nucleus should be referred to as a single entity containing both types of neurons (Jackson & Crossman, 1983; Kang & Kitai, 1990).

Preliminary evidence indicates that different PPTg functions may be associated with subpopulations of cells within the nucleus (Spann & Grofova, 1989). For example, the MLR probably corresponds to the pars compacta region of the PPTg since effective sites are found within clusters of ACh cells (Garcia-Rill, Houser, Skinner, Smith, & Woodward, 1987) and the lowest threshold sites are dorsolateral to the superior cerebellar peduncle in the region of the cuneiform nucleus (Coles, Iles, & Nicolopoulous-Stournaras, 1989). In contrast, reward appears to be mediated in a region that is ventral to the superior cerebellar peduncle and medial to the dense cluster of ACh cells (Bechara & van der Kooy, 1989; Olmstead & Franklin, 1993). Motor functioning may also be mediated through a subpopulation of PPTg neurons because disruption of simple motor tasks is attributed to lesions of non-ACh cells in the region (Dunbar et al., 1992).

Even if distinct regions within the PPTg mediate different processes, it is likely that information from different sources is integrated within the nucleus. PPTg function, therefore, may be related to motor, motivational, and cognitive processes. For example, the PPTg could mediate the ability to attend to motivational cues and to produce appropriate motor responses to these cues. In general, investigations of PPTg function assess its role in a single phenomenon, but interpretations of behavioural results should consider the possibility that more than one function may be affected by experimental manipulations of the PPTg.

The present experiment examined the role of the four structures which constitute the neural circuit of drug reward (Koob & Bloom, 1988) in the

development of a CPP to morphine. Although the results are not definitive they suggest that the PPTg is the only site within this circuit that is critically involved in the effect. It is possible that the rewarding effect of morphine involves the mesolimbic DA system, the VS, or the VP, but these structures are not necessary components in systems mediating a morphine-induced CPP. This implies that morphine's rewarding effect may be mediated through more than one system, although it appears that these systems converge at the level of the PPTg.

EXPERIMENT 4

Effects of Lesions of the PAG, the Hippocampus, the LA, or the CPu on the Development of a CPP to Morphine

Results from Experiment 3 imply that neural structures which are not part of the neural circuit of drug reward may be involved in the development of a CPP to morphine. Previous evidence from both anatomical and behavioural studies suggest that the PAG, hippocampus, LA, and CPu may mediate processes that contribute to the effect.

The PAG appears to be involved in morphine's rewarding effect because morphine injections into the region produce a CPP (Experiments 1 and 2) and (Motta & Brandao, 1993; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982). In addition, blockade of opioid receptors in the region attenuates a morphine-induced CPP (Experiment 2) and disrupts responding for intravenous heroin (Corrigall & Vaccarino, 1988). Given these results, the report that PAG lesions do not affect a morphine-induced CPP is surprising (Bechara & van der Kooy, 1989). To clarify the role of the PAG in morphine's rewarding effect, the present study re-examined the effect of PAG lesions on the development of a CPP to morphine.

The hippocampus is composed of a group of cortical fields organized along vertical and horizontal planes (Amaral & Witter, 1989; Andersen, Bliss, & Skrede, 1971). The entorhinal cortical region of the hippocampus receives extensive inputs from all cortical sensory association areas (Kosel, Van Hoesen, & West, 1981). From the entorhinal cortex, information is transmitted, via the perforant path, to other regions of the hippocampal formation (Kohler, 1985, 1988; Ruth, Collier, & Routtenberg, 1988; Steward, 1976). These anatomical connections suggest that

the hippocampus acquires information about the relationships among sensory stimuli (Hirsh, 1974; O'Keefe & Nadel, 1978; Sutherland & Rudy, 1989). Hippocampal lesions produce deficits in a variety of learning and memory tasks (Squire, 1992); results of these studies are interpreted as evidence that the hippocampus is a critical component in a memory system referred to as spatial (O'Keefe & Nadel, 1979), configural (Sutherland & Rudy, 1989), working (Olton, Becker, & Handelmann, 1979), or declarative (Squire, 1986). All of these theories posit that the hippocampus is necessary for complex associative learning, particular that which requires animals to remember relationships among two or more stimuli. In order to express a CPP, animals must remember the place and cues associated with drug administration. If the hippocampus mediates these processes, lesions of the region may block a morphine-induced CPP.

The basolateral region of the amygdala, which includes the LA, receives extensive inputs from neural sites associated with reward, most notably the VTA and the NAS (Beckstead, Domesick, & Nauta, 1979; Fallon & Moore, 1978; Kelley, Domesick, & Nauta, 1982; Ottersen, 1980; Phillipson, 1979; Woolf & Butcher, 1982). Afferents to the LA also originate in cortical areas associated with the processing of sensory information (Beckstead, 1979; Ottersen, 1982; Turner & Zimmer, 1984; Woolf & Butcher, 1982). Convergence of rewarding and sensory information within the amygdala suggests that the region mediates the formation of stimulus-reward associations (Jones & Mishkin, 1972; Gaffan, Gaffan, & Harrison, 1989). This hypothesis is supported by findings that lesions of the basolateral amygdala disrupt the acquisition of responding for a conditioned reinforcer (Burns, Robbins, & Everitt, 1993; Cador, Robbins, & Everitt, 1989; Everitt, Cador, & Robbins, 1989) and block the development of a CPP to food

(Everitt, Morris, O'Brien, & Robbins, 1991). Lesion-induced deficits have been attributed to destruction of LA neurons because lesions confined to the LA block a CPP to amphetamine (Hiroi & White, 1991) or to food (McDonald & White, 1993). If the ability to associate neutral stimuli with morphine's rewarding effect also depends on the LA, LA lesions should block a morphine-induced CPP.

The CPu receives topographically organized sensory inputs from the neocortex (McGeorge & Faull, 1989; Nauta, Pritz, & Lasek, 1974; Veening, Cornelissen, & Lieven, 1980) and related thalamic nuclei (Buchwald, Price, Vernon, & Hull, 1973; Jones & Leavitt, 1974). The CPu also has reciprocal connections with structures of the motor system (Gerfen, 1985; Gerfen, Herkenham, & Thibault, 1987), suggesting that it may be involved in sensorimotor integration (Robbins, Giardini, Jones, Reading, & Sahakian, 1990). Behavioural evidence supports this proposal: CPu lesions impair visual discrimination learning in the water maze task (McDonald & White, 1994; Mishkin & Petrie, 1984; Packard & McGaugh, 1992; Whishaw, Mittleman, Bunch, & Dunnett, 1987) and disrupt acquisition of motor responses to a cued contingency (Cook & Kesner, 1988; McDonald & White, 1993; Packard, Hirsh, & White, 1989; Schwartzbaum & Donovick, 1968). If the expression of a CPP is determined by approach responses to sensory stimuli, CPu lesions should disrupt a morphine-induced CPP.

The evidence cited in this section indicates that the PAG, the hippocampus, the LA, and the CPu may mediate processes that contribute to an opiate-induced CPP. Experiment 4 examined this possibility be testing whether lesions of each site disrupt the development of a CPP to morphine. To test whether lesion-induced deficits are altered by drug administration, lesioned animals were re-tested for a CPP in a drugged state.

Methods

Subjects

One hundred and twenty four subjects were used in the experiment.

Surgical Procedures

Bilateral lesions of the PAG, the CPu, the LA, and the hippocampus were made in different groups of subjects. In the forthcoming description, coordinates for each lesion are given as mm anterior (+) or posterior (-) to bregma, mm lateral to bregma, and mm ventral to the skull surface, according to the atlas of Paxinos and Watson (1986).

PAG Lesions

PAG lesions were induced by infusing 0.5 *u*l of 0.1 M NMDA over five minutes at coordinates -6.0, 0.5, 6.0: sham (n = 14) and lesion (n = 13).

Hippocampal Lesions

Hippocampal lesions were induced using a protocol that produces behavioural deficits in memory tasks (McDonald & White, 1993). Neurotoxic-induced lesions were produced by injecting 0.4 *u*l of a solution containing 2 *u*g colchicine and 0.1 *u*g kainic acid per 0.5 *u*l of vehicle at three sites: -3.3, 1.5, -3.7; -4.8, 3.2, 4.2; -5.8, 5.0, 7.5. All injections were made over eight minutes: sham (n = 8) and lesion (n = 8).

In a separate experiment, the fornix was lesioned by passing radio-frequency current (6 mA for 40 sec) through an electrode at two sites with 0.8 mm exposed tip: sham (n = 12) and lesion (n = 10). Coordinates were -1.5, 0.8, 4.5 and -1.5, 2.2, 4.5. Radio frequency lesions were employed in this study because

they produce more complete destruction of the fornix than lesions induced by electrolytic current (Hiroi & White, 1991).

LA Lesions

Two separate groups of animals received lesions of the LA. In the first, 0.3 *ul* of 0.25 M NMDA solution was injected over 6 minutes at coordinates -3.5, 5.5, 8.0: sham (n = 11) and lesion (n = 9). In the second group, electrolytic lesions were produced by passing anodal current (1.5 mA for 20 sec) through an electrode with 0.5 mm exposed tip: sham (n = 12) and lesion (n = 11). Coordinates were -3.5, 5.5, 8.5. Because neuronal damage induced by electrolytic current is more discrete in size and location, the LA can be more selectively destroyed using this method than by neurotoxic infusion. On the other hand, electrolytic lesions destroy fibers of passage whereas NMDA does not. The role of the LA in behavioural processes, therefore, is best examined using both techniques.

CPu Lesions

CPu lesions were induced by infusing 0.3 *ul* of 0.25 M NMDA over 6 minutes at two sites: +1.5, 3.2, 4.4 and -0.2, 4.0, 4.8, sham (n = 8) and lesion (n = 8).

Behavioural Procedures

Subjects underwent six alternating conditioning sessions, three with morphine (2 mg/kg) and three with saline, using the protocol described in the General Methods.

Results

PAG Lesions

Histology

Figure 24 illustrates the location and extent of neuronal damage induced by NMDA infusions into the PAG. PAG lesions were concentrated in the anterior and dorsal regions of the nucleus. In some animals, minimal damage was apparent in the overlying superior colliculus, but in most cases cell loss or gliosis was not present beyond the boundaries of the PAG. Subjects were discarded from the analyses if neuronal damage extended through the medial PAG to the border of the acqueduct and it appeared that the neurotoxin infusion had entered the aqueduct.

Behavioural Results

Figure 25 shows that PAG lesions blocked the development of a CPP to morphine. Shams developed a significant preference for the drug-paired compartment [$F(1,26) = 43.02$; $p < 0.01$] whereas lesioned animals did not [$F(1,26) = 0.05$; $p > 0.05$]. Furthermore, the size of the CPP was significantly reduced by PAG lesions [$F(1,52) = 32.8$; $p < 0.05$].

In Figure 26, the behavioural data from PAG lesioned animals is presented as time spent in, and entries to, drug- and saline-paired compartments at two minute intervals. PAG lesioned animals did not express a CPP during the first [$F(1,12) = 0.26$; $p > 0.05$] or second [$F(1,12) = 0.001$; $p > 0.05$] 10 minutes of testing. Lesioned animals did not enter the drug-paired compartment more often than the saline-paired compartment during the first [$F(1,12) = 0.013$; $p > 0.05$] or second [$F(1,13) = 0.92$; $p > 0.05$] half of the test.

Figure 24

Schematic representation of NMDA-induced lesions of the PAG. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

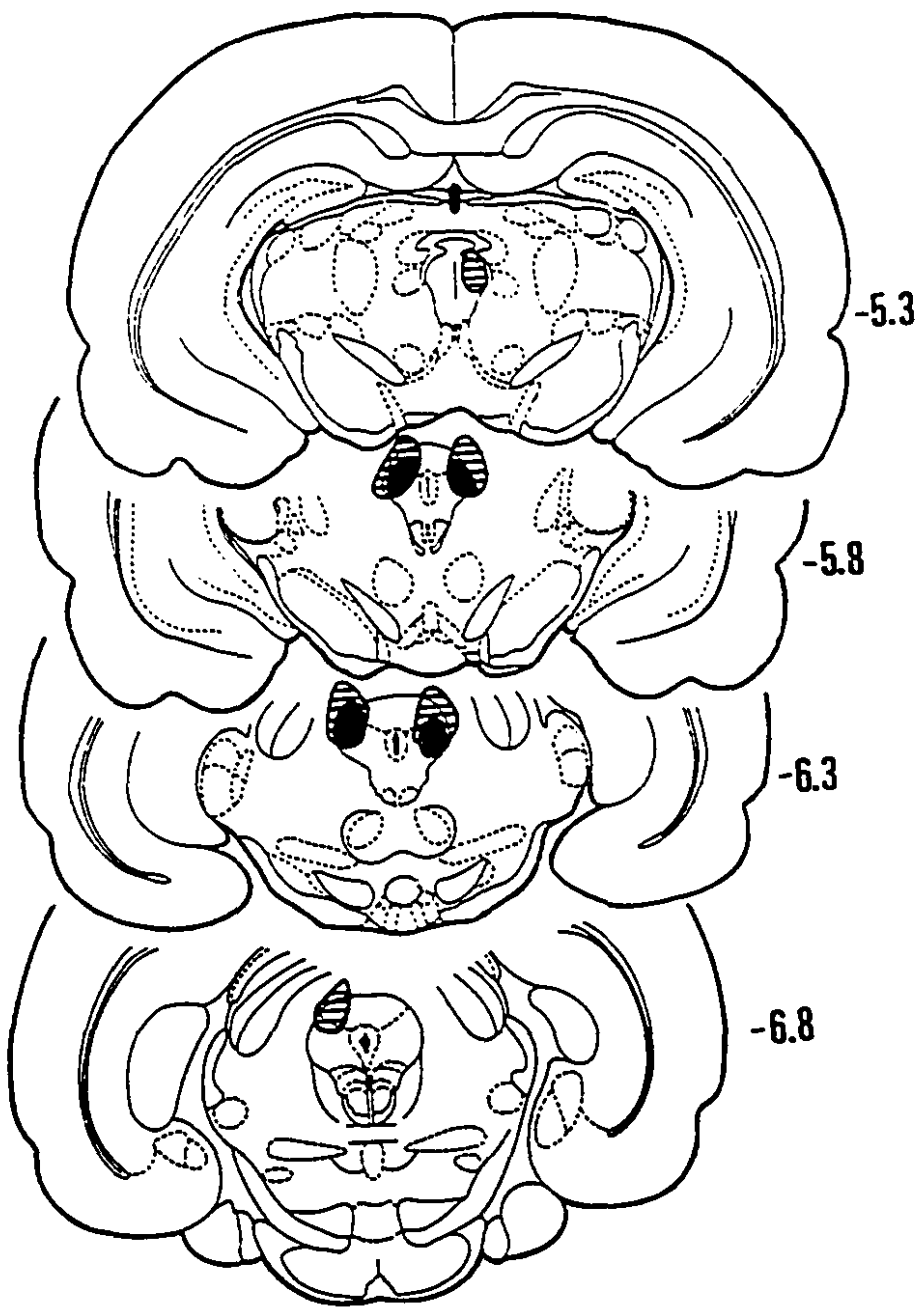

-5.3
-5.8
-6.3
-6.8

Figure 25

Effects of NMDA-induced lesions of the PAG on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.

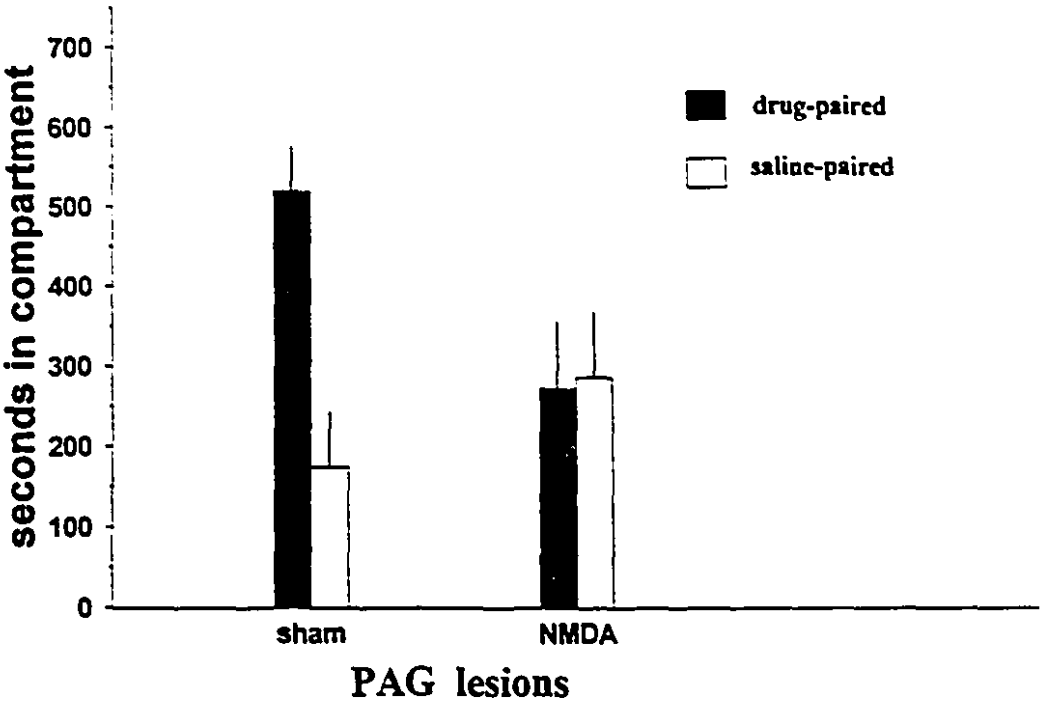
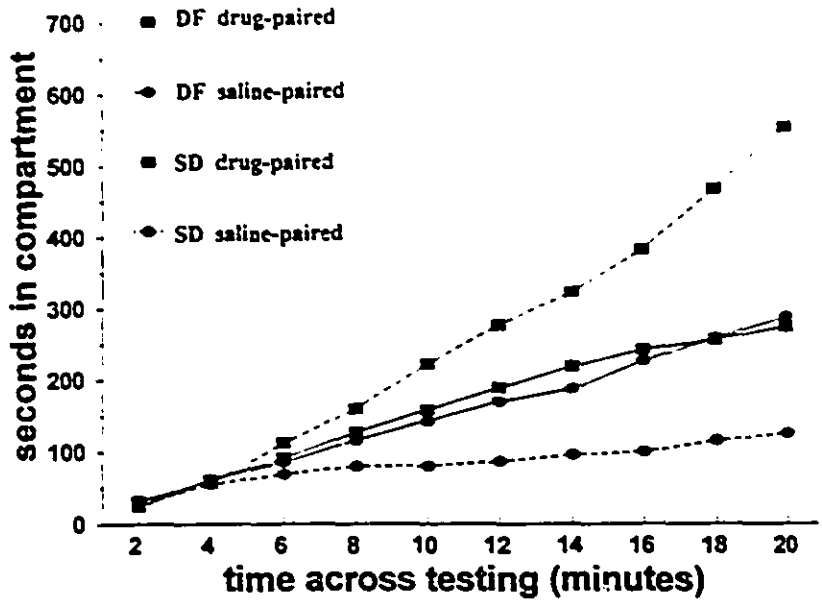
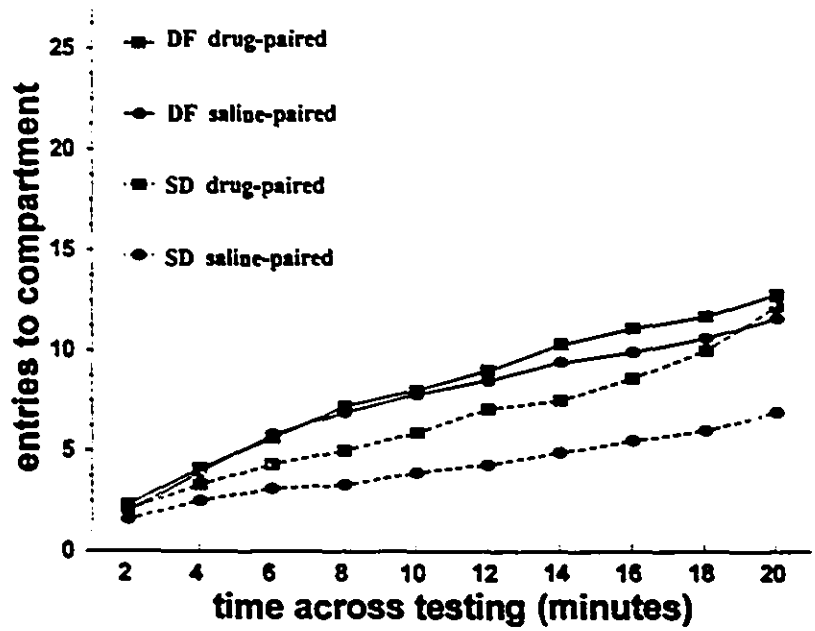

seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NMDA
PAG lesions

Figure 26

Time course of the CPP exhibited by animals with PAG lesions during drug free (DF) (solid lines) and state dependent (SD) (dotted lines) tests. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals in each test. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals in each test. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
seconds in compartment
700
600
500
400
300
200
100
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
entries to compartment
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

Seven of the thirteen PAG lesioned animals were tested for a CPP in a drugged state. It can be seen in Figure 26 that following morphine administration, these animals displayed a CPP [$F(1,6) = 14.8$; $p < 0.01$]. Compared to the drug free test, difference scores of lesioned animals were significantly larger during the state dependent test [$F(1,6) = 13.0$; $p < 0.05$]. During the first 10 minutes of the state dependent test, PAG lesioned animals did not express a CPP [$F(1,6) = 3.2$; $p > 0.05$], but they did during the second 10 minutes of testing [$F(1,6) = 13.2$; $p < 0.01$]. The size of the CPP was significantly larger in the second half of the state dependent test [$F(1,6) = 16.01$; $p < 0.01$]. PAG lesioned animals made more entries to the drug-paired than to the saline-paired compartment during both halves of the state dependent test (first 10 minutes [$F(1,6) = 13.1$; $p < 0.05$] and second 10 minutes [$F(1,6) = 8.5$; $p < 0.05$]).

Hippocampal Lesions

Histology

The location and extent of neurotoxic-induced lesions of the hippocampus are presented in Figure 27. Cell loss and gliosis was evident in CA1, CA3, and dentate gyrus regions of the hippocampal formation. In all cases, the dorsal hippocampus was completely destroyed. Neuronal damage in the ventral hippocampus varied from 25% to 75% of the region. In half of the animals, cell loss and gliosis were visible in the entorhinal cortex, the cingulum, and the subiculum. Six of the eight lesioned animals sustained unilateral damage in the overlying neocortex.

Figure 28 illustrates the location and extent of damage produced by radio frequency lesions of the fornix. In this group, neuronal destruction was more

Figure 27

Schematic representation of neurotoxin-induced lesions of the hippocampus. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

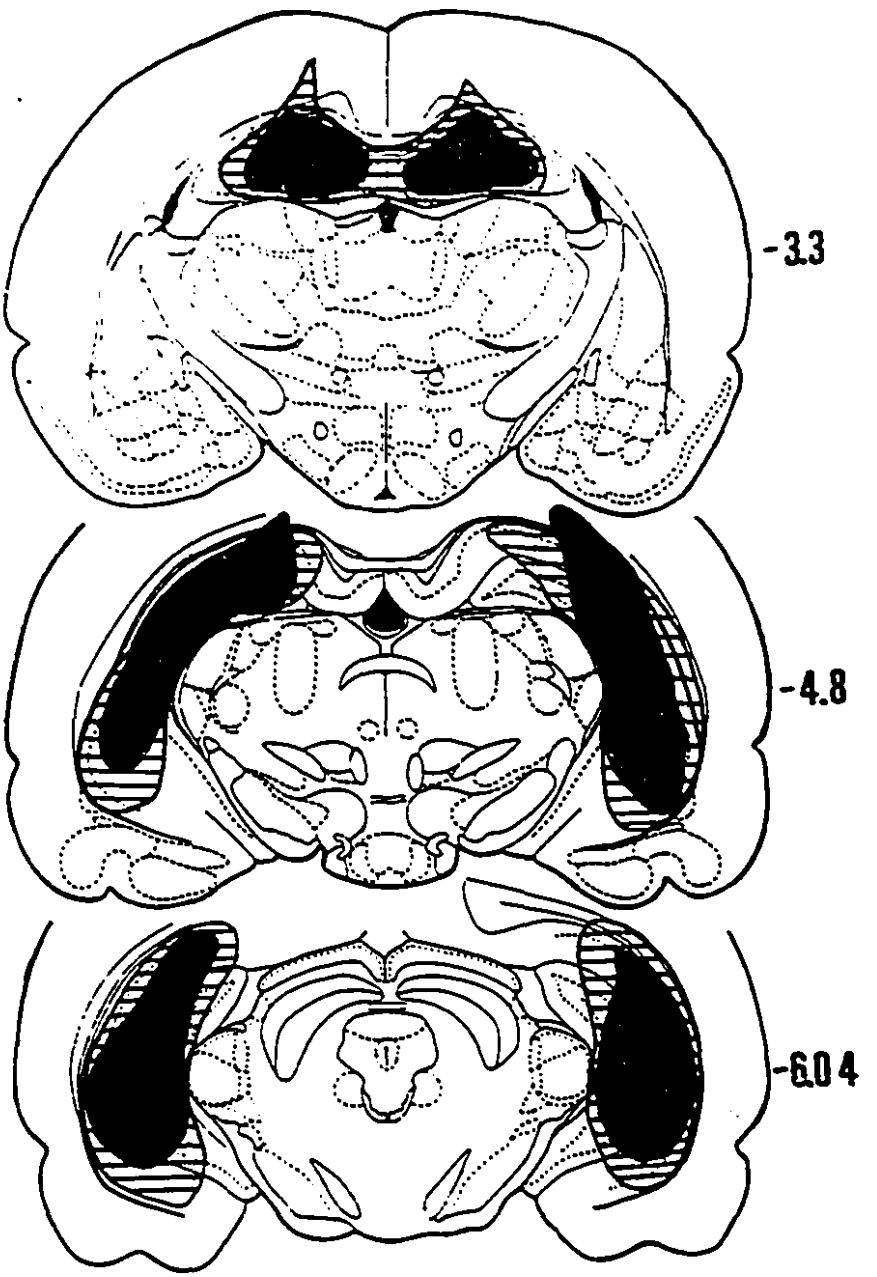
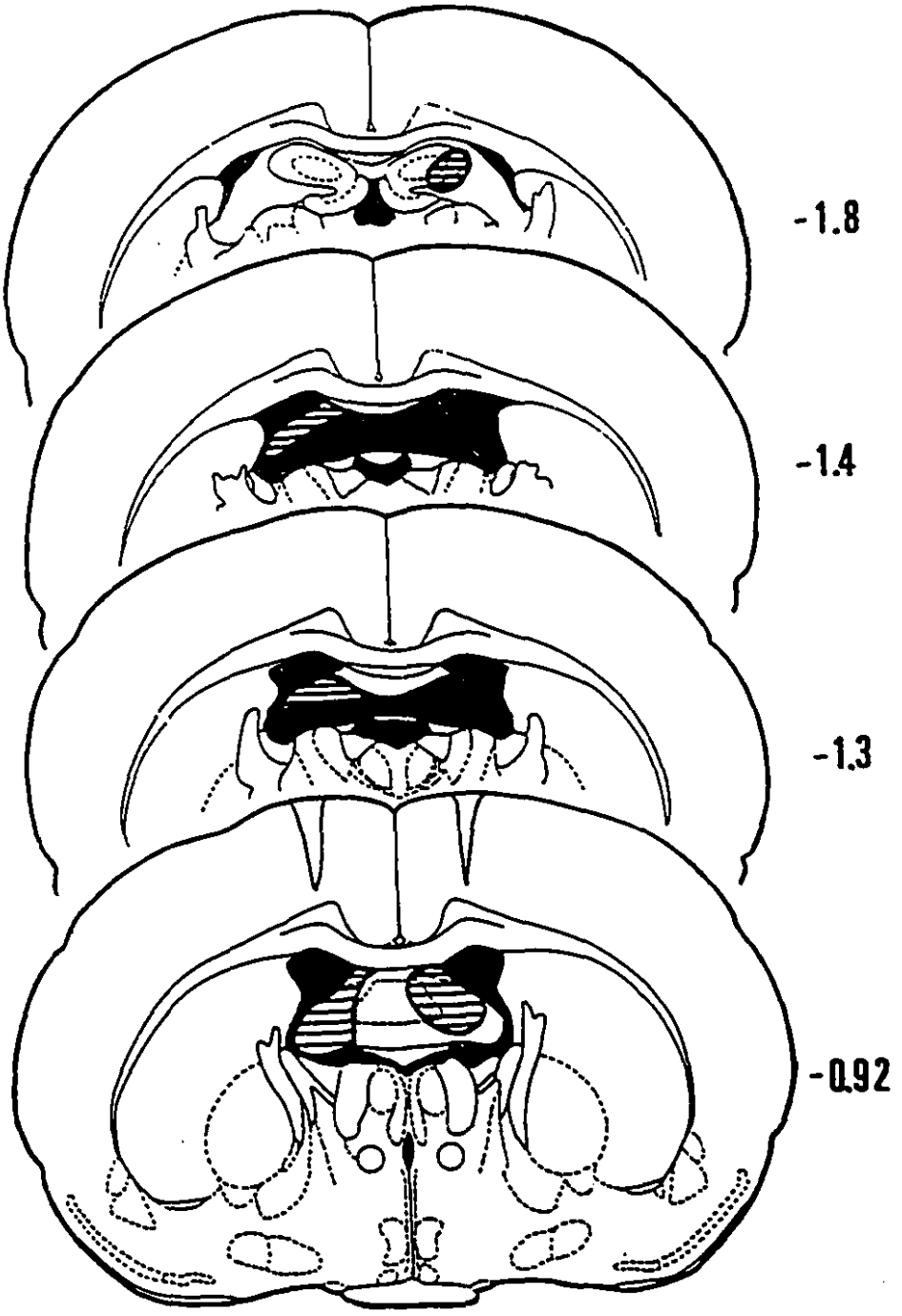

-3.3
-4.8
-6.04

Figure 28

Schematic representation of radio-frequency induced lesions of the fornix. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).


-1.8
-1.4
-1.3
-0.92

discrete than damage produced by neurotoxin infusions into the hippocampus. Medial and lateral portions of the fimbria-fornix complex were destroyed but, in all cases, the subiculum and cingulum were spared. Some animals sustained minimal damage to the entorhinal cortex.

Behavioural Results

It can be seen in Figure 29 that neurotoxin-induced lesions of the hippocampus did not disrupt a morphine-induced CPP, whereas radio frequency lesions of the fornix reduced the size of the CPP. Animals receiving sham and neurotoxin infusions into the hippocampus displayed a preference for the morphine-paired compartment (sham [$F(1,14) = 10.5$; $p < 0.01$] and lesion [$F(1,14) = 5.6$; $p < 0.05$]). The size of the CPP did not differ between these two groups [$F(1,28) = 0.6$; $p > 0.05$].

Following radio-frequency lesions of the fornix, shams developed a CPP for the drug-paired compartment [$F(1,22) = 16.9$; $p < 0.01$], whereas lesioned animals did not [$F(1,22) = 2.07$; $p > 0.05$]. Nonetheless, the size of the CPP was not significantly different for sham and lesioned groups [$F(1,44) = 2.19$; $p > 0.05$].

Animals with fornix lesions were re-tested for a CPP following morphine administration. Figure 30 shows data from the drug free and state dependent tests as time spent in, and entries to, drug- and saline-paired compartments at two minute intervals.

During drug free testing, animals with fornix lesions did not display a CPP during the first [$F(1,9) = 0.45$; $p > 0.05$] or second [$F(1,9) = 0.42$; $p > 0.05$] 10 minutes of testing. These animals did not make more entries to the drug-paired compartment during either half of the test, ([$F(1,9) = 0.01$; $p > 0.05$] and [$F(1,9) = 0.01$; $p > 0.05$]). The total number of entries made by lesioned animals

Figure 29

Effects of lesions of the hippocampus induced by neurotoxin infusion or radio-frequency current (fornix) on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.

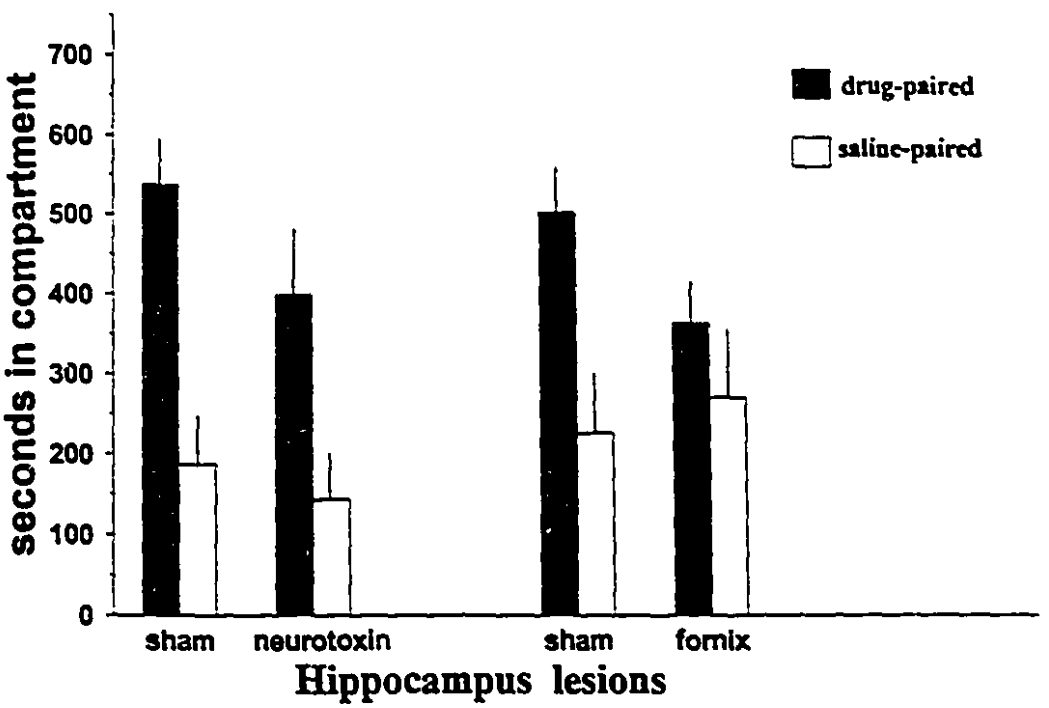
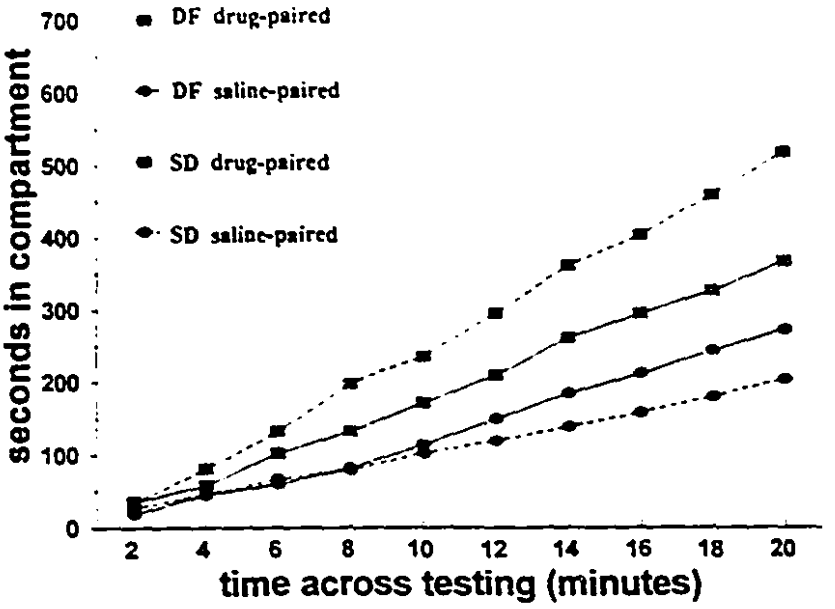
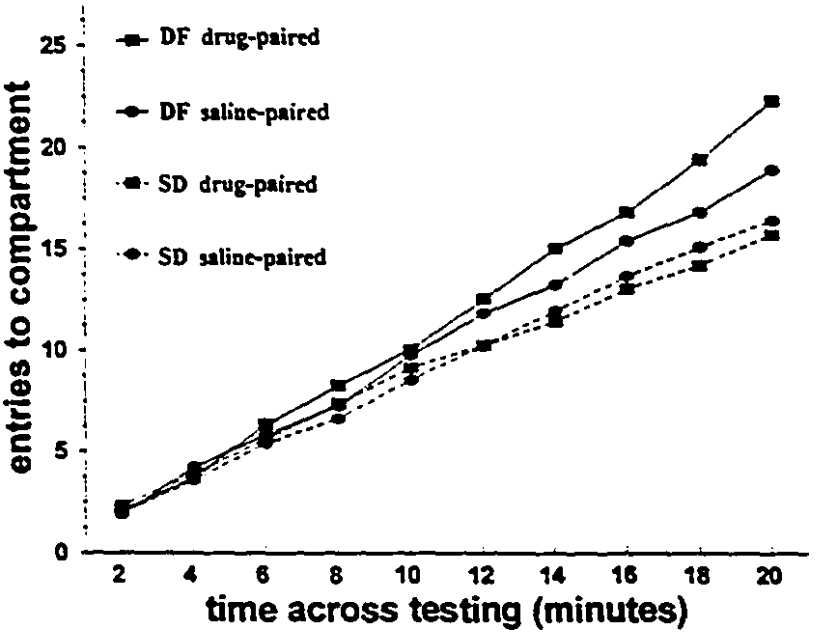

seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
neurotoxin
sham
fornix
Hippocampus lesions

Figure 30

Time course of the CPP exhibited by animals with fornix lesions during drug free (DF) (solid lines) and state dependent (SD) (dotted lines) tests. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals in each test. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals in each test. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
seconds in compartment
700
600
500
400
300
200
100
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
entries to compartment
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)

(mean = 34.9) was signifantly higher than that of their sham lesioned controls (mean = 20.4), [$F(1,44) = 15.8$; $p < 0.05$].

Following morphine administration, fornix lesioned animals displayed a significant preference for the drug-paired compartment [$F(1,9) = 5.6$; $p < 0.05$]. Difference scores were significantly larger during state dependent than during drug free testing [$F(1.9) = 6.4$; $p < 0.05$]. In the first 10 minutes of the state dependent test, fornix lesioned animals did not exhibit a CPP [$F(1,9) = 2.95$; $p > 0.05$], but in the second 10 minutes of testing they did [$F(1,9) = 5.19$; $p < 0.05$]. Nonetheless, the preference for the drug-paired compartment was not significantly different in the two halves of the test [$F(1,9) = 0.29$; $p > 0.05$]. Fornix lesioned animals did not make more entries to the drug-paired compartment during the first [$F(1,9) = 0.005$; $p > 0.05$] or second [$F(1,9] = 0.05$; $p > 0.05$] 10 minutes of the state dependent test.

LA Lesions

Histology

The location and extent of neuronal damage produced by NMDA infusions into the LA is presented in Figure 31. Neurotoxic infusions destroyed the LA and also produced substantial damage of the basolateral nucleus. In most cases, gliosis was present in central and basomedial nuclei. Two animals also sustained damage in the CPu.

Figure 32 illustrates the location and extent of neuronal damage in the LA induced by electrolytic current. Lesions were restricted to the target site with the exception of one animal who sustained damage in the basomedial nucleus.

Figure 31

Schematic representation of NMDA-induced lesions of the LA. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

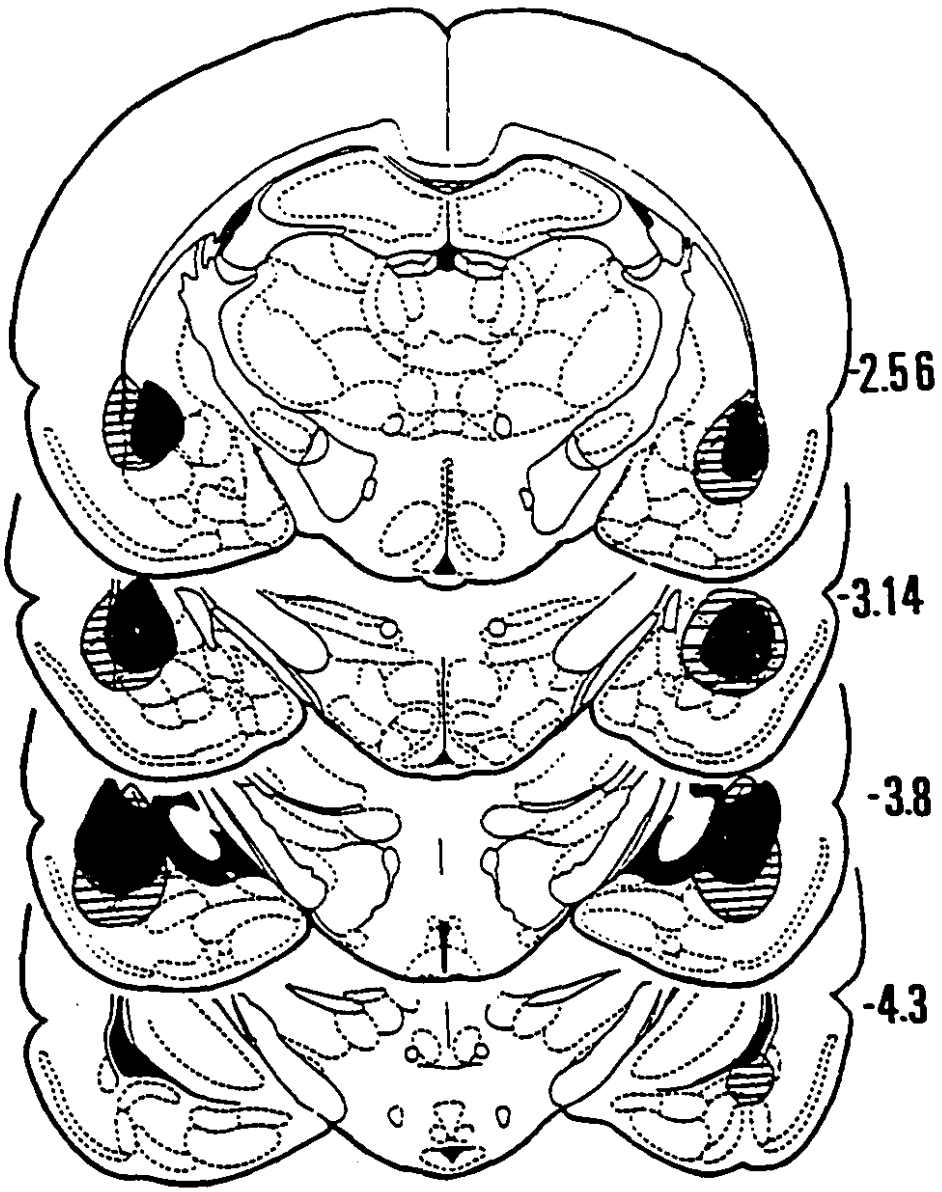
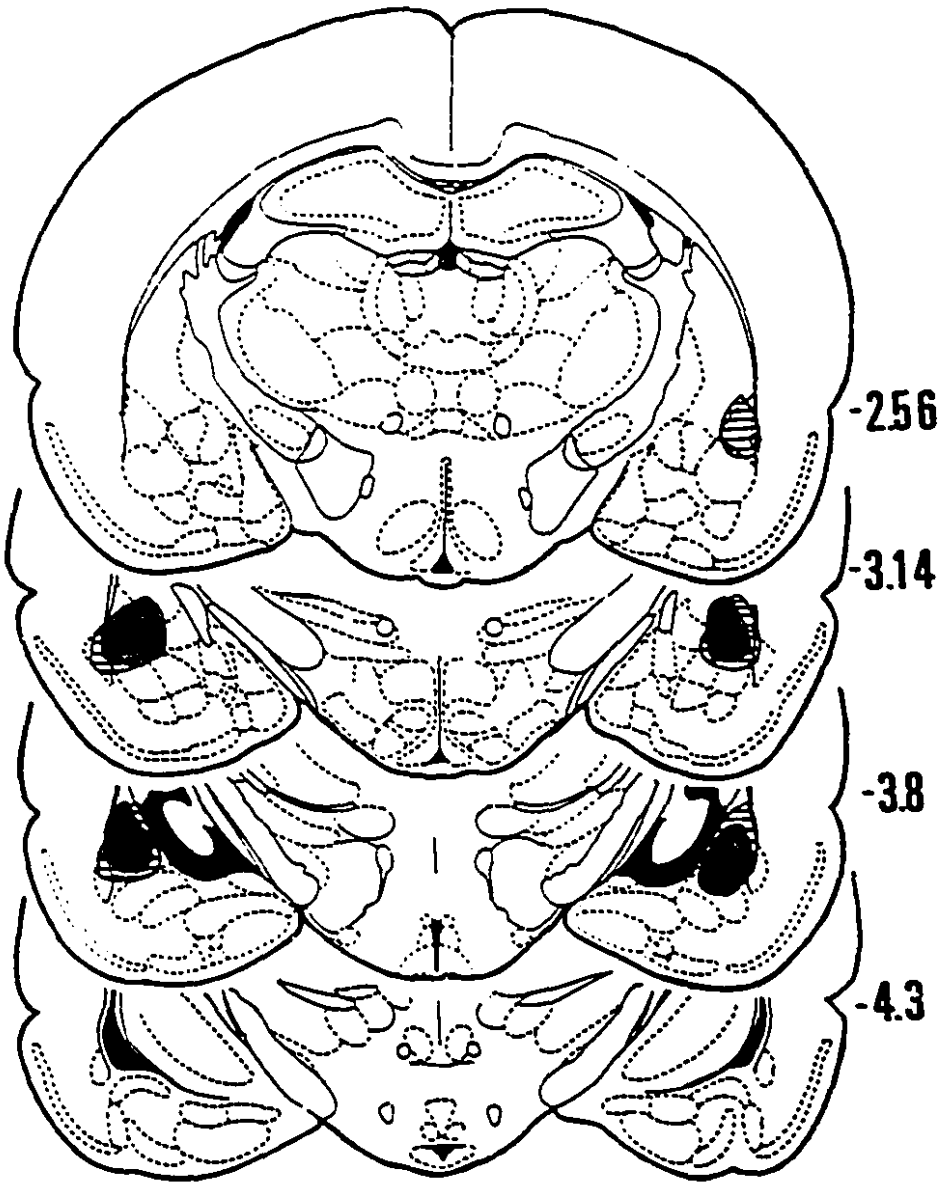

-2.56
-3.14
-3.8
-4.3

Figure 32

Schematic representation of electrolytic-induced lesions of the LA. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).


-2.56
-3.14
-3.8
-4.3

Behavioural Results

It can be seen in Figure 33 that LA lesions did not affect the development of a CPP to morphine. Following NMDA-induced lesions, sham and lesioned animals developed a preference for the drug-paired compartment (sham [$F(1,20) = 18.03$; $p < 0.01$] and lesion [$F(1,20) = 26.9$; $p < 0.01$]). The size of the CPP did not differ between sham and lesioned groups [$F(1,40) = 0.9$; $p > 0.05$].

Similarly, lesions induced by electrolytic current were ineffective. Sham (N=12) and lesioned (N=11) animals developed a preference for the drug-paired compartment (sham [$F(1,22) = 11.1$; $p < 0.01$] and lesion [$F(1,22) = 21.7$; $p < 0.01$]). There was no difference in the size of the CPP expressed by sham and lesioned animals [$F(1,44) = 1.0$; $p > 0.05$].

CPu Lesions

Histology

Figure 34 illustrates the location and extent of CPu damage induced by NMDA. Neurotoxic infusions produced neuronal damage in the dorsolateral CPu at both anterior and posterior regions of the nucleus. With the exception of one animal, there was no evidence of cell loss or gliosis in the medial or ventral CPu. In that animal, damage was present in the border between the CPu and the dorsolateral region of the NAS. Adjacent septal nuclei were spared in all animals.

Behavioural Results

As shown in Figure 35, CPu lesions had no effect on the development of a CPP to morphine. Following CPu lesions, sham and lesioned animals displayed a significant preference for the drug-paried compartment (sham [$F(1,14) = 6.2$; $p < 0.05$] and lesion [$F(1,14) = 7.8$; $p < 0.05$]). There was no difference in the size

Figure 33

Effects of lesions of the LA induced by NMDA infusions or electrolytic current on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.

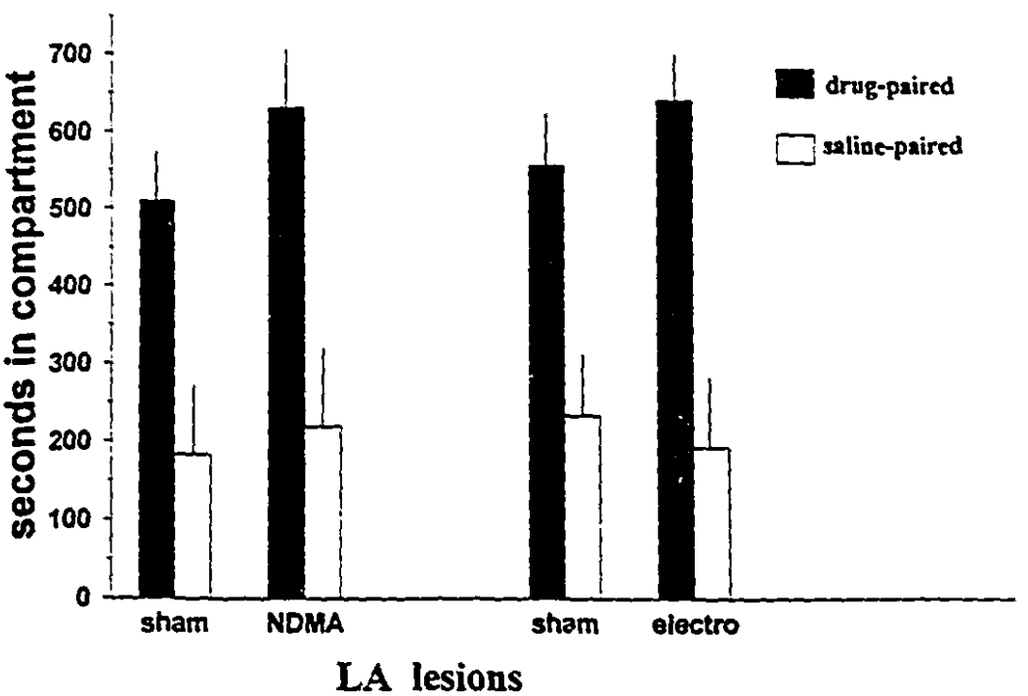

seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NDMA
sham
electro
LA lesions

Figure 34

Schematic representation of NMDA-induced lesions of the CPu. Solid areas indicate minimum, and hatched areas indicate maximum, extent of damage. Numbers to the right refer to anterior-posterior coordinates from bregma, according to the atlas of Paxinos and Watson (1986).

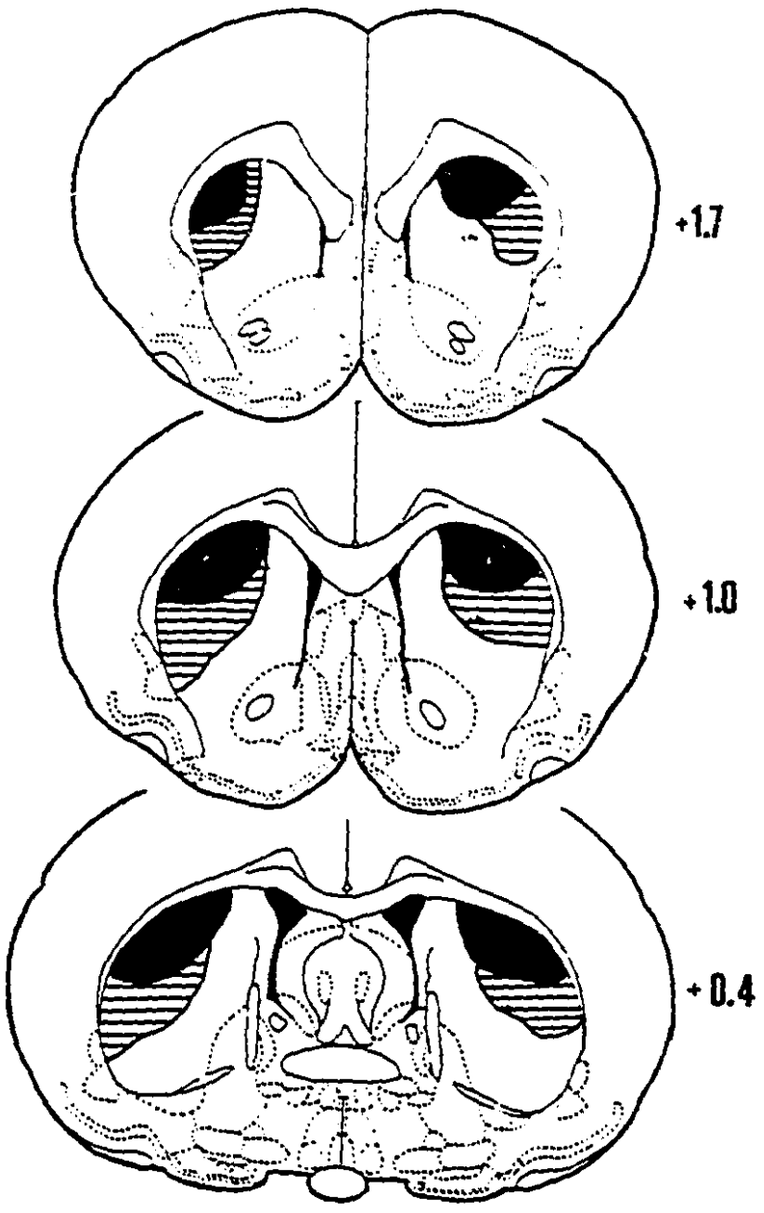
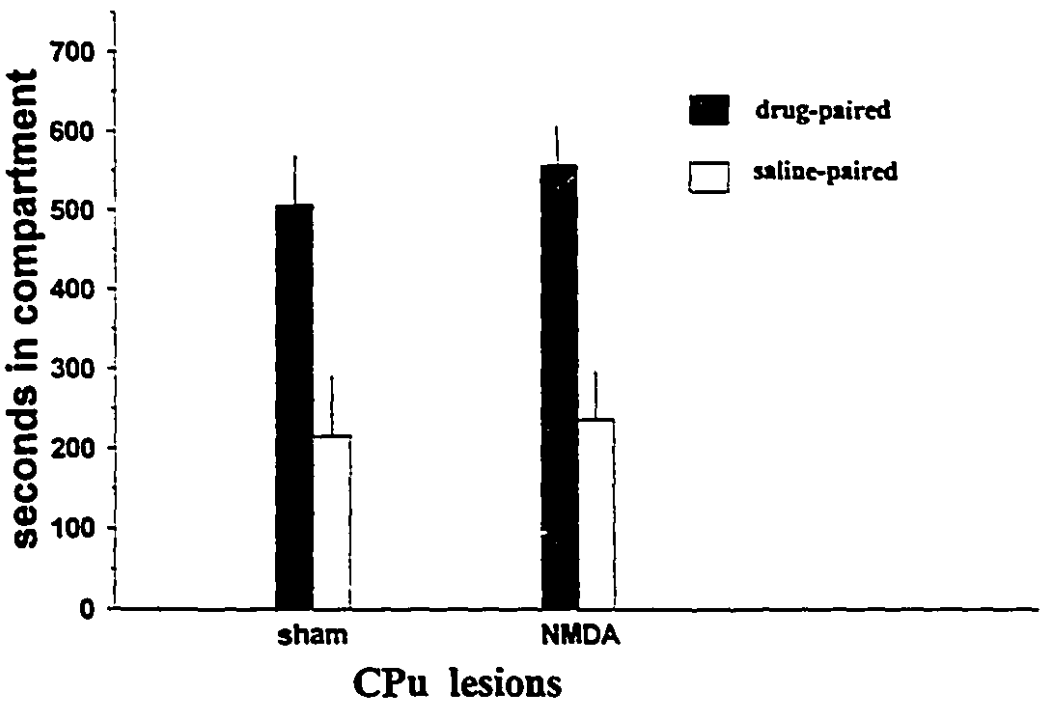

+1.7
+1.0
+0.4

Figure 35

Effects of NMDA-induced lesions of the CPu on a morphine-induced CPP. Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for sham and lesioned groups.


seconds in compartment
700
600
500
400
300
200
100
0
drug-paired
saline-paired
sham
NMDA
CPu lesions

of the CPP expressed by sham and lesioned animals [$F(1,28) = 0.006$; $p > 0.05$]. The total number of entries (entries to drug-paired plus entries to saline-paired compartments) was significantly reduced by CPu lesions (mean entries sham = 22.4; mean entries lesion = 13.1; [$F(1,28) = 4.81$; $p < 0.05$]).

Discussion

In the present set of experiments a morphine-induced CPP was blocked by PAG lesions and reduced by fornix lesions. When animals with PAG or fornix lesions were re-tested in a drugged state, however, they both displayed a significant preference for the morphine-paired compartment. Lesions of the LA, the CPu, or the hippocampus had no effect on the development of a CPP to morphine.

PAG and Morphine-induced CPP

The effect of PAG lesions on a morphine-induced CPP was examined in the present study because results from Experiments 1 and 2 suggest that the region is involved in mediating morphine's rewarding effect. The finding that PAG lesions block a morphine-induced CPP appears to support this suggestion. It contradicts previous evidence that PAG lesions have no effect on a CPP to morphine (Bechara & van der Kooy, 1989), however the two sets of lesions may not be comparable. In Bechara and van der Kooy's study, lesions destroyed the ventromedial PAG at the posterior end of the nucleus. Lesions in the present study were concentrated in the dorsolateral regions of the PAG, 2 mm anterior to the lesions in Bechara and van der Kooy's study. Given that functional divisions of the PAG are apparent along its rostrocaudal axis (Bandler & Shipley, 1994),

different lesion sites in the two studies may account for the discrepancy in behavioural results.

The interpretation that PAG lesions attenuate the rewarding effect of morphine is problematic because lesioned animals exhibited a CPP in a drugged state. The expression of a CPP during state dependent testing indicates that lesioned animals are capable of experiencing the rewarding effect of morphine and of associating this effect with salient environmental cues. The results of the state dependent test, and the nature of deficit induced by PAG lesions, must be interpreted in light of current theories of state dependent learning.

State Dependent Learning

Animals that are trained in one physiological state and tested in another may show partial or no signs of former learning (Overton, 1973, 1978). This indicates that learning is more easily retrieved under the conditions in which it was acquired. State dependent learning may be regarded as a failure to retrieve information that was learned in a drugged state (Overton, 1978; Spear, 1973).

Previously, it has been shown that state dependent learning does not influence the development of a CPP to morphine (Mucha & Iversen, 1984; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982). The fact that undrugged animals reliably express a CPP to morphine indicates that they are capable of retrieving information about the relationship between drug administration and environmental cues in a different state from the one in which the association was acquired. Although drug administration is the most salient cue that animals use during CPP testing (Bozarth, 1987a), the size of the CPP does not differ in drug free and state dependent tests (Mucha & Iversen, 1984).

In the present study, PAG and fornix lesioned animals did not display a CPP during drug free testing; during state dependent testing, however, both groups exhibited a significant preference for the drug-paired compartment. These results suggest that PAG and fornix lesions both impair the ability to retrieve information about the relationship between conditioned rewarding effects and environmental cues. Lesioned animals show a CPP during state dependent testing because the drug administration induces physiological effects which may act as a cue (Overton, 1985; Spear, 1973) that signals or retrieves the association between conditioned and unconditioned stimuli (Bouton & Bolles, 1985). The fact that both groups of lesioned animals exhibit a CPP in the second, but not the first, ten minutes of state dependent testing supports this idea; drug-induced effects should be more pronounced in the second half of the test when drug absorption has occured.

The suggestion that PAG lesions reduce a morphine-induced CPP by producing a deficit in cue retrieval is difficult to defend because PAG function is not generally associated with mnemonic processes. If the region is involved in this function, it is probably mediated through reciprocal connections between the PAG and the cortex. PAG efferents terminate in the medial prefrontal (Eberhart, Morrell, Krieger, & Pfaff, 1985; Herrero, Insausti, & Gonzalo, 1991) and entorhinal (Swanson, Kohler, & Bjorklund, 1987) cortical regions. Cortical afferents are organized topographically within the PAG, suggesting that the nucleus may integrate cortical activity (Bandler & Shipley, 1994). The PAG does not receive direct projections from the hippocampus, but target sites of hippocampal output, including the medial preoptic area, dorsomedial nucleus, and ventromedial nucleus of the thalamus, send fibers to the PAG (Marchand & Hagino, 1983).

It is also possible that the PAG is not directly involved in memory related processes, but provides some information that is necessary for memory retrieval. Evidence for this hypothesis is provided by findings from anatomical and behavioural investigations of the PAG. The PAG has a role in antinociception, vocalization, and defensive reactions [for review see (Bandler & Depaulis, 1991)], and is associated with sensory and autonomic responses, including arterial pressure, respiration, and vasoconstrictor tone (Bandler, Carrive, & Zhang, 1991). Anatomical evidence indicates that the PAG is connected with limbic forebrain sites associated with emotion (Holstege, 1991; Nauta, 1958; Li, Matsuzaki, Shinonaga, & Mizuno, 1993), and with neural structures that process somatosensory information (Jurgens, 1994; Li, Matsuzaki, Shinonaga, & Mizuno, 1993).

Given these associations, it has been suggested that the PAG integrates somatic and sensory components of defensive and protective reactions (Carrive, 1993) and that it mediates adaptive responses to emotional stimuli (Bandler & Shipley, 1994). In particular, somatosensory information from the spinal cord and affective information from the forebrain may be integrated at the level of the PAG (Cameron, Khan, Westlund, Cliffer, & Willis, 1995; Cameron, Khan, Westlund, & Willis, 1995). These hypotheses suggest that the PAG is part of an emotional motor system that controls behavioural responses to stimuli with an affective component (Holstege, 1990, 1992) and that the region is an interface between sensory and motivational systems (Beitz, 1994).

If the PAG mediates the integration of somatosensory and affective responses, PAG lesions may disrupt the ability to retrieve information about the relationship between sensory and affective properties of the drug that they acquired during conditioning. PAG lesioned animals probably express a CPP

following morphine administration because the interoceptive cues induced by drug administration are more pronounced than conditioned drug effects experienced during drug free testing. If the PAG integrates sensory information, the cue that retrieves the US-CS relationship during state dependent testing is probably related to somatosensory properties induced by morphine administration.

Hippocampus and Morphine-induced CPP

The suggestion that fornix lesions produce a deficit in cue retrieval is not difficult to reconcile with contemporary theories of hippocampal function which suggest that the region contributes to the processing of mnemonic information (Hirsh, 1974; O'Keefe & Nadel, 1979; Olton, Becker, & Handelmann, 1979; Squire, 1986; Sutherland & Rudy, 1989; Teyler, 1985). The present study was not designed to assess the validity of competing theories. Nonetheless, results are consistent with the contextual retrieval theory of hippocampal function (Hirsh, 1974). According to Hirsh, the hippocampus does not mediate the formation of the US-CS association but provides the context for its retrieval. Fornix lesioned animals do not express a CPP because the relationship between conditioned and unconditioned stimuli that they learned during conditioning can not be transferred from a storage system to a system that controls behavioural output. Drug administration reinstates the CPP because internal affective states can serve as contextual cues (Hirsh, 1974).

It is interesting to note that fornix lesions do not block a CPP to amphetamine (Hiroi & White, 1991) or to food in a conditioned cue task (McDonald & White, 1993). When animals are conditioned with these stimuli, therefore, retrieval of the US-CS association does not depend on the hippocampus. Given

that fornix lesions reduce a CPP to morphine, but not to amphetamine, the ability to respond to learned stimulus-reward associations must be mediated by different neural systems following conditioning with these two drugs.

In the absence of data from the state dependent test, results of the drug free test could be taken as support for the spatial mapping theory of hippocampal function (O'Keefe & Nadel, 1979). O'Keefe and Nadel's theory posits that the hippocampus acquires information about the relationship among stimuli in the environment (O'Keefe & Nadel, 1979). It was based on findings that hippocampal cells respond to spatial location (Yaksh, 1979), and that fornix lesions block place, but not cue, learning (Jarrard, 1980; O'Keefe, Nadel, Keightley, & Dill, 1975). Spatial mapping theory might predict that fornix lesions were effective in the present study because the expression of a CPP depends on the ability to return to a particular place. The theory can not adequately explain the present findings, however, because fornix lesioned animals show a CPP during state dependent testing.

It is unlikely that fornix lesions reduce a morphine-induced CPP by affecting the ability to detect the rewarding properties of the drug. Similar to PAG lesioned animals, animals with fornix lesions must have experienced morphine's rewarding effect during conditioning and state dependent testing. Moreover, hippocampal lesions do not impair the ability to discriminate reward magnitude (Peinando-Manzano, 1994) or to use morphine as a discriminative cue (Skinner et al., 1994).

Consistent with previous reports (Douglas, 1967; Douglas & Pribram, 1966), fornix lesions produced hyperactivity during testing. Although fornix lesioned animals entered (and exited) drug- and saline-paired compartments more often

than control animals, this can not account for the reduced CPP because fornix lesions do not attenuate a CPP to amphetamine (Hiroi & White, 1991) or to food in a conditioned cue task (McDonald & White, 1993).

In contrast to radio frequency lesions of the fornix, neurotoxic-induced lesions of the hippocampus did not affect a morphine-induced CPP. These findings are in agreement with reports that damage to the fornix has more pronounced effects in behavioural tasks than lesions of the hippocampus that destroy up to 85% of the structure (Jarrard, 1980). Because neurotoxic infusions spared portions of the hippocampus and radio frequency lesions destroyed the fornix in its entirety, it is likely that fornix lesions more effectively disrupted communication between the hippocampus and other brain regions.

The results of the present study indicate that lesions of the PAG and the fornix reduce a morphine-induced CPP because they produce a deficit in cue retrieval. Although the deficits are similar, they may be related to different stimuli. PAG lesions may produce a deficit in the ability to evaluate sensory or interoceptive cues, whereas fornix lesions probably produce a deficit in the ability to integrate external or environmental cues. If this is true, fornix lesioned animals may express a CPP when they are provided with another salient environmental cue during testing. The same cue may not reinstate a CPP in PAG lesioned animals; these animals may require drug-induced sensory cues to retrieve the association between conditioned and unconditioned stimuli.

LA and Morphine-induced CPP

One of the most unexpected findings in the present study was that LA lesions did not attenuate a morphine-induced CPP. Previous evidence indicates

that LA lesions block a CPP to food (Everitt, Morris, O'Brien, & Robbins, 1991; McDonald & White, 1993) and to amphetamine (Hiroi & White, 1991), as well as the acquisition of responding for a conditioned reinforcer (Burns, Robbins, & Everitt, 1993; Cador, Robbins, & Everitt, 1989; Everitt, Cador, & Robbins, 1989). The present results are not necessarily inconsistent with the abundance of data indicating that the LA is involved in the formation of stimulus-reward associations (Jones & Mishkin, 1972; Gaffan, Gaffan, & Harrison, 1989). Rather, they suggest that the development of a CPP to morphine is not mediated through the same neural substrate that mediates a CPP to other appetitive stimuli. It is possible that LA lesions were ineffective in the present study because the rewarding effect of morphine may be mediated through more than one system. This notion is supported by evidence from Experiment 1 that a CPP is produced by opioid-induced activity in two different systems. In any case, neither morphine reward nor the ability to associate this effect with neutral stimuli depend on processes that occur within the LA.

CPu and Morphine-induced CPP

The finding that CPu lesions have no effect on the development of a CPP to morphine suggest that the region does not mediate opiate-induced reward. Although CPu lesions were not complete, they destroyed lateral portions of the nucleus that have been associated with stimulus-response learning (McDonald & White, 1993; Mitchell & Hall, 1988; Packard, Hirsh, & White, 1989; Reading, Dunnett, & Robbins, 1991). These results suggest that the expression of a CPP to morphine does not depend on the ability to make a motor response to sensory cues because CPu lesions impair this behaviour (Carli, Evenden, & Robbins, 1985;

McDonald & White, 1993; Packard, Hirsh, & White, 1989; Reading, Dunnett, & Robbins, 1991). It appears, therefore, that animals acquire and express a CPP to morphine via a mechanism that is independent of sensorimotor responding.

During testing, CPu lesioned animals displayed thigmotaxic behaviour: they stayed close to compartment walls, often huddled in a corner and, over the 20 minutes of testing, made fewer compartment entries than did their sham lesioned controls. Thigmotaxia following CPu lesions has been observed in previous studies (Whishaw, Mittleman, Bunch, & Dunnett, 1987; McDonald & White, 1994), although the underlying mechanism producing the behaviour is unknown. The fact that CPu lesioned animals in the present study displayed the same behaviour indicates that the lesions were effective.

The results from Experiment 4 indicate that lesions of the PAG or the fornix block the preference that animals display for a morphine-paired compartment during drug free testing. The finding that PAG and fornix lesioned animals exhibit a CPP when they are tested in a drugged state suggests that the two lesions produce a deficit in cue retrieval. A disruption in the expression of a CPP, therefore, does not necessarily indicate that the manipulation reduced the rewarding properties of the stimulus. Finally, the present results suggest that the mediation of a morphine-induced CPP involves neither the LA nor the CPu.

GENERAL DISCUSSION

The empirical investigations in this thesis were designed to identify the neural substrates which are both necessary and sufficient for the development of a CPP to morphine. The ultimate purpose of the studies was to elucidate the neural mechanisms underlying the rewarding effect of opiates. In Experiment 1, the VTA and the PAG were confirmed as sites where the morphine's rewarding effect is initiated because injections of morphine into either site produced a CPP. Results from Experiment 2 showing that antagonism of opioid receptors in the VTA or the PAG block a CPP to systemic morphine verified that opiate-induced reward is mediated via opioid receptors in these sites. An analysis of behavioural responding during CPP testing revealed that animals conditioned with intra-VTA and intra-PAG morphine injections exhibited a CPP in different ways. That is, the time at which the two groups expressed a CPP and their pattern of entries to drug- and saline-paired compartments were not the same. These findings suggest that the CPPs induced by intra-VTA and intra-PAG morphine may be produced by different mechanisms.

Experiments 3 and 4 examined which neural sites are involved in the mediation of morphine-induced reward by testing whether lesions of various structures blocked the development of a CPP to morphine. Findings from these studies indicate that lesions of the VS, the PPTg, the PAG, or the fornix reduce the preference animals exhibit for a morphine-paired compartment. These results, however, do not specify what role each of these structures has in the effect. The question is complicated by the fact that the expression of a CPP depends on a number of processes: the stimulus must be rewarding; animals must associate rewarding effects with salient environmental cues; they must remember this

association at a later time (test day); they must be capable of discriminating reward-paired cues from those associated with neutral stimuli; and finally, they must be capable of exhibiting approach behaviour to the conditioned cues. Disruption of one or more of these processes will be manifested as a reduced preference for the drug-paired compartment. It is possible, therefore, that the mechanisms by which lesions of the VS, the PPTg, the PAG, or the fornix attenuate a CPP differ.

In Experiment 3, it was suggested that the rewarding effect of morphine is blocked by PPTg lesions and attenuated by VS lesions. On the other hand, results from Experiment 4 indicated that PAG and fornix lesions appear to produce a deficit in the ability to retrieve information about the relationship between conditioned and unconditioned stimuli. Finally, based on the analyses of behavioural data presented in this thesis, it was proposed that the development of a CPP to morphine may be produced through more than one neural mechanism, and that the expression of a CPP may be influenced by processes that are not necessarily related to the rewarding properties of the drug.

Differences Between Neural Substrates Mediating a CPP to Opiates and Stimulants

The idea that a CPP may be produced through more than one mechanism is supported by evidence that the development of a CPP to opiates and stimulants involve different neural substrates. Results from the present set of experiments suggest that a morphine-induced CPP is disrupted by lesions that destroy most of the VS, the PPTg, the PAG, or the fornix, but not by lesions of the mesolimbic DA system, the caudal VS, or the LA. In contrast, a CPP induced by amphetamine

is blocked by lesions of the caudal VS (Olmstead & Franklin, 1995), the LA (Hiroi & White, 1991), or the mesolimbic DA system (Spyraki, Fibiger, & Phillips, 1982c), but not by lesions of the fornix (Hiroi & White, 1991). These differences are consistent with previous evidence indicating that neural substrates mediating the development of a CPP to opiates and stimulants differ. For example, pretreatment with serotonin, DA, or glutamatergic antagonists block a CPP to amphetamine, but have no effect on a morphine-induced CPP (Kruszewska, Romandini, & Samanin, 1986; Layer, Uretsky, & Wallace, 1993; Mackey & van der Kooy, 1985).

There are at least three explanations for the differences in neural substrates mediating an opiate- and a stimulant-induced CPP. First, the rewarding effects of the two drugs may be produced through different neural substrates. If this is true, administration of either opiates or stimulants would elicit approach behaviour, but they may not induce the same affective state. This interpretation suggests that there is more than one affective state capable of eliciting approach behaviour. It also implies that reward is not induced by a unique neural event or mechanism.

A second possibility is that the rewarding effects of opiates and stimulants are mediated by the same underlying mechanism, but that the two drugs produce physiological effects which differentially influence behavioural responding. For example, physiological effects induced by the two drugs could produce changes in locomotor, sensory, or discriminative abilities that might interact with behavioural measures of reward. It seems probable that morphine and amphetamine produce different physiological states because animals are capable of using interoceptive cues to discriminate between them (Gianutsos & Lal, 1976; Hernandez, Holohean, & Appel, 1978; Shannon & Holtzman, 1976).

Finally, the neural substrates underlying the development of a CPP to opiates and stimulants may differ because the two drugs could strengthen different associations. In the CPP paradigm, drug- and saline-paired compartments are distinguished by at least three salient stimuli: odour, texture, and colour. During conditioning, animals may associate the rewarding effect of the drug with a single cue, with some combination of cues, or with the contextual stimuli. Contextual conditioning may be important in the development of a CPP because conditioned stimuli are not restricted to a single sensory modality, they are continuously present, they are not delivered in a precise time dependent manner, and they predict the general situation of the drug-paired stimuli, but not its onset. It is possible that opiates and stimulants enhance conditioning to different sets of cues, for example to simple or contextual cues.

Because simple and contextual stimuli compete for associative strength (Rescorla & Wanger, 1972) or attention (Mackintosh, 1975), behaviour on test day will be controlled by the conditioned stimulus (simple or contextual) which has acquired the strongest association with the unconditioned stimulus. This suggests that animals may express a preference for the drug-paired compartment because they are approaching either discrete or contextual cues. The experimenter can not predict which cues animals will use in a particular test, although paradigms have been developed to preferentially assess one type of learning over the other (McDonald & White, 1993; Morris, 1981, 1984; Nadel & MacDonald, 1980; Packard, Hirsh, & White, 1989).

There is evidence that classical conditioning to simple and contextual stimuli are mediated by the LA and the hippocampus respectively (Selden, Everitt, Jarrard, & Robbins, 1991). Given that lesions of the LA block a CPP to

amphetamine but not to morphine and that fornix lesions have the opposite effect (Experiment 4) (Hiroi & White, 1991), morphine administration may increase conditioning to contextual cues whereas stimulant administration may increase conditioning to simple cues. In support of this hypothesis, amphetamine and other DA agonists potentiate responding to a discrete cue when they are injected systemically (Beninger, Hanson, & Phillips, 1980; Robbins, 1978; Robbins, Watson, Gaskin, & Ennis, 1983) or directly into the VS (Taylor & Robbins, 1984). In contrast, systemic (Robbins, Watson, Gaskin, & Ennis, 1983), intra-VTA (Kelley & Delfs, 1991), or intra-VS (Cunningham & Kelley, 1992) injections of opioid agonists do not. The question of whether opiates increase conditioning to contextual cues could be tested by examining whether morphine administration enhances learning processes in contextual conditioning tasks.

CPP as a Measure of Reward

The suggestion that the development of a CPP may be produced by more than one mechanism raises the questions of what does the CPP paradigm measure, and is this paradigm a valid measure of reward? The use of the CPP paradigm as a measure of reward is based on two premises: 1) When neutral stimuli are paired with the presentation of reward, they gain the ability to elicit behavioural effects originally associated with the reward (Pavlov, 1927). 2) Rewarding stimuli are characterized by their ability to elicit approach behaviour (Glickman & Schiff, 1967; Schneirla, 1959; Young, 1959). The process whereby neutral stimuli acquire properties that elicit approach behaviour is referred to as incentive motivational learning (Bindra, 1969, 1974). While accepting that a

stimulus must be rewarding to produce a CPP, it may be more appropriate to consider the CPP paradigm as a measure of incentive motivation.

The use of the CPP paradigm has been criticized because behavioural responding during CPP testing does not strictly conform to principles of either classical or operant conditioning (Wise, 1989). Miller and Konorski (1928) originally pointed out that the laws of classical conditioning may not adequately explain operant responding. This viewpoint was later promoted by a number of learning theorists including Schlossberg (1937), Mowrer (1947), and Rescorla and Solomon (1967). Incentive Motivation theory (Bindra, 1968, 1978), along with other theoretical positions (Bolles, 1972; Hull, 1930; Spence, 1956), describes operant responding in terms of underlying classical conditioning processes. A description of the CPP paradigm as a measure of incentive motivation, therefore, assumes that the development of a CPP is mediated by a process that is common to both classical and operant responding.

Some authors have challenged the use of the CPP paradigm (Heinrichs & Martinez, 1986; Scoles & Siegel, 1986; Swerdlow & Koob, 1984) because procedural differences between studies complicate interpretations of their results. For example, the use of balanced versus unbalanced protocols, two versus three box compartments, novel versus familiar environments, equal versus unequal exposure to compartments, and the inclusion or exclusion of appropriate control groups have differed across studies. Although the criticism is valid, it does not apply to comparisons between studies presented in this thesis because a consistent protocol was used throughout all of the experiments.

Finally, it has been argued that the CPP paradigm is only valid as a confirmation of results from the self-administration paradigm (Wise, 1989). That

is, stimuli that produce a CPP are considered rewarding if, and only if, animals will also perform an operant response for them. In general, drugs that induce a CPP will be self-administered (Carr, Fibiger, & Phillips, 1989), indicating that mechanisms underlying the rewarding effect of a particular stimulus are similar in both paradigms. On the other hand, behavioural demands and learned associations are different in the CPP and self-administration paradigms. It is possible, therefore, that processes which mediate responding in the two tests could differentially influenced by factors that are not related to reward. Because of this, it may be instructive to examine the rewarding effect of a single stimulus in both CPP and self-administration paradigms. Employing more than one paradigm is important because most tests are not pure measures of a single variable and drugs may produce different artifacts that will interfere with behavioural measures (Miller, 1960).

The present investigation employed the CPP as a measure of reward because (as outlined in the General Introduction) it offers a number of advantages over the self-administration paradigm (Carr, Fibiger, & Phillips, 1989; Swerdlow, Gilbert, & Koob, 1989; van der Kooy, 1987). In addition to pointing out the problems with interpreting data from CPP studies, the analyses in this thesis have furthered the understanding of neural mechanisms underlying the rewarding effect of opiates. Moreover, the use of a single paradigm throughout an extended series of studies provided the opportunity to characterize the psychological mechanisms underlying a morphine-induced CPP. This analysis indicates that the development of a CPP to morphine is a complex phenomenon involving an interaction between a number of neural sites. An obvious extension of the present investigation would be to use the same general procedure (identification of neural sites which are

necessary and/or sufficient) to investigate the neural mechanisms underlying the rewarding effect of opiates in the self-administration paradigm. Undoubtedly, convergence of evidence from a variety of paradigms will yield a more complete understanding of the behaviour in question and the function of different neural mechanism in its mediation.

Behavioural Characteristics of a Morphine-induced CPP

Based on the evidence that intra-VTA and intra-PAG morphine injections produced different patterns of behavioural responding, it was suggested that the CPPs induced by these two injections are mediated by different mechanisms. This idea is difficult to evaluate without an understanding of the psychological processes that produce a CPP, and the manner in which these factors are reflected in behavioural responding. In an attempt to characterize the behaviours which contribute to a morphine-induced CPP, behavioural responding exhibited by control animals during CPP testing was re-examined. The data from sham lesioned animals in Experiments 3 and 4 were combined and analyzed as one group (N = 115). Although these animals served as controls for different experiments, they all underwent the same conditioning protocol (2 mg/kg morphine X 3 pairings). A subpopulation of these animals (N = 30) were tested for a CPP in a drugged state. Behavioural results of the drug free and state dependent tests are shown in Figure 36A. For purposes of comparison, the data is presented in Figure 36B along with data from the microinjection and lesion studies.

Data Analysis

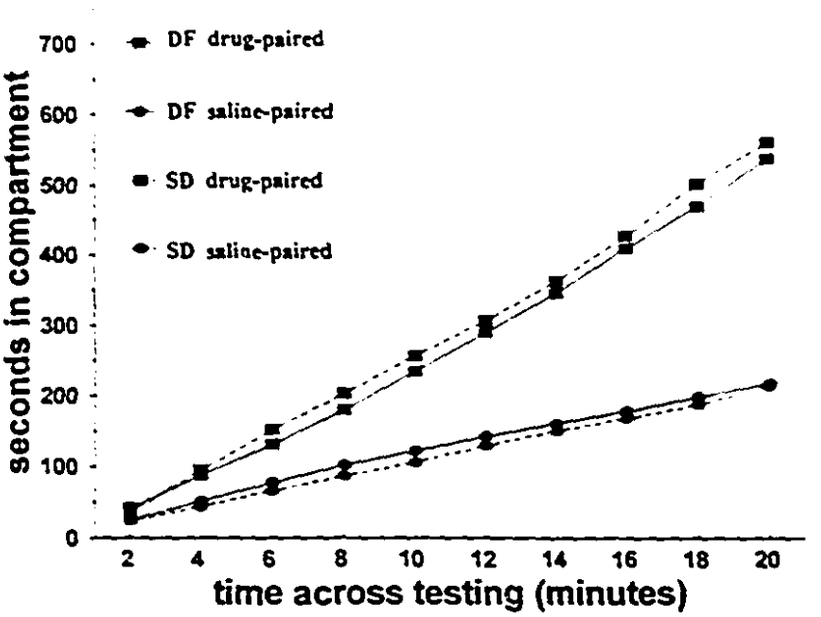
Data from the drug free and state dependent tests were analyzed using the procedures described in the General Methods. This analysis revealed that sham lesioned animals exhibited a significant preference for the drug-paired

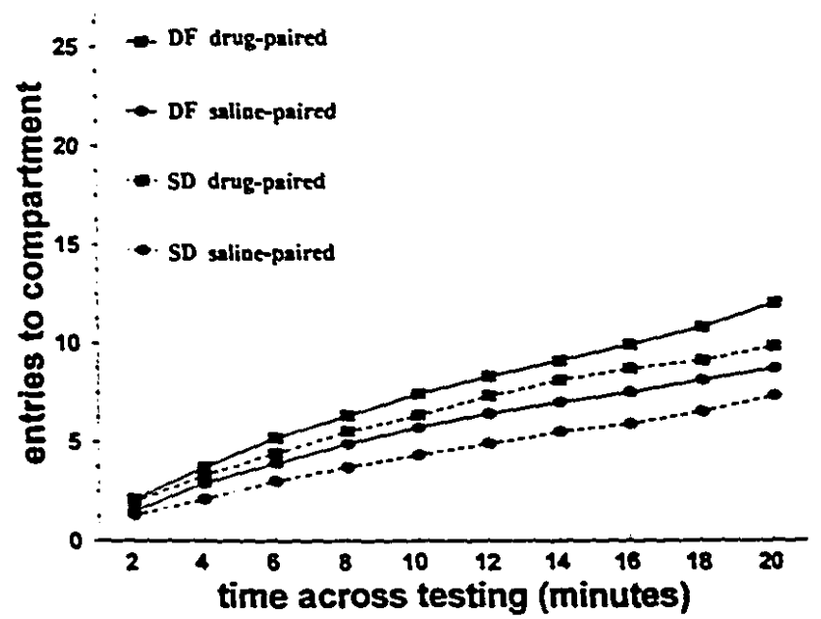
Figure 36A

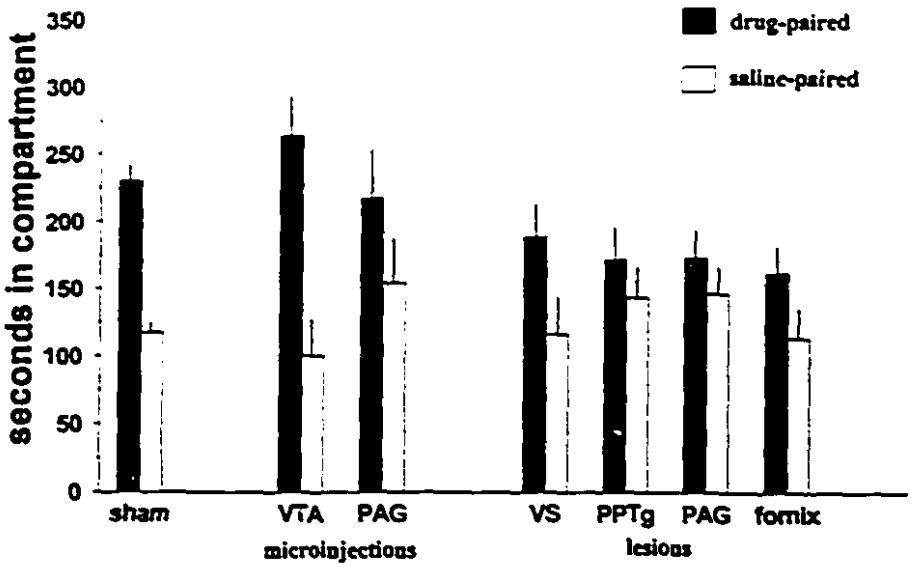
Time course of the CPP exhibited by sham lesioned animals from Experiments 3 and 4 during drug free (DF) (solid lines) and state dependent (SD) (dotted lines) tests. Top panel: Cumulative time spent in drug-paired and saline-paired compartments at 2 minute intervals in each test. Bottom panel: Cumulative entries to drug-paired and saline-paired compartments at 2 minute intervals in each test. Drug-paired compartment = square symbols; saline-paired compartment = circular symbols.

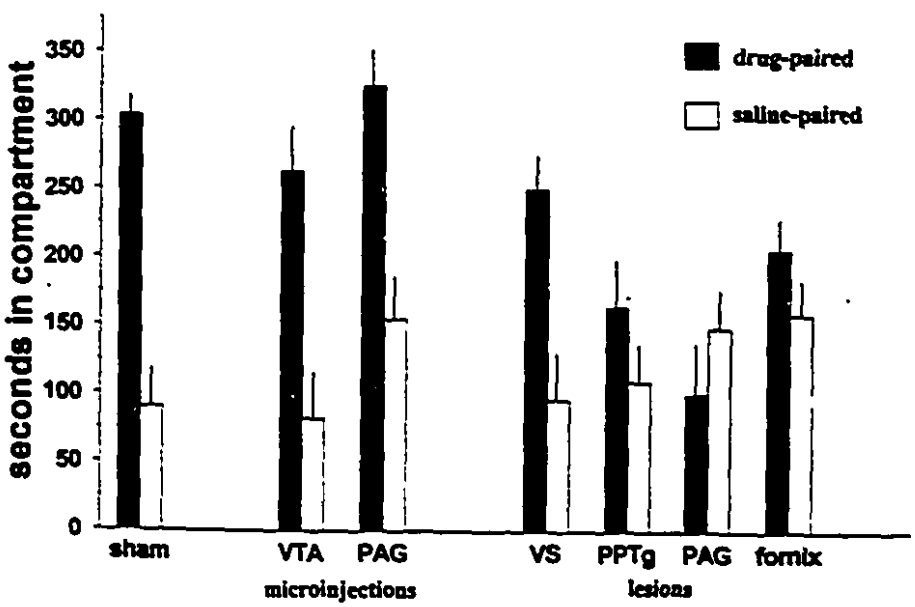
Figure 36B

Time in compartments during the first (top panel) and second (bottom panel) ten minutes of testing for the combined group of sham lesioned animals, the two groups for which morphine microinjections produced a CPP (VTA and PAG), and the groups for which lesions disrupted a morphine-induced CPP (VS, PPTg, PAG, fornix). Bars represent mean number of seconds (+ SEM) spent in drug-paired and saline-paired compartments for each group.


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
seconds in compartment
700
600
500
400
300
200
100
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


DF drug-paired
DF saline-paired
SD drug-paired
SD saline-paired
entries to compartment
25
20
15
10
5
0
2
4
6
8
10
12
14
16
18
20
time across testing (minutes)


drug-paired
saline-paired
seconds in compartment
350
300
250
200
150
100
50
0
sham
VTA
PAG
microinjections
VS
PPTg
PAG
fornix
lesions


drug-paired
saline-paired
seconds in compartment
350
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VS
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PAG
fornix
lesions

compartment over the 20 minute drug free test [$F(1,114) = 200.94$; $p < 0.01$]. The effect was apparent during the first [$F(1,114) = 135.1$; $p < 0.01$] as well as the second [$F(1,114) = 169.7$; $p < 0.01$] half of the test. The size of the CPP was larger in the second than in the first ten minutes of testing [$F(1,114) = 24.8$; $p < 0.01$].

The number of entries that animals made to the drug- and saline-paired compartments did not differ during the first 10 minutes of testing [$F(1,114) = 1.75$; $p = 0.19$]. During the second 10 minutes, however, they made more entries to the drug-paired than to the saline-paired compartment [$F(1,114) = 65.2$; $p < 0.01$]. Sham lesioned animals made 13.8 (SEM = 0.87) total entries (entries to drug-paired plus entries to saline-paired compartments) in the first half of the test and 6.9 (SEM = 0.83) total entries in the second half of the test. The difference in number of total entries was significantly reduced in the second 10 minutes of testing [$F(1,114) = 18.87$; $p < 0.05$]

On test day, animals are placed in the tunnel and allowed to freely explore the three compartments. If they have no bias for either compartment at the beginning of the test, 50% should enter the drug-paired compartment first and 50% should enter the saline-paired compartment first. Because drug administration was randomly paired with compartment, the distribution of entries to the black and white compartments should also be 50% and 50%. Seventy-eight of the 115 animals (68%) made their first entry to the drug-paired compartment whereas 45% and 55% made their first entry to the black and white compartments respectively. Using a Chi Square test to compare the observed and expected frequencies of the first compartment entry, it was determined that sham lesioned animals displayed

a significant bias to make their first entry to the drug-paired compartment [$X^2(1) = 14.62$; $p < 0.01$].

When 30 of these animals were tested in a drugged state, they exhibited a CPP [$F(1,29) = 20.55$; $p < 0.01$], the size of which was not significantly different from their drug free CPP [$F(1,29) = 0.3$; $p > 0.05$]. Sham lesioned animals expressed a CPP during the first [$F(1,29) = 20.55$; $p < 0.01$] and second [$F(1,29) = 16.06$; $p < 0.01$] half of the state dependent test. Unlike the drug free test, the size of the CPP did not differ between the first and second 10 minutes of testing [$F(1,29) = 0.04$; $p > 0.05$].

In the drugged state, animals entered the drug-paired compartment more often than the saline-paired compartment during the first [$F(1,29) = 11.33$; $p < 0.01$] as well as the second [$F(1,29) = 6.8$; $p < 0.01$] 10 minutes of testing. Twenty-one animals (70%) made their first entry to the drug-paired compartment. This did not represent a significant bias [$X^2(1) = 2.4$; $p > 0.05$], but it should be noted that the proportion of first entries to the drug-paired compartments was almost identical to that of the first, drug free test (70% and 68% respectively). The lack of significance in the state dependent test is probably related to the reduced power from the smaller sample size (115 versus 30).

Time in Compartments

As expected, the combined groups of sham lesioned animals displayed a significant preference for the morphine-paired compartment during drug free testing. It was more surprising to find that the size of the CPP increased across the test. This is contrary to general learning principles suggesting that animals would initially approach drug-paired cues and, in the absence of primary reward, the response would extinguish. As discussed in Experiment 1, morphine's

paradoxical effects may produce ambivalent responses to the drug-paired cues. If this is true, sham lesioned animals might show a smaller CPP in the initial test period because they associate the morphine-paired compartment with aversive effects of the drug. The increase in the size of the CPP over testing may reflect conflict behaviour or an increased latency to approach conditioned cues, a response that is typically associated with stimuli that are both rewarding and aversive (Cazala, 1986, 1990; Cazala, Bendani, & Zielinski, 1985; David & Cazala, 1994; Leibman, 1985).

It is also possible that animals show a larger preference for the drug-paired compartment during the latter portion of the test because they have learned the relationship between entering the drug-paired compartment and experiencing conditioned rewarding effects. During conditioning, salient environmental cues are paired with drug administration, but animals are confined to one compartment and therefore restricted from making the response (approach behaviour) that is used to assess reward. They must acquire this behaviour on test day. Whether animals enter the drug-paired compartment by approaching salient cues or through exploratory behaviour, the drug-paired stimuli should elicit conditioned rewarding effects such that the animals' tendency to subsequently approach and maintain contact with them is strengthened.

The fact that animals exhibit a smaller CPP during initial portions of the test could also be due to latent inhibition. Latent inhibition is a process whereby pre-exposure to a stimulus without consequences impairs learning about the stimulus upon subsequent presentations of it. Experimental conditions on the test day resemble those of the habituation day in that tunnels to the compartments are open and no drug is administered. If animals remember that under these

conditions the compartments have no affective valence, their preference for the drug-paired compartment may be delayed until they learn that the previously neutral cues elicit conditioned rewarding effects. The possibility that latent inhibition contributes to the time at which sham lesioned animals express a CPP could be tested by examining the time course of a CPP in animals which were not habituated to the CPP apparatus prior to conditioning.

Entries to Compartments

Most CPP studies do not assess the number of entries that animals make to drug- and saline-paired compartments, but the analyses presented in this thesis suggest that it may be instructive to do so. Although compartment entries are not related to the rewarding value of the stimulus (Bardo, Miller, & Neisewander, 1984; Crowder & Hutto, 1992), the pattern of entries that animals make may reveal factors that contribute to or influence the expression of a CPP.

The finding that a significant number of sham lesioned animals made their first entry to the drug-paired compartment indicates that the drug-paired cues had acquired incentive motivational properties during conditioning sessions. This does not necessarily contradict the hypothesis that animals learn the relationship between drug-paired cues and conditioned rewarding effects on test day. The first compartment entry that animals make probably reflects an unconditioned approach response to conditioned rewarding cues, but this tendency is weak and is only detected with a very large sample. When animals enter the drug-paired compartment (either by approaching conditioned cues or through exploratory behaviour), they presumably experience conditioned reward which reinforces the tendency to approach and maintain contact with the drug-paired stimuli. Even animals who do not make their first entry to the drug-paired compartment will

express a significant preference for the drug-paired compartment over the testing period. The idea that the tendency to enter the drug-paired compartment is increased when animals have learned the relationship between this behaviour and the experience of reward is supported by evidence from an operant place conditioning study (Crowder & Hutto, 1992): When the presentation of the rewarding stimulus is contingent upon animals entering the correct compartment, they are more likely to make their first entry to the drug-paired compartment than if they are conditioned using the standard CPP protocol.

An analysis of the entries to drug-paired versus saline-paired compartments revealed that sham lesioned animals made the same number of entries to the two compartments in the first half of the test. During the second half of the test, however, they made more entries to the drug-paired than to the saline-paired compartment. This finding is consistent with the suggestion that animals learn the relationship between entering the drug-paired compartment and experiencing conditioned reward over the testing period. That is, they begin to enter the drug-paired compartment because they are learning to approach cues that elicit conditioned rewarding effects. The hypothesis is supported by evidence that there is no difference in the number of entries to drug- and saline-paired compartments across a fifteen minute test, but on subsequent test days, animals make more entries to the drug-paired than to the saline-paired compartment (Bardo, Miller, & Neisewander, 1984).

The fact that animals make more entries to one compartment than the other indicates that they can discriminate between the stimuli that differentiate the two compartments. Manipulations that disrupt discriminative abilities, therefore, should block the increased entries to the drug-paired compartment. The same

manipulation may not reduce the size of the preference for the drug-paired compartment because animals could increase the amount of time they spend in the drug-paired compartment on each entry.

As suggested in Experiment 1, the total number of compartment entries over testing is a gross estimate of conditioned locomotor activity. The present analysis revealed that the total number of entries made by sham lesioned animals was reduced in the second ten minutes of testing. Given that increased locomotor activity may cause animals to exit the drug-paired compartment, conditioned hyperactivity could interfere with the expression of a CPP. That is, a high level of activity may reduce the apparent size of preference for the drug-paired compartment. On the other hand, animals conditioned with intra-VTA morphine in Experiment 1 exhibited the highest activity level, but also the largest CPP. Furthermore, manipulations that produce an increase in activity do not block a CPP (Hiroi & White, 1991; McDonald & White, 1993). It is unlikely, therefore, that the increased preference for the drug-paired compartment in the second half of the test is due simply to reduced locomotor activity.

State Dependent Testing

Following morphine administration, sham lesioned animals displayed a significant preference for the drug-paired compartment. Consistent with previous reports (Mucha & Iversen, 1984), the size of the CPP did not vary between drug free and state dependent tests. Nonetheless, the behaviours exhibited by animals in the drugged and undrugged tests differed in two ways. First, the magnitude of preference for the drug-paired compartment did not increase across the state dependent test. Second, when they were tested in a drugged state, sham lesioned

animals made more entries to the drug-paired than to the saline-paired compartment in the first ten minutes of testing.

The differences between drug free and state dependent tests are difficult to interpret because the two tests were not counterbalanced: the state dependent test was always conducted the day after the drug free test. Thus, changes in behavioural responding may be due to the presence of the drug, repeated exposure to the testing environment, or some combination of these two effects. Data from the state dependent test, however, are consistent with the effects of repeated tests (Bardo, Miller, & Neisewander, 1984) in that sham lesioned animals made more entries to the drug-paired compartment in the second CPP test. This finding also supports the suggestion that animals learn to associate entering the drug-paired compartment with conditioned rewarding effects on test day. That is, animals may show a larger preference for, and increased entries to, the drug-paired compartment during initial portions of the second (state dependent) test because they have learned to approach the drug-paired cues during the first (drug free) test. The question of how drug administration and repeated exposure to the testing environment influence the expression of a CPP must be examined by comparing behavioural responding in drug free and state dependent tests that are counterbalanced.

In summary, an analysis of behavioural responding during CPP testing reveals that sham lesioned animals exhibit an increasing preference for the drug-paired compartment during testing. They also make more entries to the drug-paired than to the saline-paired compartment in the latter half of the test. Although the explanations proposed to account for these effects are speculative, the phenomena are robust and have implications that should be considered in CPP

studies. Because the preference for the morphine-paired compartment is larger during the second half of the test, CPP studies that test for only 10 minutes may not detect a CPP when one is present. The duration of testing, therefore, may be a critical variable in determining the results of CPP tests. Furthermore, it may be important to examine the time course of different variables during CPP testing when new drugs are being screened for their rewarding effects.

Behavioural Characteristics of the CPPs Induced by Intra-cerebral Morphine Injections

Results from Experiments 1 and 2 demonstrate that morphine may act at two sites (the VTA and the PAG) to produce a CPP. The behavioural characteristics of the CPPs induced by morphine injections into the VTA and the PAG were examined in Experiment 1. This analysis revealed that animals conditioned with intra-VTA morphine displayed a preference for the drug-paired compartment throughout the 20 minute test, and made more entries to the drug-paired than to the saline-paired compartment during both halves of the test. In contrast, animals conditioned with intra-PAG morphine exhibited a CPP during the second but not the first ten minutes of testing, and their entries to the drug- and saline-paired compartments did not differ during either portion of the test. These findings suggest that the CPPs induced by intra-VTA and intra-PAG morphine injections may be produced via different mechanisms.

Given that systemic morphine administration acts at both the VTA and the PAG, the CPP exhibited by sham lesioned animals should reflect behavioural characteristics of both intra-VTA and intra-PAG morphine groups. This appears to be the case in that, similar to animals conditioned with intra-PAG morphine,

sham lesioned animals exhibited an increasing preference for the morphine-paired compartment during testing, and similar to animals conditioned with intra-VTA morphine, they made more entries to the drug-paired than to the saline-paired compartment.

The notion that intra-VTA and intra-PAG morphine injections produce a CPP via different mechanisms is supported by evidence that morphine injections into the two sites have different effects on motivated behaviours. For example, morphine injections into the VTA increase responding for electrical stimulation of the LH and enhance feeding responses induced by LH stimulation, whereas intra-PAG injections of morphine suppress both responses (Broekkamp et al., 1976; Jenck, Gratton, & Wise, 1986; Jenck, Quirion, & Wise, 1987). Moreover, in most cases, anatomical and behavioural studies do not suggest that the VTA and the PAG share a common function. In addition to its role in opiate-induced reward, the VTA is a part of the neural system that mediates the rewarding effect of stimulants [for review see (Pulvirenti, Swerdlow, Hubner, & Koob, 1991)] and food (Jenck, Gratton, & Wise, 1986; Jenck, Quirion, & Wise, 1987). The VTA has also been implicated in the initiation of forward locomotion (Holmes, Bozarth, & Wise, 1983; Holmes & Wise, 1985; Joyce & Iversen, 1979). A fundamental characteristic of all rewards is their ability to initiate forward locomotion (Glickman & Schiff, 1967) or approach behaviour (Young, 1959). It has therefore been suggested that the rewarding and locomotor activating effects of a stimulus are mediated through the same neural substrates (Glickman & Schiff, 1967; Wise & Bozarth, 1987). Given its role in these functions, the VTA appears to be a component in the neural systems that mediate processes of reward and positive reinforcement (Wise & Rompre, 1989).

In contrast to these results, the PAG does not appear to be involved in the rewarding effect of stimulants (Corrigall & Vaccarino, 1988) or of food (Jenck, Gratton, & Wise, 1986; Jenck, Quirion, & Wise, 1987). Rather, PAG function is associated with defensive reactions (Abrahams, Hilton, & Zbrozyna, 1960; Bandler & Carrive, 1988; Depaulis, Keay, & Bandler, 1992) and behavioural responses to stimuli with an aversive component (Holstege, 1990, 1992). Given that morphine injections into the PAG inhibit or attenuate responses to aversive stimuli (Jenck, Schmidtt, & Karli, 1983; Manning, Morgan, & Franklin, 1994; Mayer & Price, 1976; Pert & Yaksh, 1974; Yaksh, Yeung, & Rudy, 1976), they may produce a preference for the drug-paired compartment because they block the aversive aspects of CPP conditioning. If this is true, morphine injections into the PAG produce a CPP via a mechanism of negative reinforcement.

Morphine injections into the VTA and the PAG may produce a CPP by processes of positive and negative reinforcement respectively, but blockade of opioid receptors in either site eliminated a CPP to systemic morphine (Experiment 2). The finding that opioid-induced activity in both the VTA and the PAG is necessary for the rewarding effect of systemically administered opiates is not necessarily in conflict with the notion that intra-VTA and intra-PAG morphine injections produce a CPP via different mechanisms. Structures which mediate opiate-induced antinociception are connected such that injections of an opioid agent into one site stimulate endogenous opioid release in the other sites (Ma & Han, 1991a, 1991b, 1992; Ma, Shi, & Han, 1992). Processes within the VTA and the PAG that mediate morphine's rewarding effect may have the same relationship to each other. If this is true, opioid-induced activity in the VTA and PAG may

make different, albeit necessary, contributions to the CPP exhibited by animals conditioned with systemic morphine.

Behavioural Characteristics of a Morphine-induced CPP Following Different CNS Lesions

In Experiments 3 and 4, lesions of the PPTg, the PAG, or the fornix blocked the preference animals exhibit for a morphine-paired environment. Lesions that destroyed a significant portion of the VS reduced, but did not eliminate, this effect. When they were tested in a drugged state, animals with PAG or fornix lesions exhibited a CPP. These changes in behavioural responding following different CNS lesions suggest that the mechanisms by which lesions disrupt a CPP may differ. A comparison of the CPPs expressed by lesioned animals with the CPP expressed by sham lesioned animals may provide some insight into this issue.

Animals with large VS lesions exhibited a CPP but, unlike sham lesioned animals, the size of the effect did not increase during testing. Furthermore, VS lesioned animals did not make more entries to the drug-paired than to the saline-paired compartment in either the first or the second half of the test. The fact that animals with VS lesions do not appear to spend more time in, or make more entries to, the drug-paired compartment over the testing period suggests that VS lesions may interfere with the ability to learn the relationship between entering the drug-paired compartment and experiencing conditioned reward. In particular, VS lesions may disrupt the ability to discriminate drug- and saline-paired cues. In Experiment 3, it was suggested that VS lesions attenuate the rewarding properties of the stimulus. The reduction in preference for a morphine-paired compartment following VS lesions could be due to motivational and/or discriminative deficits.

Unfortunately animals with VS lesions did not undergo a state dependent test. It is not known, therefore, whether they would exhibit different behavioural characteristics when tested in a drugged state.

PPTg lesions completely eliminated the preference for a morphine-paired compartment in both drug free and state dependent tests. Because the effects of PPTg lesions were so dramatic, it is difficult to determine how they reduce a CPP. That is, what is the nature of deficit induced by PPTg lesions? The fact that animals with PPTg lesions do not display a CPP when they are tested in a drugged state suggests that they may not be capable of experiencing morphine's rewarding effect. It is also possible that animals with PPTg lesions do not exhibit a CPP because they are unable to discriminate between drug- and saline-paired compartments. The latter suggestion is supported by evidence that PPTg lesions do not affect the level of responding for a conditioned reinforcer, but appear to disrupt the ability to discriminate between stimuli associated with reward and non-reward (Inglis, Dunbar, & Winn, 1994).

The idea that PPTg lesions produce a deficit in discriminative abilities is difficult to defend because animals with PPTg lesions develop a CPP when they are drug dependent or food deprived (Bechara, Harrington, Nader, & van der Kooy, 1992; Bechara & van der Kooy, 1992a, 1992b; Nader, Bechara, Roberts, & van der Kooy, 1994). On the other hand, food deprivation does not eliminate deficits in second order conditioning (Inglis, Dunbar, & Winn, 1994) or performance in the radial arm maze (Dellu, Mayo, Cherkaoui, LeMoal, & Simon, 1991; Lepore, 1993). Although animals with PPTg lesions can discriminate rewarding from neutral stimuli (Inglis, Dunbar, & Winn, 1994), they may be unable to detect subtle changes in reward magnitude. When animals are drug naive or sated and are tested for the

development of a CPP, the differences between conditions of reward and non-reward are small. In these tests, PPTg lesioned animals exhibit deficits. When they are drug dependent or food deprived, however, the affective difference between rewarding and neutral stimuli is amplified. Under these conditions, PPTg lesions animals are capable of discriminating stimuli and will express a CPP.

The initial observation that PAG and fornix lesions block a morphine-induced CPP could be taken as evidence that the two lesions reduce the rewarding effect of the drug. The finding that PAG and fornix lesions animals exhibit a CPP when they are tested in a drugged state argues against this interpretation. Rather, these data indicate that both lesions produce a deficit in cue retrieval. Given that the hippocampus is involved in acquisition of information about the relationship among sensory stimuli (Hirsh, 1974; O'Keefe & Nadel, 1978; Sutherland & Rudy, 1989), fornix lesions may disrupt a CPP because animals are unable to relate information about the external, sensory, stimuli that are part of the CPP apparatus. In contrast, the PAG may be involved in the processing of somatosensory information (Jurgens, 1994; Li, Matsuzaki, Shinonaga, & Mizuno, 1993), and the production of defensive reactions to aversive stimuli (Holstege, 1990, 1992). PAG lesions, therefore, may block a CPP because animals are unable to integrate information about the sensory and affective properties of the conditioned stimuli.

One striking feature of the CPP exhibited by fornix lesioned animals is that 90% of the animals made their first entry to the drug-paired compartment during state dependent testing. In a previous section of this discussion, it was suggest that the first compartment entry reflects an approach response to conditioned cues. The present data appear to indicate that fornix lesions enhance the response. This interpretation is consistent with the contextual retrieval theory of hippocampal

function which suggests that a learning system based on the hippocampus competes with a neural system mediating stimulus-response associations (Hirsh, 1974). The theory explicitly states that in the absence of a hippocampus, behavioural responding is controlled by stimulus-response learning. If this is true, fornix lesioned animals would be more likely to enter the drug-paired compartment because their behaviour is controlled by an approach response to conditioned cues. The fact that animals show an exaggerated approach response to conditioned cues during state dependent, but not drug free, testing may be related to the presentation of the cue (drug administration) which allows animals to retrieve information about conditioned reward and drug-paired stimuli.

The discussion in this section focused on the changes in behavioural responses during CPP testing following different experimental manipulations. The analyses suggest that opioid-induced activity in the VTA and the PAG produce a CPP through different processes, and that lesions of the VS, the PPTg, the PAG, and the fornix reduce a CPP to morphine by disrupting different mechanisms. By inference, each of these structures has a different function in the development of a CPP to morphine. Nonetheless, even if the VTA, the PAG, the VS, the PPTg, and the hippocampus make different contributions to an opiate-induced CPP, these regions do not function in isolation. Rather, they are components of neural systems that mediate processes of reward, reinforcement, learning, and motivation. It is the interaction of these factors which produces the observed preference for a morphine-paired environment.

Neural Mechanisms Underlying a CPP Induced by Morphine

The preceding discussion emphasized that the CPP paradigm is not simply a measure of reward, and that the development of a CPP may be influenced by factors that are not related to reward. Because of these factors, results from CPP studies are difficult to interpret in terms of reward. Nonetheless, experimental evidence from the present set of experiments may provide some insight into the neural substrates which mediate the rewarding effect of morphine.

The findings that intra-VTA or intra-PAG morphine injections produce a CPP indicate that morphine's rewarding effect may be initiated at these sites. Results showing that lesions of the PPTg completely eliminate a CPP to systemic morphine appears to indicate that the PPTg is part of a neural system that mediates opiate-induced reward. The rewarding effect of intra-PAG morphine may be transmitted directly to the PPTg because the PAG sends strong projections to the PPTg (Beitz, 1994). Holstege (1991) has argued that the functions of the PAG and the PPTg are closely linked and that both structures are part of the emotional motor system which originates in the limbic system and controls behavioural responses to stimuli with an affective component. One function of this system is to transmit information from neural sites associated with emotion to motor reflex pathways and, to a limited extent, directly to motor neurons. This implies that the brainstem structures including the PAG and the PPTg mediate the limbic system control of motor output. The possibility that PAG projections to the PPTg mediate opiate-induced reward could be examined by testing whether lesions that disconnect the two structures disrupt the development of a CPP to morphine.

In the absence of anatomical evidence that VTA efferents terminate in the PPTg, it must be concluded that intra-VTA morphine injections produce their

rewarding effect via another route. One possibility is that the effect is mediated through the putative neural circuit of drug reward (Koob & Bloom, 1988), that is via DA projections from the VTA to the VS, VS projections to the VP, and VP projections to the PPTg. In support of this suggestion, a CPP induced by intra-VTA *u* agonist injections is blocked by mesolimbic DA lesions (Shippenberg, Bals-Kubik, & Herz, 1993). These results do not contradict the finding that mesolimbic DA lesions have no effect on a CPP to systemic morphine (Experiment 3). According to the present hypothesis, systemic morphine may produce a CPP through more than one system; destruction of DA projections from the VTA to the VS may not block a morphine-induced CPP because a neural system that includes the PAG and its projections to the PPTg also mediates morphine reward and is capable of supporting a CPP. Similarly, lesions of the VS or the VP may have been ineffective for the same reason. It is important to note, however, that the neural systems mediating opiate-induced reward in either the VTA or the PAG are not completely independent because blockade of opioid receptors in either site eliminated a CPP to systemic morphine (Experiment 2).

The CPP paradigm is often considered to be a simple, effective, and reliable method of assessing the rewarding properties of stimuli (Carr, Fibiger, & Phillips, 1989; van der Kooy, 1987). Despite the fact that the development of a CPP to morphine has been investigated extensively (Beach, 1957; Bechara & van der Kooy, 1989; Bozarth & Wise, 1981b; Katz & Gormezano, 1978; Kumar, 1972; Mucha & Iversen, 1984; Mucha, van der Kooy, O'Shaughnessy, & Bucenieks, 1982; Phillips & LePiane, 1980; Rossi & Reid, 1976; Sherman, Pickman, Rice, Liebeskind, & Holman, 1980; Shippenberg & Herz, 1987; van der Kooy, Mucha, O'Shaughnessy, & Bucenieks, 1982; White, Sklar, & Amit, 1977), neural substrates

and mechanisms underlying the effect have remained elusive. The findings and analyses in this thesis suggest that the lack of consensus may be related to the possibilities that opiate-induced reward is mediated by several neural sites, that behavioural measures of reward are influenced by other physiological states, and that the development of a CPP depends on processes that are not related to reward.

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