THE EFFECTS OF MORPHINE AND NALOXONE ON AVERSIVELY CONDITIONED HEART RATE RESPONSES OF THE RAT

by

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INTRODUCTION

The discovery of opiate receptors and the subsequent isolation of endogenous opioid peptides has generated an enormous amount of research concerning the functioning of the opiate system and its role in behavioral processes. A number of previous studies have made it clear that the endogenous opiate system is involved in the perception of pain (Watkins and Mayer, 1982). Other research has indicated that endogenous opiates have a variety of other functions as well . Recent studies have shown that opiates are involved in memory and learning processes and in the regulation of autonomic function (e.g Feldberg and Wei, 1978; Holaday and Ward, 1982; Mauk, Warren and Thompson, 1982). The purpose of the present set of experiments was to provide new information on the involvement of opiates in the control of classically conditioned and reflex changes in heart rate to aversive stimulation.

Evidence implies the existence of at least three different types of opiate receptor (mu, delta and kappa) and three distinct ligand classes (Lord, Waterfield, Kosterlitz and Hughes, 1977). Two of the ligand classes possess receptor specificities that correspond to a particular receptor type, while ligands of the third class appear to bind to all three receptor types with equal affinity (Lord

et al.,1977).

Ultimately, one would like to assign specific physiological or behavioral functions to each receptor and ligand type. Thus, in a subsequent section, the effects of various opiates on learning and memory are discussed in view of recent evidence that suggests the existence of a functionally diverse endogenous opiate system.

Endogenous Opioid Compounds in the CNS

The discovery of stereospecific opiate binding in the brain led to speculation about the existence of endogenous ligands. Early attempts to isolate ligands by treating brain extracts with antisera raised against morphine met with failure, which suggested that endogenous opioids were chemically distinct from morphine (Goldstein, 1973). However, in the middle 1970's several groups of researchers were sucessful in obtaining crude brain extracts that possessed opiate activity in the guinea pig ileum and the mouse vas deferens. Hughes (1975) and Hughes, Morgan and Fothergill (1975) derived crude extracts from the pig brain that inhibited contractions of muscle twitches in the mouse vas deferens and the guinea pig ileum. The activity of the extract could be antagonised with naloxone, implying that activity at opiate receptor sites was responsible for the extract's effect in the assays. It is interesting to

note that in the mouse vas deferens it took more naloxone to antagonize the effect of the extract's opiate activity than was required to reverse the effects of an equivalent concentration of morphine. In retrospect, it is clear that this was due to the predominance of delta receptors in the mouse vas deferens.

Hughes (1975) found that the brain extract lost all activity when it was treated with carboxypeptidase A, an enzyme that cleaves amino acids from the carboxy-terminal end of proteins. This was strong evidence that the active compound in the extract was a peptide. The primary structure of the endogenous opiate was soon determined by Hughes, Smith and Kosterlitz (1975). The first four amino acid assignments were Tyr Gly Gly Phe. Hughes et al. (1975) were unable to make the assignment of the final amino acid because their sequence data indicated that the last amino acid was either Leu or Met. Hughes et al. (1975) synthesized Tyr Gly Gly Phe Met and Tyr Gly Gly Phe Leu and found that both peptides exerted opiate activity in the mouse vas deferens assay. In addition, the mass spectrum of a mixture of met- and leu-enkephalin matched the spectrum obtained from the purified extract. Hughes et al. (1975) pointed out that the pituitary hormone beta-lipotropin (beta-LPH) contained the met-enkephalin sequence at its amino-terminal end. The C-fragment of beta-LPH which is

called beta-endorphin, was soon found to be at least as active as met-enkephalin in the mouse vas deferens assay. The larger peptide was isolated from the anterior and intermediate pituitary and was also found within the CNS (Miller and Cuatrecasas, 1978).

The two enkephalins isolated by Hughes et al. (1975) were found to be heterogeneously distributed within the CNS of the rat. This was clear before their structures had been elucidated, since Hughes (1975) showed that extracts obtained from different regions of the rat brain had different activities in the mouse vas deferens. Hughes (1975) found that extracts from the striatum possessed the most opiate activity and that the mid-brain, the pons and the medulla all exhibited a moderate amount of opiate activity. The hippocampal and the cerebellar extracts were without significant opiate activity.

Immunocytochemical methods have been employed to map the distribution of the enkephalins in the CNS. In general, there is overlap between the distribution of the enkephalins and opiate receptors in the brain, but there are a number of exceptions. In the rat, enkephalin-like immunoreactivity was present in the substantia gelatinosa of the Vth nerve, the nucleus tractus solitarius, the area postrema and the nucleus ambiguus (Simantov, Kuhar, Uhl and Snyder, 1977). In the brainstem there were reactive fibers in the locus

coeruleus, near the floor of the fourth ventricle and more anteriorly, within the parabrachial nucleus. In the thalamus, enkephalin-like immunoreactivity was localized in the mid-line nuclei and in the medial thalamic nucleus in particular. Heavy staining was also detected in the globus pallidus, while staining was much less intense in the caudate-putamen (Simantov et al., 1977).

Sar, Stumpf, Miller, Chang and Cuatrecasas (1978) used antisera raised against met-enkephalin and leu-enkephalin to map their distributions within the CNS. For the most part, the distributions of the two peptides overlapped. There was heavy staining in the substantia gelatinosa of the spinal cord, the amygdala, the globus pallidus, the CA2 region of the hippocampus, the periventricular nucleus, the periaqueductal gray, the locus coeruleus, the nucleus of the solitary tract, the dorsal motor nucleus of the vagus and the nucleus commissuralis. It is interesting to note that the caudate-putamen which contained a high density of opiate receptors, exhibited little enkephalin-like immunoreactivity. This was explained by the presence of enkephalinergic terminals in the caudate-putamen that originated in the globus pallidus. The central nucleus of the amygdala, which contains immunopositive cell bodies, projected enkephalinergic fibers to the interstitial nucleus via the stria terminalis (Uhl,

Kuhar and Snyder, 1978).

The presence of enkephalin, as well as opiate receptors in the medial thalamus, the periaqueductal gray and the periventricular region of the thalamus supports the idea that enkephalin is involved in the inhibition of nociception, which is consistent with other work that has shown that stimulation or the application of opiate agonists into these sites produces analgesia (Jacquet and Laschka, 1976).

The presence of the enkephalins in a number of medullary nuclei (Sar et al., 1978) suggests that the enkephalinergic system is involved in the regulation of autonomic activity. Several investigators have suggested that the presence of opioid peptides and opiate receptors in the amygdala and the hippocampus imply a role for these structures in the mood altering effects of opiates (e.g. Rodgers, 1978; Simantov et al., 1976).

Evidence for the Existence of Three Opiate Systems

Although beta-endorphin contains the met-enkephalin sequence at its amino terminal end, the distributions of met-and leu-enkephalin do not appear to be related to the distribution of beta-endorphin in the CNS (Watson, Akil, Richard and Barchas, 1978). In the years that immediately followed the isolation and characterization of the opiate

peptides it was suggested that beta-endorphin was the precursor for met-enkephalin, and that a peptide similar to beta-endorphin was the precursor for leu-enkephalin. This hypothesis was questioned because beta-endorphin could not be detected in a number of CNS sites that were known to contain the enkephalins. Another speculation was that the enkephalins were derived from fragments of beta-endorphin-like precursors that made their way from the pituitary to the CNS via the circulation. The fact that hypophysectomy had no effect on CNS levels of met- or leu-enkephalins appeared to rule out this hypothesis (Miller and Cuatrecasas, 1978). These discrepencies were cleared up by the application of nucleic acid sequencing methods to the study of opioid peptide precursors.

It has been shown that mRNA derived from pituitary cells codes for a large precursor containing the sequences for ACTH, alpha MSH, and beta LPH which contains the beta-endorphin sequence (Nakashani, Inoe, Kita, Nakamura, Chang, Cohen and Nama, 1979). This molecule, called pro-opiomelanocortin (POMC) did not contain the sequence for leu-enkephalin. In subsequent experiments by the same group, the primary structure of the precursor of met- and leu-enkephalin was deduced using purified mRNA from bovine adrenal cells. The precursor, which was named proenkephalin A, encoded for five molecules of met-enkephalin and for one

leu-enkephalin molecule. The met- and leu- enkephalin residues were flanked by basic amino acids which are believed to be probable sites of cleavage for a trypsin-like enzyme (Gubler, Kilpatrick, Seeburg, Gage, and Udenfriend, 1982; Noda, Furatani, Takahashi, Toyosato, Hirose, Inayama, Nakanishi and Numa, 1982). These experiments have firmly established the existence of separate precursors for beta-endorphin and the enkephalins and explained why their distributions failed to overlap in the CNS.

Recently, proenkephalin B, the precursor of dynorphin and alpha neo-endorphin, has been isolated and characterized (Kakadani, Furutani, Takahashi, Noda, Morimoto, Hirose, Asai, Inayama, Nakanishi and Numa, 1982). Peptides that derive from proenkephalin B exhibit pharmacological activity that is characteristic of kappa receptor agonists (Corbett et al., 1982).

Taken together, the morphological, pharmacological and molecular biological evidence suggest that there may be three distinct endogenous opiate systems. There are three different opiate peptide classes and each class has its own idiosyncratic receptor specificity. A reasonable working hypothesis is that each of the three opiate peptide and receptor types has a distinct function. The enkephalins are specific for the delta-receptor, whereas alpha-neo-endorphin and dynorphin are specific to the kappa receptor. Beta

endorphin is somewhat less specific, in that it binds with equal affinity to the mu, the delta and the kappa receptor. It seems likely that there may be a specific physiological function for each type of opiate and opiate receptor. In the next section evidence that suggests the existence of three distinct types of opiate receptor is discussed.

Opiate Receptors

About ten years ago stereospecific opiate receptors were found in the brain of the rat (Pert and Snyder, 1973). Labelled naloxone bound to the membrane fraction of rat brain homogenates and was displaced by levo- but not dextro-isomers of various opiate agonists (Pert and Snyder, 1973). Autoradiographic methods were subsequently employed (Atweh and Kuhar, 1977a, 1977b, 1977c; Herkenham and Pert, 1982) to map the distribution of opiate receptors within the mammalian central nervous system (CNS). In general, there was a positive correlation between sites that bound labelled opiate ligands and sites at which morphine or electrical stimulation produced analgesia. This supported the idea that opiate receptors mediated the analgesic effects of opioid alkaloids.

In the rat, the periventricular gray, the substantia gelatinosa of both the (spinal) trigeminal nucleus and the spinal cord, the caudate-putamen, and the medial nucleus of the thalamus all contained high to moderately high densities

of opiate receptors (Atweh and Kuhar, 1977a, 1977b, 1977c; Herkenham and Pert, 1982; Pert, Kuhar and Snyder, 1975;).

The microinjection of morphine in the caudate-putamen or the periaqueductal gray (PAG) of the rat produced analgesia in the rat, as measured by a number of behavioral tests (Jacquet and Lajtha, 1976; Lewis and Gebhart, 1977; Sharp, Garnett and Cicero, 1974). These findings suggested that the PAG was a major site of analgesic activity for both morphine and electrical stimulation (Jacquet and Lajtha, 1976; Liebeskind, Mayer and Akil, 1974; Pert and Walker, 1976). Other areas in the CNS were found to contain high densities of opiate receptors, but appeared not to be involved in nociception. For example, opiate receptors have been localized in the dorsal motor nucleus of the vagus and the nucleus ambiguus (Atweh and Kuhar, 1977b). In addition, opiate receptors have been localized in the following sites which have no apparent role in the anti-nociceptive effects of opiates: the nucleus tractus solitarius; the superior colliculus; the ventral nucleus of the lateral geniculate; the amygdaloid complex; the subfornical organ and areas adjacent to the anterior olfactory nucleus (Atweh and Kuhar, 1977a,b; Herkenham and Pert, 1982).

The fact that opiate receptors are found in areas of the mammalian CNS that appear not to be directly involved in the perception of pain suggests that opiates have numerous

physiological and behavioral effects that are independent of their analgesic activity. It seems probable that the presence of opiate receptors in medullary nuclei may account for some of the effects of opiates on autonomic function, and that opiate binding in the limbic system may help explain the effects of opiates on behavior and emotionality.

The idea that certain regions of the CNS are involved in the analgesic effects of opiates, while other regions are involved in the modulation of emotional behavior was supported by the findings of Gallagher, Kapp, McNall and Pascoe (1981) who have shown that the injection of levorphanol in the caudate nucleus, which produces analgesia in the rat (Jacquet and Lajtha, 1973), had no effect on aversively conditioned heart rate (HR) responses in the rabbit. Levorphanol did, however, attenuate HR conditioned responses (CRs) when it was injected into the central nucleus of the amygdala. Rodgers (1978) and Gallagher et al. (1981) suggested that opiate receptors in the central nucleus of the amygdala may be involved in opiate effects on learned and unlearned behaviors in a way that is not directly related to pain perception.

Opiate receptors in the nucleus tractus solitarius (NTS) have been shown to be located pre-synaptically on primary visceral afferents. This suggested that opiates can

influence afferent input to autonomic nuclei through a presynaptic mechanism (Atweh, Murrin and Kuhar, 1978). The presence of opiate receptors in the NTS may account for the action of opiates on cough reflexes and respiration (Atweh, Murrin and Kuhar, 1978). Recent evidence suggests that morphine produces bradycardia in the rat by acting at the mu-receptor (Holaday and Ward, 1982). Given the presence of pre-ganglionic cardio-inhibitory fibers in the dorsal motor nucleus of the vagus and the nucleus ambiguus (Nosaka, Yamamoto and Yasunaga, 1979), and the abundance of opiate receptors in each nucleus, it seems probable that opiates produce bradycardia by exerting their effects at one of these sites.

In summary, the above studies indicate that opiate receptors are not homogeneous in terms of physiological functions. There are well defined areas in the rodent CNS where opiate receptors clearly mediate the anti-nociceptive activity of opiate agonists. In addition, there are other sites at which opiates exert behavioral and autonomic effects apparently unrelated to pain perception.

Opiate Receptor Sub-Types

Recent evidence suggests that there may be at least three sub-types of opiate receptor: the mu-receptor, the delta-receptor and the kappa-receptor. For example, Martin, Eades, Thompson, Huppler and Gilbert (1976) have shown that

three different types of opiate alkaloid produce qualitatively different autonomic and behavioral effects. Compounds related to morphine produce inhibition of a flexor reflex, pupillary constriction, behavioral suppression and a biphasic tachycardia-bradycardia in the dog. Compounds related to ethylketocyclazocine (EKC) produce pupillary dilation and sedation, while compounds related to nalorphine produce manic behavior and tachycardia (Martin et al. 1976). Martin et al. (1976) suggested that the three different classes of opiate agonists acted at three different opiate receptors: the mu-receptor (morphine); the kappa receptor (EKC); and the sigma receptor (n-allylnorcyclazocine).

Additional evidence for the existence of multiple opiate receptors has come from experiments which have compared the activities of opiate agonists in the mouse vas deferens and the guinea pig ileum bioassays (Corbett, Paterson, McKnight, Magnan and Kosterlitz, 1982; Lord et al. 1977). To the extent that opiate receptors are homogeneous in the two systems, the rank order of activities of a series of opiate agonists should be the same in each system. However, if opiate receptors are different in the two systems then rank order activities of a series of opiates would be different (Lord et al., 1977). It has been shown that electrically stimulated contraction of muscle derived either from the mouse vas deferens or the guinea pig ileum

is inhibited by the presence of morphine in the incubation medium. The activity of morphine in either system can be antagonized by naloxone, implying that the inhibitory effect of morphine on muscle contraction is mediated by an opiate receptor.

Hutchinson, Kosterlitz, Waterfield and Terenius (1975) have shown that benzomorphans behave differently from morphine in the mouse vas deferens and the guinea pig ileum assays. The benzomorphans were more active in the guinea pig ileum than in the mouse vas deferens. Hutchinson et al. (1975) suggested that relative to the mouse vas deferens, the guinea pig ileum contains more receptors that were specific for benzomorphans than the mouse vas deferens. In a subsequent experiment, Lord et al. (1977) have shown that the enkephalins (see Table 1) displayed an activity profile in the mouse vas deferens and the guinea pig ileum that was different from the activity profile exhibited by morphine, supporting the idea that the opiate receptor populations in the two systems were heterogeneous. The activities of met- and leu-enkephalin in the mouse vas deferens were antagonized by naloxone, only when the naloxone concentration was increased seven-fold over the concentration that was required to reverse the effects of morphine. In the guinea pig ileum naloxone antagonized the effects of the enkephalins and normorphine with equal

Table 1. The primary structure of common opioid peptides.

BETA-ENDORPHIN

Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr Leu Phe Lys Asn Ala Ile Ile Lys Asn Ala His Lys Lys Gly Gln OH

ALPHA-ENDORPHIN

H Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr OH

GAMMA ENDORPHIN

H Tyr Gly Gly Phe Met Thr Ser Glu Lys Ser Gln Thr Pro Leu Val Thr Leu OH

METHIONINE ENKEPHALIN

Tyr Gly Gly Phe Met

Table 1. (Continued)

LEUCINE ENKEPALIN

Tyr Gly Gly Phe Leu

ALPHA NEO-ENDORPHIN

H Tyr Gly Gly Phe Leu Arg Lys TyrPro Lys OH

DYNORPHIN

H Tyr Gly Gly Phe Leu Arg Arg Ile Arg Pro Lys Leu Lys Trp Asp Asn Gln OH

efficacy. However, the effect of EKC on muscle contraction in the guinea pig ileum was antagonized by a four-fold increase in the concentration of naloxone. Thus, it appeared that the enkephalins and morphine acted at the same receptor in the guinea pig ileum, but that another receptor, which Martin et al. (1976) called the kappa-receptor, mediated the effects of compounds of the EKC class.

Lord et al. (1977) also found that 3H-naloxone was more readily displaced from rat brain membranes by morphine than by either methionine or leucine enkephalin. Conversely, met- and leu-enkephalin were more active in displacing 3H-leu-enkephalin from membranes than in displacing morphine. This suggested that the guinea pig brain possessed separate receptors for morphine and the enkephalins. Lord et al. (1977) have called the putative enkephalin receptor the delta-receptor.

Recent evidence indicated that the rabbit vas deferens primarily contains kappa receptors because delta and mu agonists were inactive in this system except at very high concentrations (Corbett, et al., 1982; Oka, Negishi, Suda, Matsumiya, Inazu and Ueki, 1981). The opiate peptide dynorphin (see Table 1) appears to be the endogenous ligand for the kappa receptor, since it inhibited contractions of the rabbit vas deferens and had little biological activity in the rat vas deferens which has few kappa receptors

(Corbett et al., 1982).

The enkephalins and the opiate alkaloids exhibit different regional binding patterns in the rat CNS. Chang, Cooper, Hazum and Cuatrecasas (1979) have shown that D-ala-leu-enkephalin, a metabolically stable analog of leu-enkephalin, binds with greater affinity to membranes derived from the frontal lobe and the striatum than to tissue homogenates derived from the brainstem, the thalamus and the hypothalamus. Morphine displays an opposite binding pattern which suggests that the frontal lobe and the striatum contain relatively more delta-receptors than the thalamus, the hypothalamus or the brain stem. The kappa receptors are localized in the deep layers (V and VI) of the guinea pig cerebral cortex and it has been suggested that the kappa receptor mediates the sedative effects of EKC-like compounds (Goodman and Snyder, 1982).

In summary, it may be noted that there are four types of evidence in favor of the existence of multiple opiate receptors: (1) different opiate agonists possess different behavioral profiles (Martin et al., 1976); (2) opiate agonists have different activity profiles when tested in parallel in the guinea pig ileum and the mouse vas deferens assays; (3) different agonists have different binding activities in brain membrane preparations; (4) naloxone (mu receptor), enkephalin analogs (delta receptor)

and benzomorphans (kappa receptor) exhibit different binding distributions in the CNS.

Opiate Alkaloids and Opioid Peptides: Behavioral Effects

It is perhaps the primary aim of behavioral pharmacology to show that a compound's behavioral effects are related to its activity at a specific receptor class. The development of relatively specific opiate agonists within the last few years has allowed initial investigations of opiate receptor involvement in learning and memory. It should be kept in mind that although the opiate agonists exhibit specific receptor binding in vitro, their specificities are not absolute. Thus, mu receptor agonists will bind to delta receptors, as well as to mu receptors, particularly when the concentration of the mu-receptor agonist is high. Therefore, the effects of an agonist on behavior can rarely be attributed to a single receptor-type. This type of analysis will have to await the development of highly specific opiate antagonists that are specific for each receptor type. Peripherally administered peptides probably act at all three opiate receptor types, and their behavioral effects may result from complex interactions among the three different receptor systems.

Active Avoidance. When a drug is administered prior to a learning trial or a series of learning trials, it is difficult to determine whether any behavioral effects

that might occur were due to its influence on memory, motivation or on the performance of the response. Thus it is not possible to demonstrate unequovically that an opiate has an effect on learning when it is administered prior to multi-trial learning.

Nevertheless, a number of reports indicate that morphine and related opiates can attenuate aversively motivated CRs, and this result is usually interpreted as a direct drug effect on learning. Given the known analgesic and the sedative effects of opiate alkaloids in humans, it is not suprising that these compounds exert an influence on aversively motivated learned behaviors in animals. In some of the studies outlined below, the most straightforward explanation of morphine's adverse effects on a learned response was that it reduced the noxiousness of the aversive reinforcing stimulus (US) , thereby retarding the development of the learned response.

Effects on established avoidance responses. In the rat, morphine impaired an established wheel turning avoidance response in a dose dependent manner. Morphine was injected after rats made 155 avoidance responses in 160 consecutive trials. (Verhave and Owen,1958). A dose of morphine (6.5 mg/kg, sc) that has been found to be analgesic in a tail-flick test (Dewey and Harris, 1975) did not affect avoidance responding, but higher doses (10 mg/kg and 20

mg/kg) impaired avoidance performance (Verhave and Owen,1958). The effect of morphine on avoidance behavior was also time-dependent. The performance decrement occurred within 20 to 40 min after administration and lasted for up to 2 hrs (Verhave and Owen, 1958).

Morphine also impaired the performance of an established pole jump avoidance response in a dose dependent manner (Cook and Weidley, 1957). In this experiment, which is widely cited as evidence of direct opiate involvement in learning, doses of morphine (4 mg/kg to 16 mg/kg) interfered with conditioned avoidance behavior, but did not affect escape from shock on trials in which the shock and the tone were presented simultaneously. On this basis, the authors argued that morphine did not alter sensitivity to shock. They did not conduct an independent test of morphine's analgesic activity, however, morphine-produced analgesia could not account for poor performance of the avoidance response on the first trial because rats had not yet experienced shock while under the influence of morphine. Thus, morphine had an effect on the performance of avoidance behavior that appeared to be unrelated to its analgesic effects. On later trials, after shock had been delivered, the avoidance decrement could have been due, in part, to the drug's analgesic effects. The results obtained by Cook and Weidley (1957) suggest that morphine can impair the

retention or performance of an established avoidance response in a manner that is somewhat independent of its analgesic activity

In a study by Domino, Karoly and Walker (1963) dogs were trained until they made 19 out of 20 successful avoidance responses in a session, and then were given 200 extinction trials. Domino et al. (1963) reported that the avoidance CR was highly resistant to extinction and that morphine (2, 4, and 8-mg/kg) failed to have any significant effect on the response 1 to 2 hr after it was injected.

Opiate effects on the development of avoidance behavior. A number of experiments have shown that morphine can attenuate the development of conditioned avoidance behaviors when it is administered prior to the first trial of a conditioning session. Banerjee (1971) demonstrated that the injection of as little as .25 mg/kg, sc of morphine disrupted the development of a pole jump avoidance response. Banerjee reported that the same dose of morphine did not alter responses to tail-pinching, but the relevant data were not presented. Others, however, have shown that doses of morphine below 1 mg/kg have little analgesic activity in the rat (Dewey and Harris, 1975). Therefore, it seems possible that the effect of morphine on the pole jump response found by Banerjee (1971) was probably not due to the drug's analgesic effect.

The effects of delta and kappa agonists on active avoidance responding are more difficult to interpret than the effects of classical opiate alkaloids. Leu-enkephalin, which is a delta receptor agonist, impaired the acquisition of a one-way active avoidance response when injected (400 ug/kg, ip.) prior to a series of avoidance trials. The peptide appeared specifically to affect learning and not performance (Rigter, Jensen, Martinez, Messing, Vasquez, Liang and McGaugh, 1980a). Rats in the experimental group were trained on Day 1 until they made at least one avoidance response and then were returned to their home cages. On Day 2, the experimental group was split into two subgroups and one subgroup was injected with saline, and the other was injected with peptide. Both subgroups were given eight additional avoidance trials. A yoked control group received an equivalent number of shocks on Day 1, but the rats were not allowed to make any avoidance responses. On Day two the yoked control group was split and injected with saline or peptide and then given eight avoidance trials. Leu-enkephalin impaired the avoidance performance of rats in the yoked control group, but not of the experimental group. The authors argued that if the peptide was affecting only performance, then the avoidance behavior of both the experimental and yoked groups should have been disrupted. Apparently, the experimental group learned the avoidance

response on the first day of training, and performance or retention of the response was not affected by the presence of the peptide on Day 2.

The results obtained by Rigter et al. (1980a) suggest that the enkephalins can alter the ability of the rat to learn an aversively motivated response. The doses of peptide used by Rigter et al. (1980a) had no effect on flinch-jump thresholds to shock, implying that the peptide's effects were not due to an alteration of shock sensitivity.

Met-enkephalin, which is a delta agonist, also impaired the acquisition of a shuttle avoidance response in Fischer F344 rats when it was administered 5 min before the first learning trial (Rigter, Hannan, Messing, Martinez, Vasquez, Jensen, Veliquette and MaGaugh, 1980b). Met-enkephalin did not alter escape latencies on the first learning trial or have any effect on inter-trial shuttling. Rigter et al. (1980b) concluded that met-enkephalin had no effect on shock sensitivity or on motor activity. The effect of leu-enkephalin was naloxone-reversible suggesting that leu-enkephalin impaired the conditioned avoidance response at the classical mu-receptor. Naloxone, in the absence of any opiate, failed to affect avoidance behavior over a wide dose range (1 mg/kg to 100 mg/kg). This finding implies that endogenous opiate activity at the mu-receptor

does not affect avoidance behavior in the situation employed by Rigter et al. (1980b).

Endogenous opiate agonists related to beta-lipotropin (beta-LPH), which bind to the mu, the delta and the kappa receptors with equal affinity (Lord et al, 1977) also influence the expression of active avoidance behaviors. Beta-endorphin facilitated the extinction of pole jump avoidance when it was injected 2 hr prior to the extinction session (DeWied, Bohus, van Ree and Urban, 1979; LeMoal, Koob, and Bloom, 1978). Alpha-endorphin enhanced avoidance responding during a block of extinction trials that was given 2 hr after the peptide was injected. Alpha-endorphin had no effect on 4 hr after it was injected (DeWied et al.,1979; LeMoal, Koob and Bloom, 1979). Morphine, in extremely small systemic doses (3 ug and 60 ug) delayed extinction, while naloxone facilitated extinction of the avoidance response. DeWied et al. (1979) suggested that the endorphins and morphine enhanced the retrieval of the avoidance response during extinction.

The results and conclusions of DeWied et al. (1979) are in contrast to findings from experiments in which opiates were found to impair aversively conditioned CRs (e.g. Cook and Weidley, 1957; Gallagher et al. 1981; Mauk et al. 1982). It should be pointed out that the associative mechanisms that are involved in the extinction of learned

behaviors are not well understood. There is no clearcut relationship between resistance to extinction and associative strength (Mackintosh, 1974). For example, extinction of a response may require an animal to learn new contingencies between stimuli and reinforcers. From this point of view, the retardation of extinction by morphine and beta-endorphin might be due to the impaired ability of animals to learn the new contingencies.

Passive Avoidance. A number of investigators have employed a single trial passive avoidance task in which opiates are administered after the learning trial in order to assess their effects on memory consolidation. The passive avoidance paradigm allows an experimenter to differentiate between a drug's possible effects on performance and its effects on memory. In this paradigm, retention tests are administered at various intervals after the drug is given. If a drug that is given after a learning trial reduces the subsequent retention of the response, then the drug may be considered to have affected memory consolidation. In experiments of this type, it is important to demonstrate that the treatment effect is dependent on the interval between the learning trial and drug administration. Most theoretical treatments of memory consolidation assume that consolidation occurs during a short interval after the learning trial (McGaugh and Stevens, 1971). For example,

one would expect that a drug given 1 min after the learning experience would have a greater effect on retention performance than a drug given 6 hr later. However, this expectation is based on the assumption that the interval between the administration of the drug and the test for retention is sufficiently long for the drug to be metabolically eliminated in the 6-hr group. If this condition is not met, then it is possible that a 6-hr group could show a performance decrement because the drug had not been eliminated at the time of the test for retention.

In Fischer 344 rats, morphine (1 mg/kg and 3 mg/kg, ip) impaired passive avoidance 72 hrs after it was injected (Messing, Jensen, Vasqez, Martinez, Spiekler and McGaugh, 1981). Since it is unlikely that morphine was still present 72 hr after its administration, these findings imply that morphine can interfere with memory consolidation. However, Messing et al. (1981) did not test the time dependency of morphine's effects, so it is possible (but unlikely) that morphine exerted a proactive effect on retention performance.

Alpha-endorphin enhanced the performance of passive avoidance at 24 and 72 hr after the initial learning trial, when it was administered 1 hr after learning or 1 hr before the retention test (Kovacs, Bohus and DeWied, 1981). One interpretation of this outcome is that alpha-endorphin

enhanced memory consolidation and retrieval.

By contrast, gamma-endorphin and des-tyrosine-gamma endorphin interfered with a passive avoidance response if injected 1 hr after learning or 1 hr before the retention test (Kovacs, Bohus and DeWied, 1981; Kovacs and DeWied, 1981). As DeWied et al. (1978) have noted, the opposite effects of gamma and alpha endorphin on avoidance performance are surprising because their primary structures differ by only a single amino acid.

The effects of alpha- and gamma-endorphin on learned behaviors may depend upon the reinforcement and/or motivational variables present in an experimental situation. For example, LeMoal et al. (1981) have shown that both alpha- and gamma- endorphin increased the rate of extinction in rats that were conditioned to run for a water reward. In avoidance conditioning studies, alpha-endorphin enhanced performance and gamma-endorphin interfered with performance. The fact that the direction of the effects of alpha and gamma endorphin on behavior may depend upon the nature of the reinforcer is one argument against the idea that opioid peptides affect performance by some general influence on learning or memory.

A number of experiments by Rigter have provided mixed results regarding the effects of delta receptor agonists on the performance of learned behaviors. Rigter

(1978) has shown that both met- and leu enkephalin reverse the effects of CO2 amnesia in the single trial passive avoidance paradigm. Peptides were effective in preventing or reversing amnesia if they were administered prior to the single acquisition trial or prior to the retention test. The anti-amnesic effects of the two peptides could not be reversed with naloxone, indicating that their effects were probably not mediated by the mu receptor.

Orienting Behaviors. A crucial question is whether morphine impairs some general learning process, or whether its effects are limited to behaviors that are aversively motivated. Izquierdo (1979) argued that morphine can impair memory in a situation that involves no painful stimulation. In this study, rats were given a series of tone presentations and their orienting responses (ORs) to the tone were recorded. Immediately after this session, an experimental group was injected with morphine and a control group was given saline. Twenty-four hrs later, the experimental group exhibited more ORs than the saline control group. These results suggested to Izquierdo (1979) that memory for tones was impaired in the morphine group. Izquierdo (1979) also found that a naloxone group showed fewer ORs on Day 2 (i.e. enhanced memory) when naloxone was administered after the first series of tone presentation. Izquierdo (1979) suggested that this indicated that the

release of endogenous opiates may attenuate memory consolidation.

Classical Conditioning. In a recent experiment involving rabbits, Mauk, Warren and Thompson (1982) have shown that intravenous (i.v.) injection of 5-mg/kg of morphine, administered after a nictitating membrane CR had been established, blocked the performance of the response. The same dose had no effect on nictitating membrane unconditioned responses to the airpuff US. Because morphine blocked the CR but not the UR Mauk et al. (1982a) argued that morphine interfered with the expression of the CR in a manner that was independent of its effects on nociception or on motor behavior. They suggested that the nictitating membrane CR was tied to fear and that morphine reduced the fear state supporting the CR. The interfering effect of morphine was reversible with naloxone suggesting that the action of morphine was mediated by the mu-receptor.

Mauk et al. (1982a) also showed that morphine given after a nictitating membrane CR had been established blocked a conditioned increase in single unit activity in the CA2 area of the hippocampus, which in previous experiments had been shown to mirror the development of the membrane CR. Mauk, Madden, Barchas and Thompson (1982) have also shown that the injection of morphiceptin, a potent opiate peptide that is specific for the mu-receptor, into

the cerebral acqueduct also blocked the peformance of an established nictitating membrane response within 5 min of injection. The effect of morphiceptin was blocked by the prior administration of naloxone (Mauk et al., 1982 b).

In a subsequent study (Lavond, Mauk, Madden, Barchas and Thompson, 1982), the central injection of morphiceptin into the IV ventricle, impaired the performance of an established decelerative conditioned HR response in the rabbit. This supported the suggestion that opiates interfere with the peformance of aversively motivated behaviors by reducing conditioned fear (Lavond et al. 1982). Lavond et al. suggested that one possible site of activity for mu receptor agonists is the periaqueductal gray, a site that may be involved in the analgesic activity of opiates (Jacquet and Lajtha, 1976).

In the rabbit, microinjections of levorphanol, a mu receptor agonist, into the central nucleus of the amygdala impaired the development of an aversively motivated heart rate (HR) response, but did not have any reported effect on unconditioned HR responses (Gallagher, Kapp, McNall and Pascoe, 1981). The fact that dextrorphan, a biologically inactive enantiomer of levorphanol, failed to have any effect on HR CRs, and that the effect of levorphanol was reversible by naloxone implied that levorphanol disrupted HR conditioning through its action at an opiate receptor.

Naloxone, in the absence of any other treatment, increased the magnitude of conditioned HR CRs, an effect which implies that the release of endogenous opiates can influence the development of aversively motivated HR CRs in the rabbit.

In summary, a number of experiments have shown that morphine can impair the performance of aversively motivated behaviors. This outcome is usually interpreted as being the result of a direct effect of the drug on a memory or learning process that is not related to morphine's known analgesic effects. It should be pointed out however, that the response decrements in a number of experiments were such that analgesia could not be ruled out as a factor. Thus, in some cases, the performance of the CR may have been impaired because morphine-produced analgesia reduced the reinforcing properties of the aversive stimulus, thereby retarding learning.

Opiate Receptor Sub-types and Learned Behaviors

The role of the mu receptor in aversively motivated learned behaviors appears to be relatively straightforward. Morphine almost invariably interferes with the performance of aversively motivated behaviors. In those situations in which a mu-receptor agonist is administered prior to behavioral testing, the effect of the drug often appears to be related to its analgesic effect. However, the fact that morphine interferes with the retention of a passive

avoidance response when it is injected shortly after learning trials (Messing et al., 1981), indicates that morphine may exert its effects in a manner unrelated to its analgesic effects or its performance effects.

Given the inconsistent effects of the opioid peptides on the performance of a number of learned behaviors, few generalizations can be made about the role of delta and kappa receptors in learning and memory processes. One problem is that the opiate peptides may bind to all three receptor types when injected systemically. Therefore, their behavioral effects may be the result of complex interactions among the three receptor types. In addition, the behavioral effects of the opiate peptides depend upon motivational factors, the type of learning task employed, genetic variables, and the route of drug administration. A clearer picture of the involvement of the kappa and delta receptor systems in learning and memory will have to await the development of specific antagonists for each receptor type, the standardization of behavioral tasks, and a more fully developed analysis of learning and memory processes.

Aim of the Study

The purpose of the present study was to provide new

information on the learning and performance effects of morphine and naloxone on a classically conditioned HR response. A classical conditioning paradigm offers a number of advantages over instrumental learning situations that have usually been employed to study the effects of opiates on learned behavior. An important aspect of a classical conditioning paradigm is that it provides the experimenter with a great deal of control over stimulus events that are presented to subjects. This feature is particularly important when one wishes to distinguish between a drug's effects on performance of a learned response from a drug's effect on learning. In an instrumental situation, a drug induced performance deficit could alter the number of reinforcers received by a drug group relative to a control group. In the classical conditioning situation groups are equated in terms of their reinforcement histories despite possible differences in performance of a learned response.

The heart rate conditioning paradigm employed in the present experiment has a number of features that make it useful in examining drug effects on learning and performance. First, factors influencing the HR CR of the restrained rat have been well characterized by Fitzgerald and coworkers (Fitzgerald, 1976; Fitzgerald and Hoffman, 1976; Fitzgerald and Martin, 1971; Fitgerald, Martin and O'Brien, 1973; Fitzgerald and Tyler, 1970). It seemed

reasonable to believe that the use of a well characterized learning situation would make the interpretation of drug effects on HR CRs less difficult. Secondly, the HR CR is rapidly established in normal rats within 10-15 CS-US pairings. This feature was important because it eliminates the possible confounding effects of drug tolerance and withdrawal that can arise when drug treatments and conditioning sessions are repeated over a series of days (e.g. Markowitz et al., 1976; Verhave et al., 1958). Finally, a number of regions in the CNS that may be involved in the HR CR have been shown to contain opiate receptors. Thus, it was expected that morphine or naloxone would exert some effect on the development or performance of HR CRs.

An additional, more general aim of the present dissertation was the development of a model system for the study of the learning and perfomance effects of opiate alkaloids and opioid peptides. Such a model might be useful in elucidating the role that each receptor sub-type plays in learning and memory processes. Of course, as was discussed in a previous section, this goal will have to await the development of highly specific opiate antagonists.

EXPERIMENT 1

Rationale

Although a number of previous experiments have demonstrated that peripheral injections of morphine can influence established avoidance behaviors, the effects of morphine on the development of classically conditioned HR responses of the rat have not been examined. The purpose of Experiment 1 was to determine if morphine had an effect on the HR CR of the restrained rat. Three doses of morphine (.25, 5 and 10 mg/kg) were employed in order to establish a rough dose-response relationship. These doses were chosen on the basis of previous work showing that doses of morphine within this range impair a variety of aversively motivated behaviors. A saline group served as an injection control. Both morphine and saline were injected prior to preconditioning presentations of the CSs. No attempt was made to distinguish between the learning and performance effects of morphine in the first experiment.

Method

Subjects

Thirty-seven male rats, 300 to 350 g, of the Sprague-Dawley strain (Charles River) were employed as subjects in the first experiment. The rats were maintained

in a temperature controlled environment on a 12-hr light-dark schedule (light: 6 AM to 6 PM) and were allowed food and water ad libitum throughout the experiment.

Apparatus

The rats were restrained in inverted U-shaped plastic holders purchased from Narco Biosystems Inc. The holders had adjustable sliding inserts that allowed the rats to be tightly restrained. The holders were placed in Industrial Acoustics sound isolation chambers 10.5 cm in front of two 8.3 cm loudspeakers. The sound isolation chamber was equipped with a 7.5 cm exhaust fan and white noise was provided by an 8.3 cm loudspeaker that was located 12 cm behind the rat. The white noise (75 dB re 20 uN/m2) and the exhaust fan served to mask extraneous auditory stimulation. Conditioned stimuli (CSs) were a 10.5-sec, 85-dB, 1-KHz tone and a 10.5-sec, 85-dB, 5 KHz tone. One CS served as the CS+ and was paired with the US at an interstimulus interval of 10 sec. The other CS served as the CS- and was always presented without shock. The frequency of the CS+ was counterbalanced so that one half of the subjects in each group received the 1-KHz tone as the CS+ and the 5-KHz tone as the CS-. The opposite relationship held for the other half of the subjects. The unconditioned stimulus (US) was a .5-sec, 175-Volt DC, shock delivered by a Massey Dickinson shock generator through two 20 ga needles inserted

subcutaneously on either side of the rat's thoracic cavity.

Heart beats were recorded on a Grass Model 5 polygraph. A microswitch was positioned above the polygraph pen so that it was triggered by the R wave of the QRS complex. Massey Dickinson logic circuits converted the output from the microswitch to +5-volt square wave pulses that were fed into an Apple // microcomputer through a California Computer Systems parallel interface card (Model 7720). An assembly language program developed by Cunningham (1982) controlled stimulus events and measured interbeat intervals (IBIs). Interbeat intervals that were less than 30 msec or greater than 150 msec were automatically scored as errors and were not included in subsequent data analyses. An average IBI for each measurement interval was calculated and stored in the Apple //'s memory. At the end of each trial IBI data and total sample time for each measurement interval were stored on magnetic disk. An offline BASIC program was used to convert interbeat interval data to HR and to sort CS+ and CS- trials into separate data files. Difference scores were also calculated by the BASIC program. In addition, an error handling routine determined whether less than 30% of a given measurement interval had been sampled. If this condition was met, data obtained in that interval were replaced by data from an appropriate adjacent interval. If the 30% sample criterion was not met for more

than two consecutive measurement intervals, then heart beat data were obtained manually from paper polygraph records.

The assembly language data acquisition and experimental programs were run on an Apple // plus microcomputer with 48 kilobytes of random access memory (RAM). The computer was equipped with a disk drive to provide permanent storage of data. A clock card was set to provide interrupts at 100-Hz and thus allowed experimental events to be timed at a resolution of 10 msec.

Procedures

In Experiment 1, separate groups received subcutaneous injections of either saline or morphine prior to conditioning. There were four different groups. One group received saline (1 ml/kg N= 9). The other three groups received either a low dose (.25 mg/kg; N=8), a medium dose (5 mg/kg; N=10) or a high dose (10 mg/kg; N=10) of morphine prior to conditioning. All drugs were dissolved in .9 % saline (1 ml/kg).

Mean interbeat intervals were recorded on each trial during two 5-sec pre-CS intervals from which an average baseline HR was calculated. Heart beats were measured during the CS+ for five consecutive 2-sec intervals and during three 2-sec post-shock intervals. Heart beat data were obtained in an identical manner during presentations of

the CS-. After interbeat intervals were converted to heart rate, difference scores were calculated by subtracting the baseline HR from the HR in each of the 2-sec measurement intervals. Difference scores were averaged across five trials for the data analyses described below.

On the day of conditioning, the rats were placed in the plastic holders and then given a subcutaneous injection of the appropriate drug. Injections were made behind the animal's head in the neck, through a hole in the top of the restrainer. The rats were then placed in the sound isolation chamber for a 15-min drug absorption period.

Immediately after the drug absorption period, the CS-alone phase of the experiment began. This phase consisted of four presentations each of the 1-KHz CS and the 5-KHz CS in a quasi-random order. The conditioning phase of the experiment immediately followed the CS-alone phase. For this phase, there were 20 presentations of the CS+ and 20 presentations of the CS-. The order of CS+ and CS- presentations varied quasi-randomly with the restriction that no more than three trials of a given type could occur consecutively with the intertrial interval for the CS-alone trials and the conditioning trials varying randomly among either 160, 180, or 200-secs (mean= 180 -secs). Immediately following the conditioning phase, the groups received six extinction trials in which the CS+ and CS- were presented in

the absence of shock.

Experimental Design and Data Analysis

Heart rate responses were initially analyzed by 4-way ANOV on morphine (saline, .25, 5 and 10mg/kg) data. The plan of the experiment involved one between-subjects factor (dose) and three within-subjects factors (type of CS, trial blocks and measurement interval), and can be viewed as a 4 x 4 x 2 x 5 (dose x trial blocks x CS-type x measurement intervals) design. Follow-up probes to the 4-way analyses were performed to determine the nature of interaction effects. For example, if there was a significant interaction involving dose, measurement interval and CS-type, the data were averaged over trials and a 3-way analysis was carried out. This general procedure for probing interaction effects was followed until it was possible to make post hoc comparisons of individual levels within a given factor. Baseline and unconditioned response data were treated in a similar manner. In the figures that follow, HR responses were averaged over the measurement-interval factor. Measurement interval was included in each statistical analysis and any significant group effects involving this factor are reported below. In addition, tone frequency was included as a between-groups factor in initial ANOVs of morphine responses. There were no significant main effects or interaction effects involving

the frequency factor and HR responses were averaged across tone frequency in the analyses presented below.

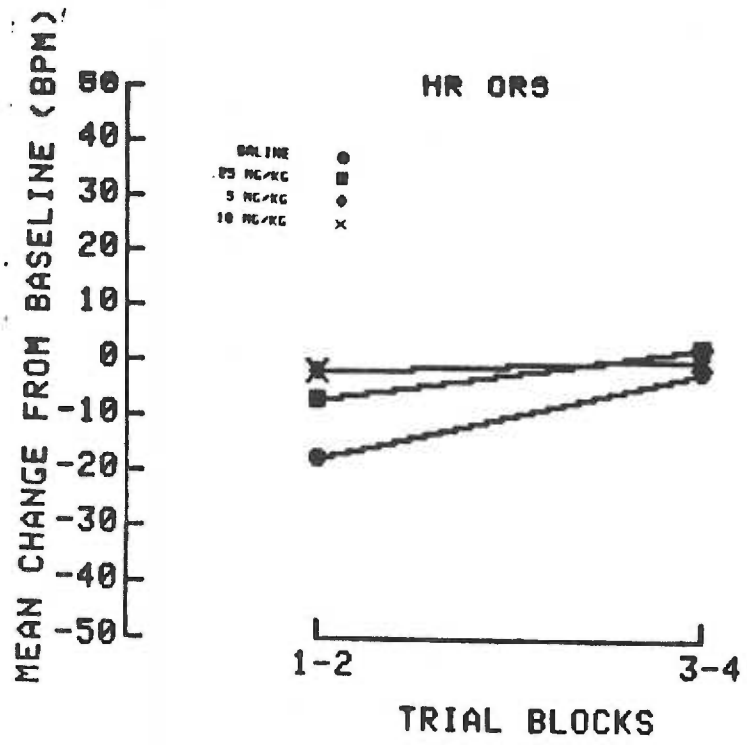
Results

Orienting Responses

Figure 1 depicts HR orienting responses (ORs) of each group averaged over both CSs and over successive blocks of 2 trials. The OR of the saline group was a deceleration that decreased in magnitude from the first to the second trial block. The ORs were also decelerative in the three morphine groups, but the magnitude of HR change was attenuated relative to the saline group.

A 4 x 2 x 2 x 5 (dose x trial blocks x type of CS x measurement interval ANOV showed that there was a significant effect due to dose, $F(3,33) = 3.25$, $p < .01$, indicating that the ORs of the groups were reliably different, and a significant effect due to trials, $F(1, 33) = 17.58$, $p < .01$, confirming that the magnitude of HR ORs changed significantly over trials. A Neuman-Keuls test demonstrated that HR OR of each group receiving morphine was significantly different from that of the saline group ($p < .05$). The effect of morphine was not dose dependent since the ORs did not differ reliably among the three morphine groups.

Figure 1. Heart rate orienting responses (ORs) of the saline and the morphine groups, averaged over CS-types and plotted in two-trial blocks.

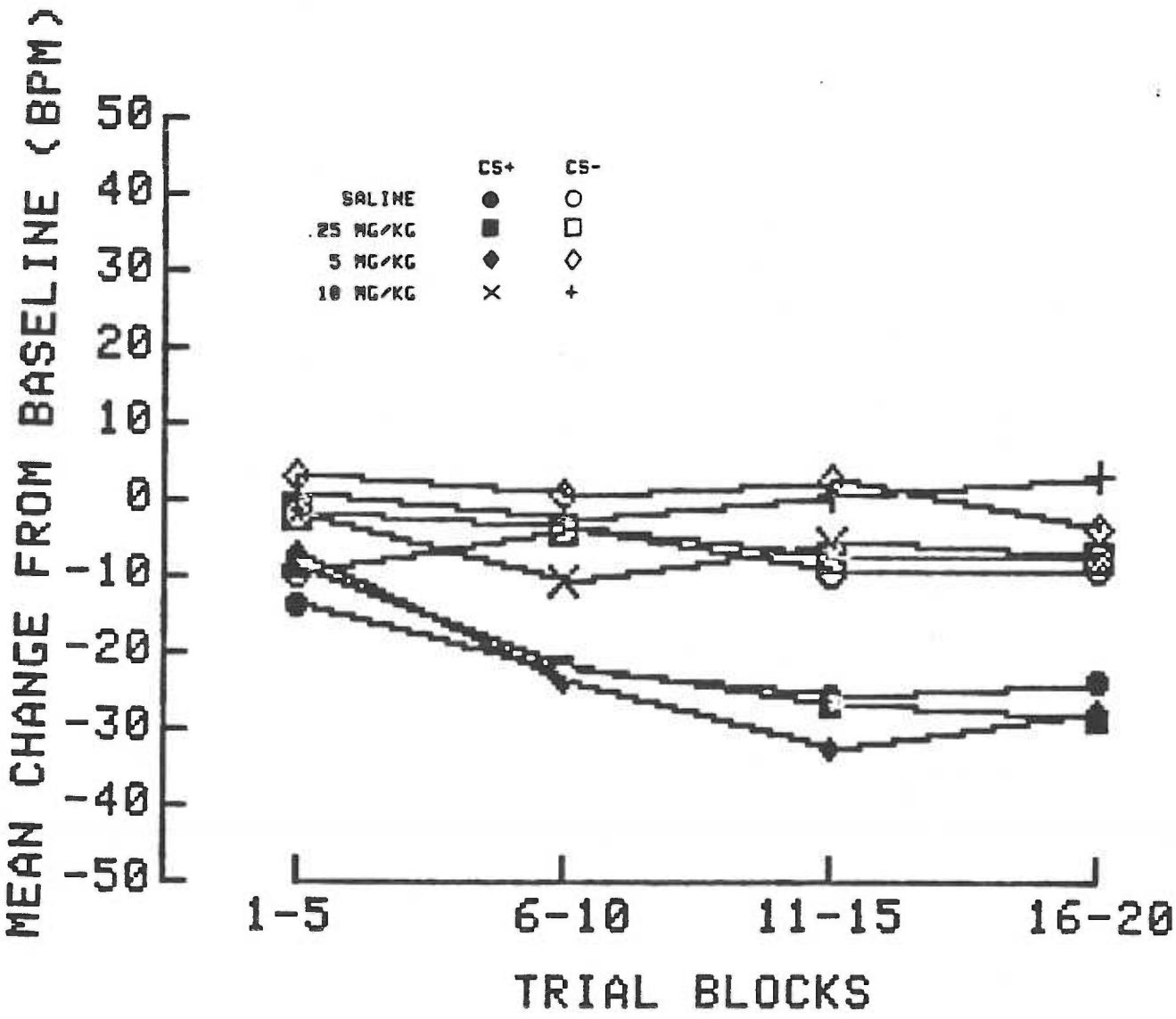

HR ORS
MEAN CHANGE FROM BASELINE (BPM)
60
40
30
20
10
0
-10
-20
-30
-40
-50
1-2
3-4
TRIAL BLOCKS

Conditioned Responses

Conditioning Phase. Figure 2 depicts the mean HR responses of each group averaged over successive blocks of five trials. It may be seen that the saline, the .25-mg/kg and the 5-mg/kg morphine groups showed the development of a decelerative HR response to CS+ over the course of conditioning and near zero responding to the CS-. Although difficult to see, the 10-mg/kg group showed a small decelerative HR response to CS+ that appeared early and remained nearly constant in magnitude over trials. The response of the 10-mg/kg group to the CS- was also near zero.

The reliability of the conditioning outcomes was tested by means of a 4 x 4 x 2 x 5 (groups x trial blocks x type of CS x measurement intervals) analysis of variance (ANOV). There was a significant type of CS effect, $F(1,33)$, $p < .01$, confirming that reliable conditioning occurred and a significant type of CS x trial blocks x measurement intervals interaction, $F(12, 396) = 7.24$, $p < .01$, indicating that conditioning developed over trials. In addition, there was a significant groups x type of CS x measurement interval interaction. This interaction was tested with separate 4 x 5 (groups x measurement intervals) ANOVs of the CS+ and the CS- data. For the CS+ data, this

Figure 2. Mean CS minus pre-CS heart rate changes of the saline and the morphine groups in successive blocks of five conditioning trials.


MEAN CHANGE FROM BASELINE (BPM)
50
40
30
20
10
0
-10
-20
-30
-40
-50
CS+
CS-
SALINE
.25 MG/KG
5 MG/KG
10 MG/KG
1-5
6-10
11-15
16-20
TRIAL BLOCKS

follow up analysis revealed a significant groups effect, $F(3,33) = 3.45$, $p < .05$, a significant measurement intervals effect and a significant groups x measurement intervals interaction, $F(4, 132) = 4.01$ $p < .01$. Follow up ANOVs at each measurement interval showed that decelerative HR responses of the 10-mg/kg group to the CS+ were significantly smaller ($p < .01$) than those of the saline, the .25-mg/kg group, or the 5 mg/kg group, during measurement intervals 2, 3, 4 and 5. The CS- analysis uncovered a significant effect due to measurement intervals, $F(12,132) = 4.01$, $p < .01$, but no significant effects involving groups.

In order to establish that CS+ and CS- responses were reliably different for each group, separate 2 x 4 (type of CS x trial blocks) ANOVs were carried out. For the saline group, there was a significant type of CS x trial blocks interaction, $F(3, 24) = 3.54$, $p < .05$, which indicated that differential responding to the CS+ and CS- developed over trials. There were significant type of CS x trialblocks effects for the .25-mg/kg group, $F(3, 21) = 4.43$, $p < .05$, and a significant type of CS effect for the 5-mg/kg $F(1,9) = 17.69$, $p < .05$, and for the 10-mg/kg group, $F(1, 9) = 5.90$, $p < .05$. Therefore, reliable HR CRs were established in each group.

Extinction Phase Heart rate CRs during

extinction, averaged over successive 2-trial blocks are shown in Figure 3. The saline, the .25-mg/kg, and the 5-mg/kg groups continued to show CRs during all of the extinction trials. The 10-mg/kg group continued to exhibit attenuated CRs. Responses to both the CS+ and the CS- showed little change over the 6 extinction trials in any group.

A 4 x 3 x 2 x 5 (groups x trial block x type of CS x measurement interval) analysis of variance revealed significant effects due to groups, $F(3,33) = 4.66$, $p < .01$, suggesting that combined responses to the CS+ and the CS- were influenced by morphine treatment in a dose dependent manner. A Neuman-Keuls test indicated that the response of the 10-mg/kg group was significantly smaller than the responses of the saline, the .25-mg/kg and the 5-mg/kg groups ($p < .05$).

Unconditioned Responses

Difference-score responses of the three morphine groups and the saline group for the three post-shock measurement intervals, averaged over four blocks of five trials each, are shown in Figure 4. It can be seen that the URs of the saline and the .25-mg/kg groups were consistent HR accelerations that increased in magnitude over trials. In contrast, the 5-mg/kg and 10-mg/kg groups exhibited HR decelerations during the first trial-block, followed by low

Figure 3. Mean CS minus pre-CS heart rate changes of the saline and the morphine groups in successive blocks of four extinction trials.

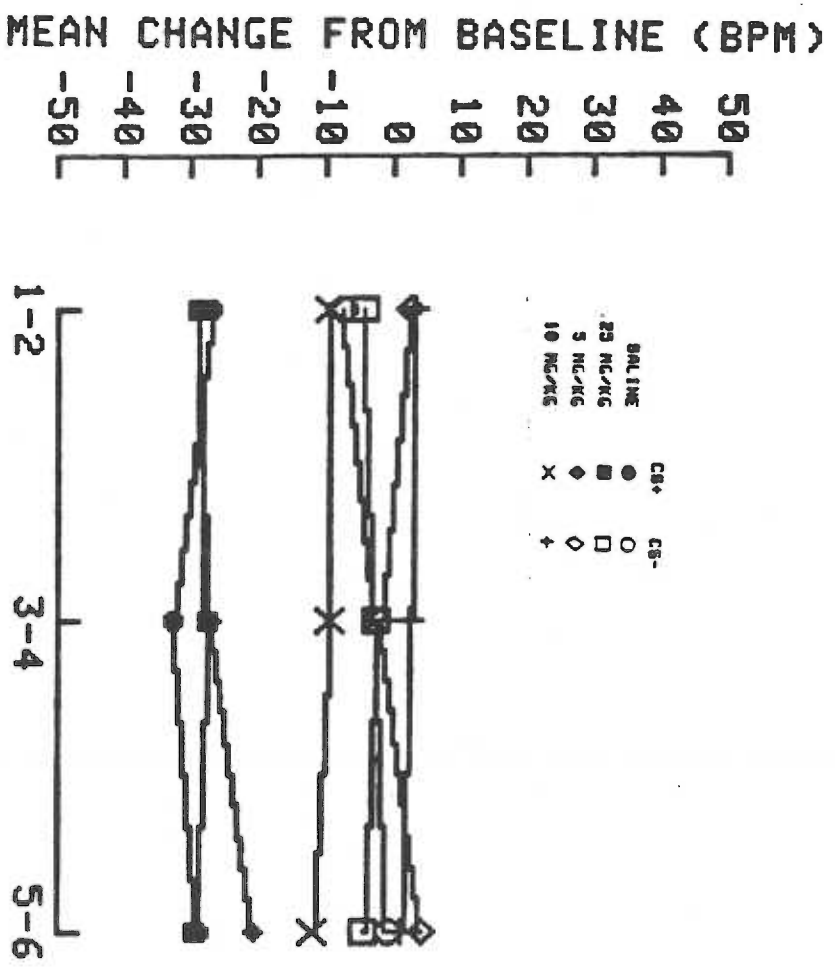
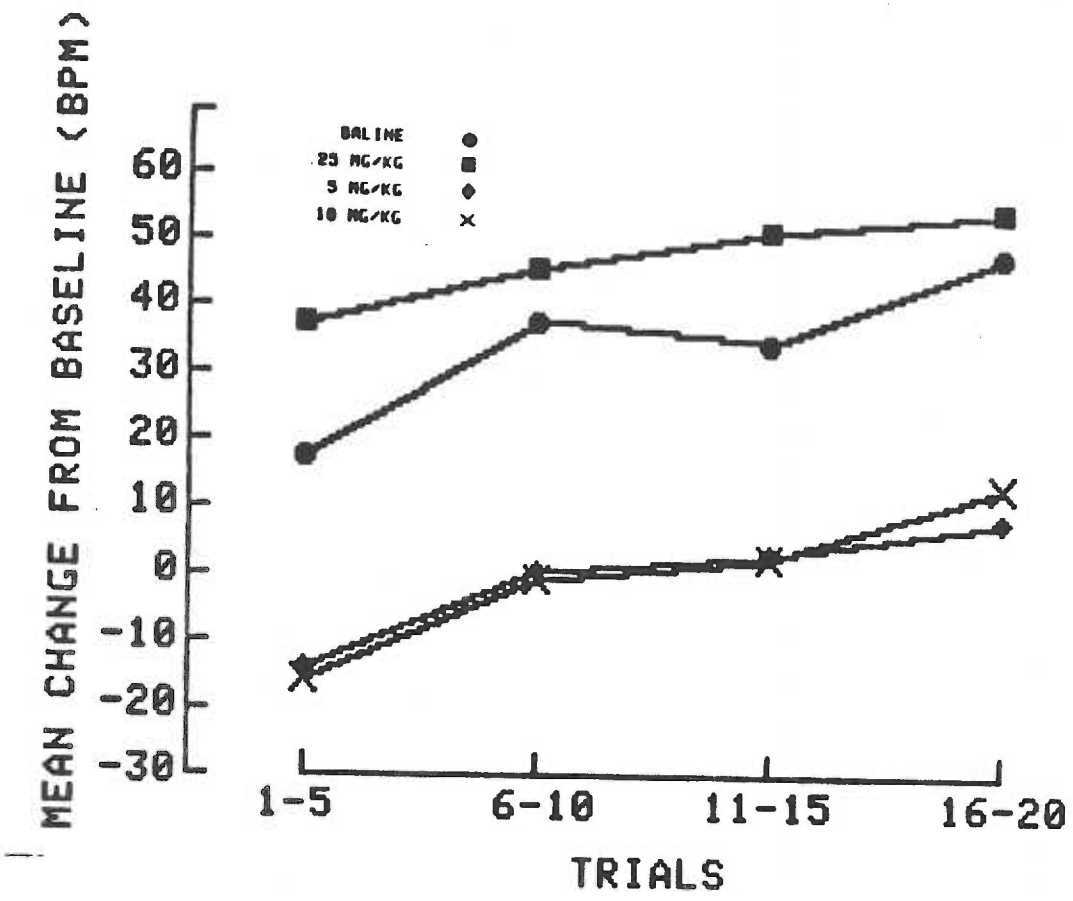

MEAN CHANGE FROM BASELINE (BPM)
-50
-40
-30
-20
-10
0
10
20
30
40
50
1-2
3-4
5-6
SALINE
25 NG/KG
5 NG/KG
1 NG/KG
CS+
CS-

Figure 4. Mean post-shock minus pre-CS heart rate URs in successive blocks of five conditioning trials.


MEAN CHANGE FROM BASELINE (BPM)
60
50
40
30
20
10
0
-10
-20
-30
BALINE
25 MG/KG
5 MG/KG
10 MG/KG
1-5
6-10
11-15
16-20
TRIALS

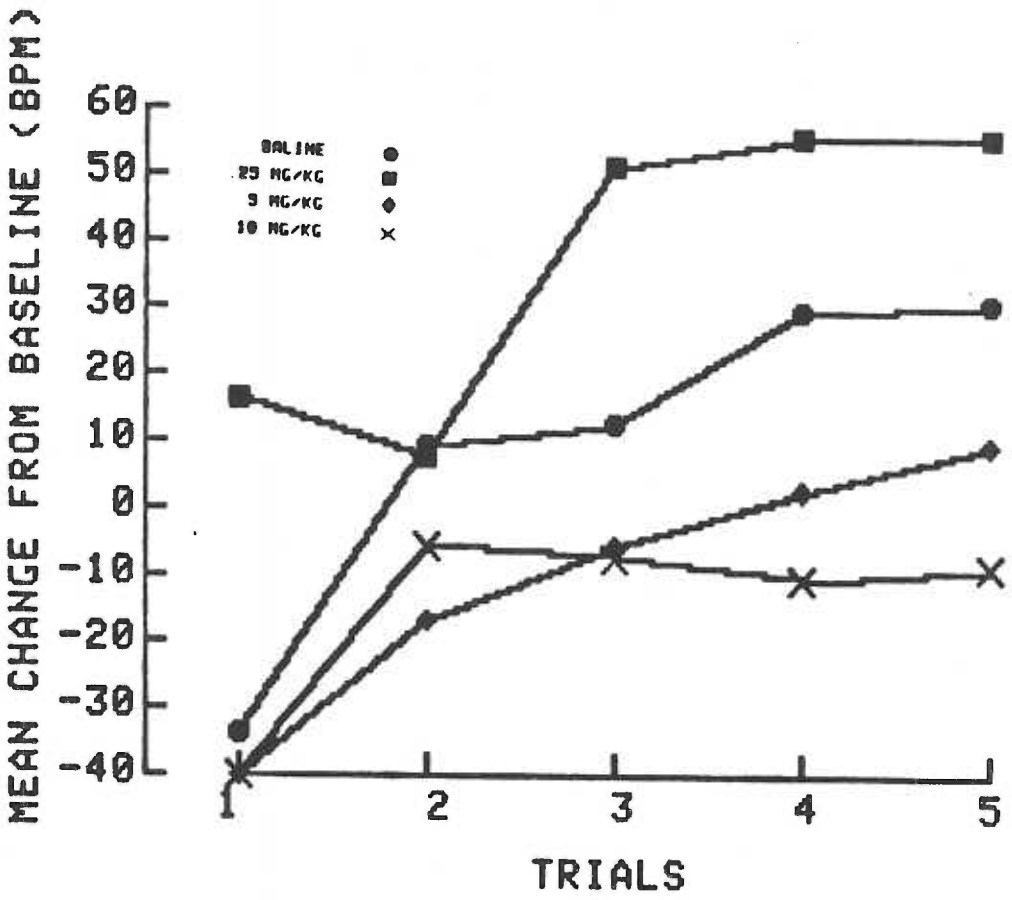
magnitude HR accelerations on the second, third and fourth trial blocks. A 4 x 4 (groups x trial blocks) ANOV revealed significant main effects due to groups $F(3,33) = 9.19$, $p < .01$, and trial blocks, $F(3,99) = 21.42$, $p < .01$. A Neuman-Keuls test showed that the HR URs of the 5-mg/kg and 10 mg-kg groups were significantly different from the HR URs of the .25-mg/kg and saline groups.

Unconditioned response data on each of the first five trials are plotted in Figure 5. These trials were analyzed separately to obtain information on the possible effects of morphine on HR URs that were not preceded by large CRs. On the first trial, HR URs were decelerative for the saline, the 5-mg/kg and the 10-mg/kg groups, but accelerative for the .25-mg/kg group. Over trials 2, 3, 4 and 5 the HR URs of the saline group changed to accelerations, whereas those of the 5-mg/kg and the 10-mg/kg groups continued to be slightly decelerative or near zero change.

A 4 x 5 (groups x trials) ANOV showed significant main effects due to groups $F(3,33) = 6.91$, $p < .01$, and trials, $F(4, 132) = 13.69$, $p < .01$. A Neuman-Keuls test indicated that the URs of the 5-mg/kg and 10-mg/kg groups were different from that of the saline and the .25-mg/kg groups ($p < .01$).

Baseline Heart Rate

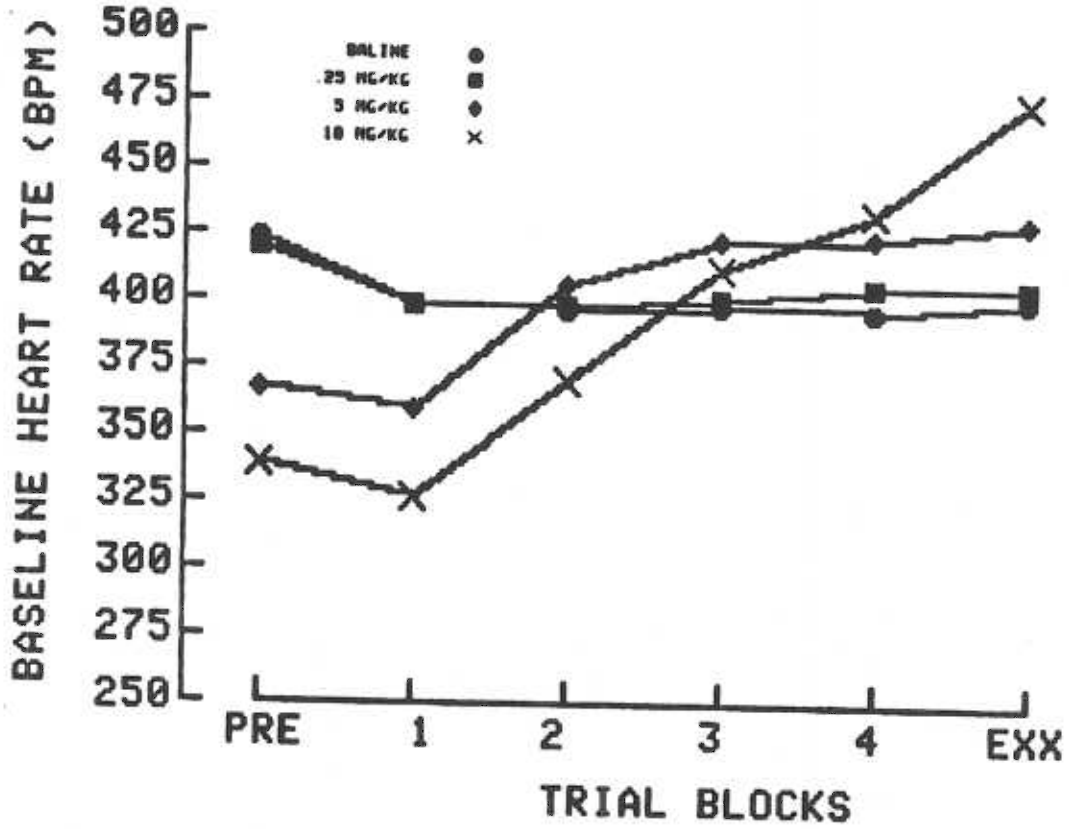
Figure 5. Mean post shock minus pre-CS heart rate changes (URs) plotted for the first five trials of conditioning.


MEAN CHANGE FROM BASELINE (BPM)
60
50
40
30
20
10
0
-10
-20
-30
-40
SALINE
25 MG/KG
5 MG/KG
10 MG/KG
1
2
3
4
5
TRIALS

Baseline HRs of each group, averaged over the pre-conditioning phase, over 5-trial blocks of the conditioning phase and over the extinction phase are presented in Figure 6. Baseline HRs of the 5-mg/kg and 10-mg/kg groups were lower than those of the saline and the .25-mg/kg groups during the pre-conditioning phase and the first trial block of conditioning. Thereafter, baseline HRs of the 5-mg/kg and 10-mg/kg groups increased to levels above those of the other groups.

A 4 x 6 (groups x trial blocks) ANOV, revealed a significant groups x blocks interaction, $F(15, 165) = 19.11$, $p < .01$. Follow up analyses revealed significant differences among the groups during pre-conditioning, $F(3, 33) = 7.13$, $p < .01$, the first conditioning trial block, $F(3, 33) = 7.06$, $p < .01$ and during extinction, $F(3,33) = 6.82$, $p < .01$. A Neuman-Keuls test on the pre-conditioning phase indicated that baseline HR in the 10-mg/kg group was significantly lower than that of the .25-mg/kg or saline groups ($p < .01$). A Neuman-Keuls test on the first trial block showed that baseline HR of the 10-mg/kg group was lower than baseline HR of the saline and .25 mg/kg groups ($p < .01$). Baseline HR of the 5-mg/kg group was also lower than that of the saline and .25-mg/kg groups ($p < .05$). Neuman-Keuls analysis on extinction revealed that baseline HR of the 10-mg/kg group was

Figure 6. Mean pre-CS heart rate of the saline and the morphine groups plotted for the preconditioning (PRE), the conditioning, and extinction (EXX) phases of Experiment 1.


BASELINE HEART RATE (BPM)
500
475
450
425
400
375
350
325
300
275
250
SALINE
.25 MG/KG
5 MG/KG
10 MG/KG
PRE
1
2
3
4
EXX
TRIAL BLOCKS

significantly higher than those of the .25-mg/kg and saline groups ($p < .01$).

Experiment 1: Discussion

Summary The groups receiving 10 mg/kg of morphine showed attenuated decelerative HR CRs. The HR CRs of groups given lower doses of morphine (.25 and 5 mg/kg) were no different from those of a saline control group. The HR URs were attenuated in the 5- and 10-mg/kg groups, but not in the .25-mg/kg group. All three doses of morphine reduced pre-conditioning orienting responses (ORs) to the CSs. Baseline HR was initially depressed in the 5 and 10-mg/kg groups relative to the saline and .25-mg/kg groups.

Orienting Responses. In Experiment 1 morphine reduced the magnitude of the decelerative HR ORs to initial presentations of the CSs. Previous work has shown that morphine and levorphanol failed to affect HR ORs in the rabbit (Gallagher et al., 1981). The present findings suggest that morphine may have produced a general reduction in reactivity, as opiates are widely known (e.g. Rodgers, 1978) to produce sedative as well as analgesic effects. There is little available evidence to suggest that morphine can impair hearing (Jaffe and Martin, 1980). In the present experiment, all of the morphine groups showed

reliable differential responding to the CSs during the conditioning phase of the experiment, suggesting that frequency discrimination was not impaired.

Conditioning Phase. The 10-mg/kg dose of morphine impaired the development of the decelerative HR CR, while, the .25-mg/kg and the 5-mg/kg doses failed to have any effect. This outcome contrasts with the findings of Banerjee (1971) who found that as little as .25 mg/kg of morphine injected prior to conditioning interfered with the acquisition of a pole jump avoidance response. These contrasting outcomes raise the possibility that HR CRs are less sensitive to the effects of opiates than other learned behaviors. Even though the 10-mg/kg group exhibited attenuated HR CRs, it did show conditioning in that HR decelerations were significantly larger to the CS+ than to the CS- from the first block of conditioning onward.

The 10-mg/kg dose of morphine could have interfered with the HR CR in a number of ways. The first possibility has to do with the known analgesic properties of morphine. Systemically and centrally administered morphine has been shown to reduce reactivity to noxious electrical and thermal stimulation in the rat. For example, 10-mg/kg and 5-mg/kg doses raised shock thresholds in a footshock titration procedure (Kornetsky and Kiplinger, 1968; Markowitz, Jacabson, Bain and Kornetsky, 1976). In addition, a number

of workers have shown that relatively low doses of morphine impaired reflex escape responses from noxious thermal stimulation (D'Amour and Smith, 1942; Dewey and Harris, 1975). Therefore, it is well established that morphine, in the dose range employed in the present experiment, can produce analgesia.

It seems likely that both the 5 and 10-mg/kg doses of morphine reduced the noxiousness of shock. In some experiments, conditioning performance has been positively related to the intensity of the US and therefore probably to the noxiousness of the US (Kamin and Brimer, 1963; Smith, 1969). Possibly, associative strength between a CS and US is stronger the more noxious or painful the US (Mackintosh, 1974). Thus, a reduction in the noxiousness of shock could explain the adverse effect of morphine on the HR CR. One problem with this account is that the 10-mg/kg group showed a CR decrement, but the 5-mg/kg group exhibited a normal HR CR. The 5-mg/kg dose may have had produced less analgesia than the 10-mg/kg dose and that is why conditioning was not impaired in the 5-mg/kg group.

A second possibility is that the HR CR of the 10-mg/kg group was reduced because of a morphine-produced decrease in conditioned fear. Mauk et al. (1982a,b) have interpreted the interfering effect of mu receptor agonists such as morphine, on the nictitating membrane CR in this

way. However, it does not seem likely that fear could mediate the nictitating membrane response because of the rather short CS-US interval (see MacAllister and MacAllister, 1971). In the HR conditioning situation, Lavond et al. (1982) have assumed that the HR CR is secondary or is at least strongly tied to a learned fear reaction, an assumption that receives little empirical support (Obrist, Sutterer and Howard, 1972).

While it is true that the effects of opiates on learned behaviors may vary as a function of the type of reinforcement (i.e. appetitive vs. aversive) employed (for example, White and Holtzman, 1983), there seems to be little to be gained by introducing the concept of conditioned fear in order to explain the effect of morphine on the HR CR. Despite the failure of the 5-mg/kg group to show a HR CR decrement, the most straightforward explanation of morphine's effects in the present experiment might be that the drug produced analgesia, and that HR CRs of the 10-mg/kg group were reduced as a direct function of a decrease in the noxiousness of the US.

A third possibility, given the presence of opiate receptors in the nucleus ambiguus and the dorsal motor nucleus of the vagus (Atweh and Kuhar, 1977b), is that the 10-mg/kg dose of morphine altered the activity of cardio-inhibitory neurons in the final output pathway.

However, if the CR decrement to morphine on the HR CR was related to its direct effects on the cardiovascular system then one might have expected both the 5-mg/kg and 10-mg/kg groups to show altered HR CRs, because both doses had quantitatively similar effects on baseline HR and HR URs. This outcome suggests that the effects of morphine on the HR CR are independent of the drug's effects on the HR UR and baseline HR.

Extinction. Conditioned HR responses showed little evidence of extinction over the six unreinforced CS+ trials. The HR CRs of each group were quite similar to those occurring during the final five conditioning trials. The saline, the .25-mg/kg and the 5-mg/kg morphine groups continued to respond to the CS+ with 20 to 30 bpm decreases in HR, and the 10-mg/kg morphine group responded with 5-10 bpm decreases in HR. These data suggest that too few extinction trials were administered to assess the effects of morphine on the extinction of the HR CR adequately.

Unconditioned Responses

The 5-mg/kg and 10-mg/kg doses of morphine had a profound effect on the direction and the overall magnitude of post-shock HR responses. On the initial conditioning trial in which shock was presented, all groups, except the one receiving .25-mg/kg of morphine responded to shock with decreases in HR. In the saline group, the initial

deceleration was replaced by accelerative HR changes by Trial 5. In the 5-mg/kg and 10-mg/kg groups the decelerative URs appeared to habituate, and were not replaced by accelerative URs. Fitzgerald and Hoffman (1976) and Fitzgerald and Teyler (1970) have previously reported that HR URs in normal restrained rats may sometimes be decelerative on initial US presentations and accelerative on later trials.

In some respects, the HR URs of the 5-mg/kg and 10-mg/kg morphine-groups resembled ORs. First, the direction of the response was originally decelerative as are HR ORs to auditory stimuli (e.g. Fitzgerald and Tyler, 1970). Second, they habituated over trials just as ORs do (Fitzgerald and Tyler, 1970; Hoffman and Fitzgerald, 1976). Although morphine produces analgesia, it does not appear to interfere with other forms of somatic sensation (Jaffe and Martin, 1980). Therefore, it seems possible that for the two highest morphine dose groups, the shock may have felt like an intense, but painless, tactile or vibratory stimulus. This interpretation could explain why the decelerative URs habituated and were not replaced by accelerative URs.

Baseline Heart Rate

The 5-mg/kg and 10-mg/kg doses of morphine produced

large bradycardias as reflected by baseline HR. This effect was evident during the pre-conditioning phase and during the first trial block of conditioning, which represented a total time of 60 to 80 min following injections. The reduction of baseline HR by morphine is in agreement with previous experiments that have shown that morphine produced bradycardia in anesthetized cats and rats (Bolme, 1979; Rashid and Waterfall, 1978). In the anesthetized rat, the morphine-produced fall in HR was due to enhanced vagal outflow, as the bradycardia was eliminated with atropine (Rashid and Waterfall, 1978). Morphine has a central site of action and can produce bradycardia when it is injected into the IVth ventricle (Bolme, 1979). A recent experiment demonstrated that the fall in HR seen after the peripheral injection of morphine was largely due to the activity of morphine at the mu receptor (Holaday and Ward, 1982). In that study, large doses (> 75-mg/kg) of morphine also induced hypotension that may be the result of its combined activity at the delta and the mu receptor (Holaday and Ward, 1982).

Following the fall in baseline HR, the HR of the 5- and 10-mg/kg groups increased back to the levels of the other groups. It does not seem likely that these increases in HR were due to the elimination of the drug. First, Mullis et al. (1979) have shown that the half-life of

10-mg/kg of morphine, injected subcutaneously, is 5 hr, and increases in baseline HR were seen in the present experiment within 1 hr of injection. If baseline HR returned to control levels because morphine fell below some threshold concentration, then one might have expected that the rates of increase in HR would have been different for the 5 and 10-mg/kg doses. Thirdly, and perhaps most importantly, the fourth study to be reported below indicated that 10-mg/kg continued to produce analgesia 120 minutes after it was injected indicating that morphine was still active. Therefore, the increase in baseline HR observed after 1 hr in the 5 and 10-mg/kg groups seems to be due to an effect of morphine that is specific to the cardiovascular system. One possibility is that the rise in baseline HR was the result of a cardiovascular compensatory mechanism.

EXPERIMENT 2

Rationale

The findings of a number of experiments have suggested that naloxone can enhance the performance of a learned response (Gallagher et al., 1981) improve and memory consolidation (Izquierdo, 1979; Messing et al., 1981). Naloxone is an opiate receptor antagonist with little biological activity of its own, and any biological effect of

naloxone is usually assumed to result from its blockade of endogenous opiate activity at an opiate receptor. Thus, when naloxone is administered prior to a series of conditioning trials, it is thought to provide an indirect measure of the effect of endogenous opiates on the performance of a learned response.

In the conditioning situation employed in the present experiments, rats were restrained and given repeated presentations of electric shocks. The release of endogenous opiates has been shown to occur in response to restraint stress (Amir and Amit, 1979), and in response to electric shock (Chance et al., 1978). Thus, it seemed likely that endogenous opiates were released during conditioning, and this raised the possibility that endogenous opiates exerted some influence on learned and unlearned HR responses. The purpose of Experiment 2 was to test indirectly whether endogenous opiates influenced HR responses during aversive Pavlovian conditioning. Three doses of naloxone (.1, 1, and 10 mg/kg) were employed in order to establish a rough dose-response relationship. Naloxone, or control injections of saline were injected prior to preconditioning presentations of the CSs.

Method

Subjects

Thirty-four male rats (280-325 g) of the Sprague-Dawley strain (Charles River) were employed as subjects in Experiment 2. Rats were maintained under the same conditions that were described for Experiment 1.

Apparatus

The apparatus, the stimulus parameters, and data collection procedure employed in Experiment 2 were identical to those in Experiment 1.

Procedure

The procedure for Experiment 2 was identical to the procedure employed in Experiment 1. In Experiment 2, one group received saline (1 ml/kg, N=9), and three groups received either .1 mg/kg (N=8), 1 mg/kg (N=9) or 10 mg/kg (N=10) of naloxone HCl dissolved in .9 % saline (1 ml/kg) prior to preconditioning presentations of the CSs.

Experimental Design and Data Analysis

The experimental design and analysis of Experiment 2 were identical to the design and analysis described for Experiment 1.

Results

Orienting Responses

Original HR responses to the CS+ andCS- combined, averaged over two-trial blocks of pre-conditioning are

depicted in Figure 7. The saline group showed a decelerative OR which was larger on the first trial block than the responses of the other groups. The HR ORs decreased in magnitude from the first to the second block of trials.

A 4 x 4 x 2 x 5 (groups x trials x type of CS x measurement intervals) ANOV revealed a significant trials effect, $F(3,90) = 15.15$, $p < .01$, which indicates that the decrease in response magnitude was reliable. There was also a significant groups x measurement intervals interaction, $F(12, 120) = 2.81$, $p < .01$. A follow-up probe of this interaction showed that the HR changes of the 10-mg/kg group were significantly smaller than those of the saline group during the first three measurement intervals ($p < .05$), and that the HR responses of the .1-mg/kg group were significantly smaller than those of the saline group in the second and third measurement intervals, ($p < .05$).

Conditioned Responses

Conditioning Phase. Heart rate responses to the CS+ and the CS- averaged over four blocks of five trials each are shown in Figure 8. As can be seen, the HR CRs to the CS+ of each group were mainly decelerative, while, responses to the CS- were low magnitude decelerations and accelerations. Figure 8 illustrates that during the first trial block there was little difference between responses to

Figure 7. Mean pre-CS minus CS heart rate changes of the saline and the naloxone groups averaged over type of CS in successive two-trial blocks of preconditioning.

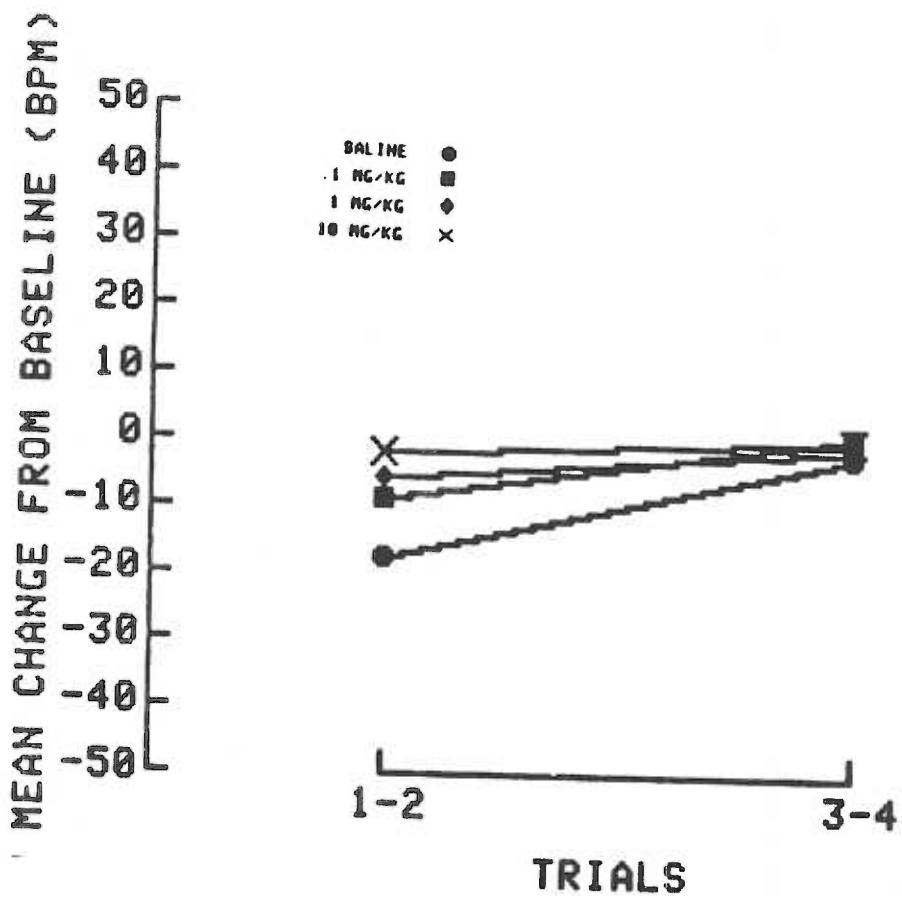
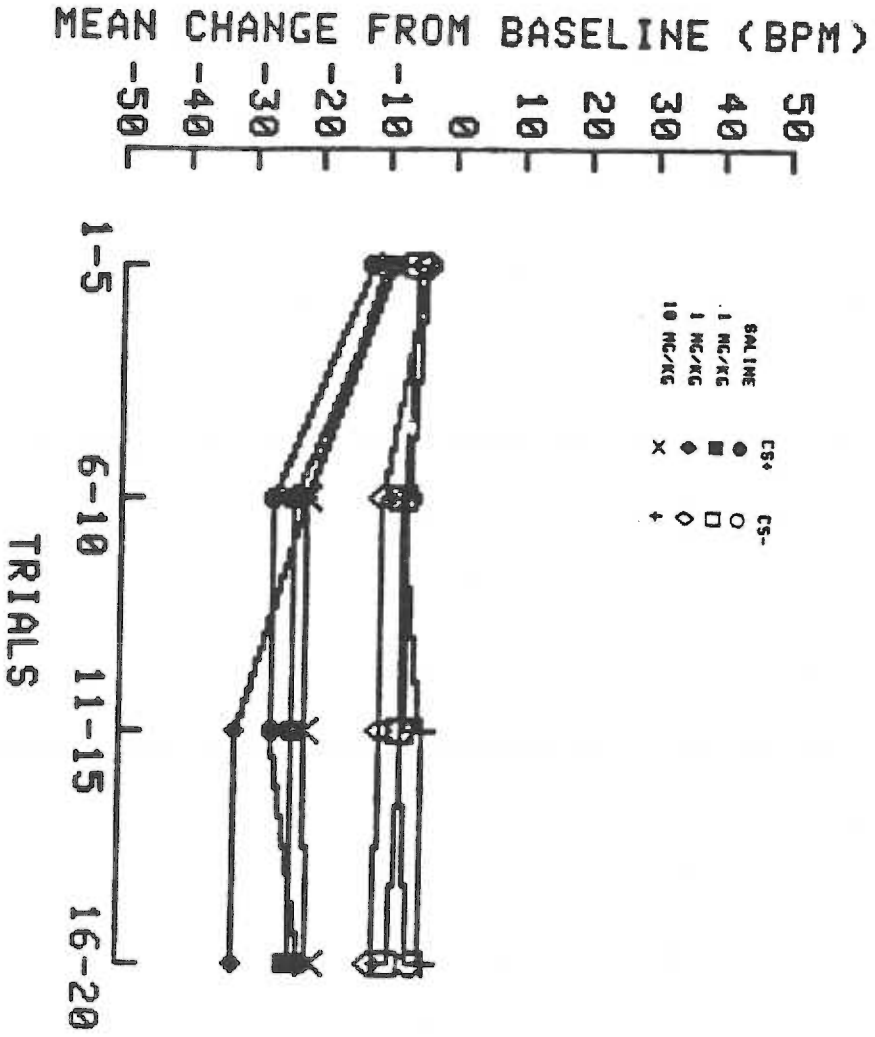

MEAN CHANGE FROM BASELINE (BPM)
50
40
30
20
10
0
-10
-20
-30
-40
-50
SALINE
.1 MG/KG
1 MG/KG
10 MG/KG
1-2
3-4
TRIALS

Figure 8. Mean pre-CS minus CS heart rate changes of the saline and the naloxone groups in successive blocks of five conditioning trials.

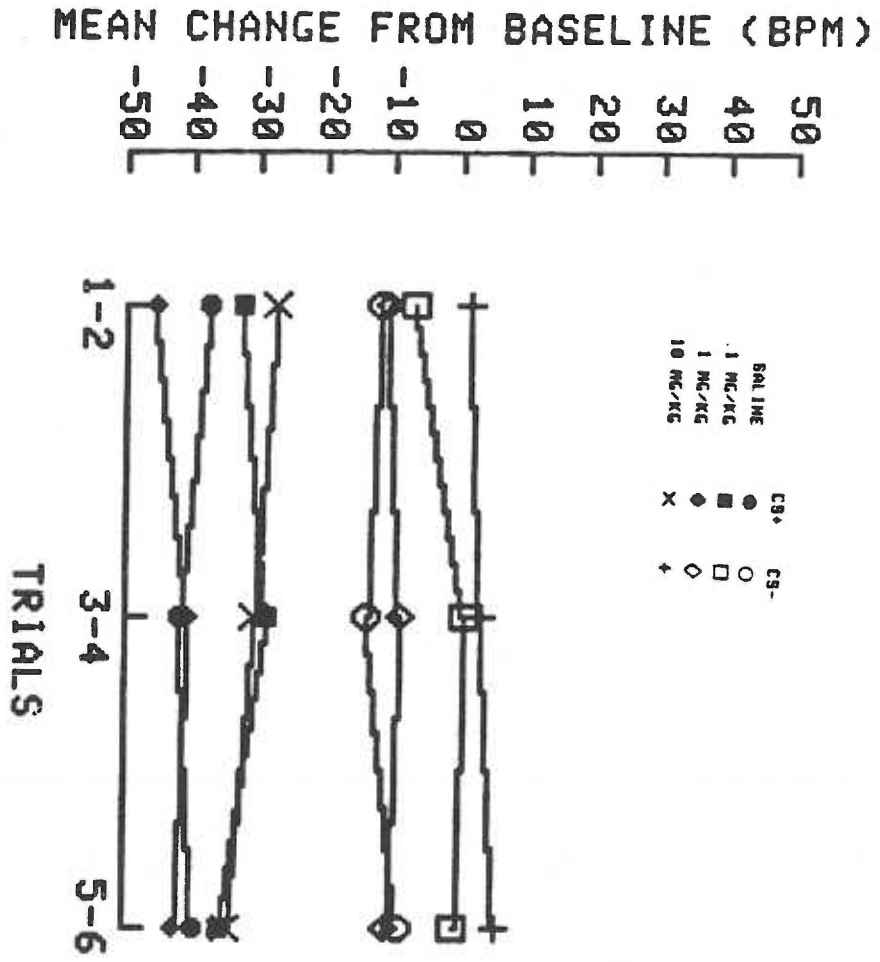

MEAN CHANGE FROM BASELINE (BPM)
-50
-40
-30
-20
-10
0
10
20
30
40
50
1-5
6-10
11-15
16-20
TRIALS
SALINE
1 MG/KG
3 MG/KG
10 MG/KG
CS+
CS-

the CS+ and to the CS-. However, responses to the CS+ and the CS- were clearly different from the second trial block onward. A comparison of the groups reveals that naloxone had no major effect on the magnitude of the HR CRs.

A 4 x 4 x 2 x 5 (groups x trial blocks x type of CS x measurement intervals) ANOV provided a significant effect due to trial blocks, $F(3, 30) = 17.89$, $p < .01$, indicating that the magnitude of HR responses changed significantly over trial blocks, a significant type of CS effect, $F(1, 30) = 38.41$, $p < .01$, and a significant interaction involving type of CS and trial blocks, $(3,90)=13.31$, $p < .01$, showing that reliable conditioning occurred. There were no significant main effects or interactions involving the groups factor (all p's > .05).

Extinction Phase. Heart rate responses to CS presentations plotted over 2-trial blocks of extinction are shown in Figure 9. It can be seen that CRs in each group were maintained throughout the extinction phase of the experiment. Figure 9 shows that HR CRs were quite similar in each group suggesting that naloxone had little effect on the performance of HR CRs during extinction. A 4 x 3 x 2 x 5 (groups x trial blocks x type of CS x measurement interval) ANOV revealed no significant effects involving groups.

Figure 9. Mean pre-CS minus CS heart rate changes of the saline and the naloxone groups in successive blocks of two extinction trials.


MEAN CHANGE FROM BASELINE (BPM)
-50
-40
-30
-20
-10
0
10
20
30
40
50
1-2
3-4
5-6
TRIALS
SALINE
.1 MG/KG
1 MG/KG
10 MG/KG
CS+
CS-

Unconditioned Responses

Heart rate responses of each group to the shock US averaged over blocks of five trials are displayed in Figure 10. The responses of all the groups were accelerative and showed little change in magnitude over the 20 conditioning trials. A 4 x 4 (groups x trial blocks) ANOV produced no significant outcomes relating to groups.

Baseline Heart Rate

Baseline HR data of each group averaged over the pre-conditioning trials, over five-trial blocks for the conditioning phase, and over the six extinction trials are shown in Figure 11. All groups showed a drop in baseline HR at the start of conditioning followed by a gradual recovery back toward preconditioning levels. A 4 x 6 (groups x trial blocks) ANOV resulted in a significant trial blocks effect, $F(5, 150) = 22.94$, $p < .01$ and a significant groups x trial blocks interaction, $F(15, 150) = 3.96$, $p < .01$. Separate 1-way ANOVs at each trial block revealed a significant group effect during extinction, $F(3, 30) = 3.04$, $p < .01$. A Neuman-Keuls test indicated that extinction baseline HR was significantly lower in the .1-mg/kg group relative to the saline, the 1-mg/kg and the 10-mg/kg groups ($p < .01$).

Figure 10. Mean pre-CS minus post shock HR changes (URs) of the saline group and the naloxone groups in successive blocks of five conditioning trials.

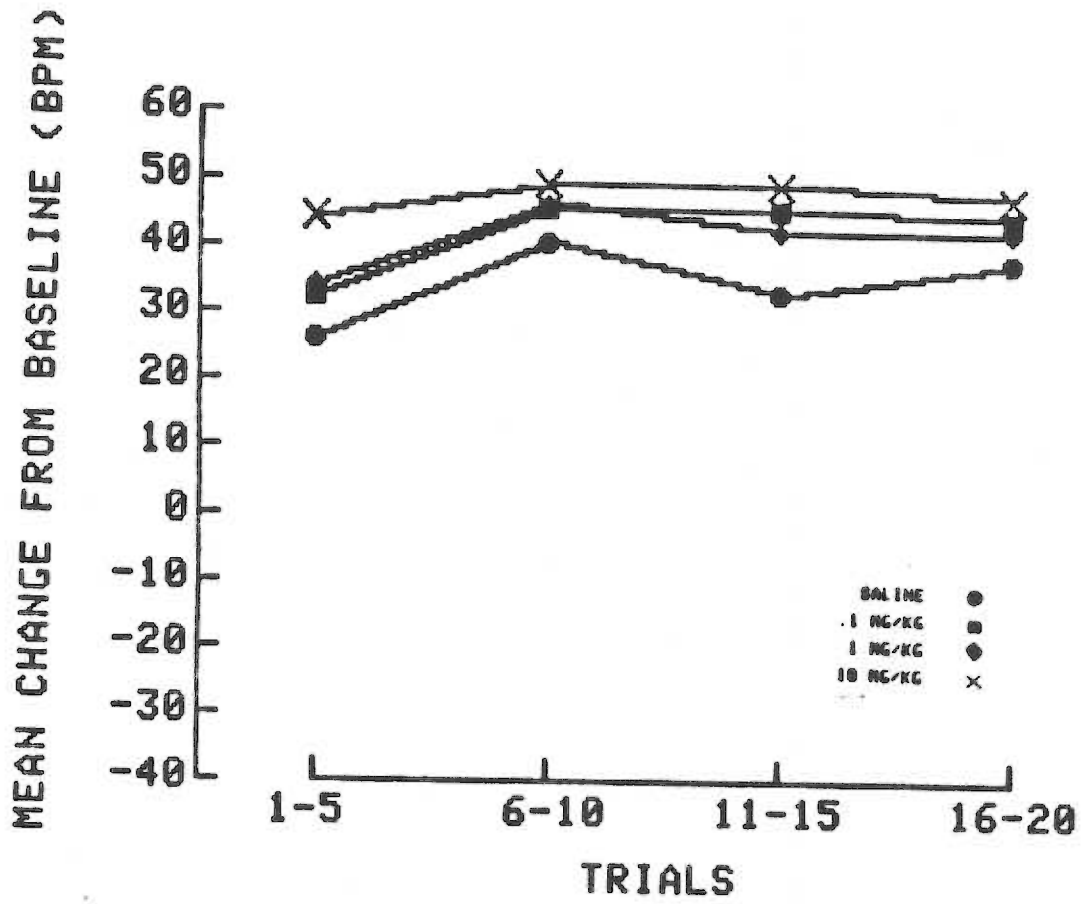
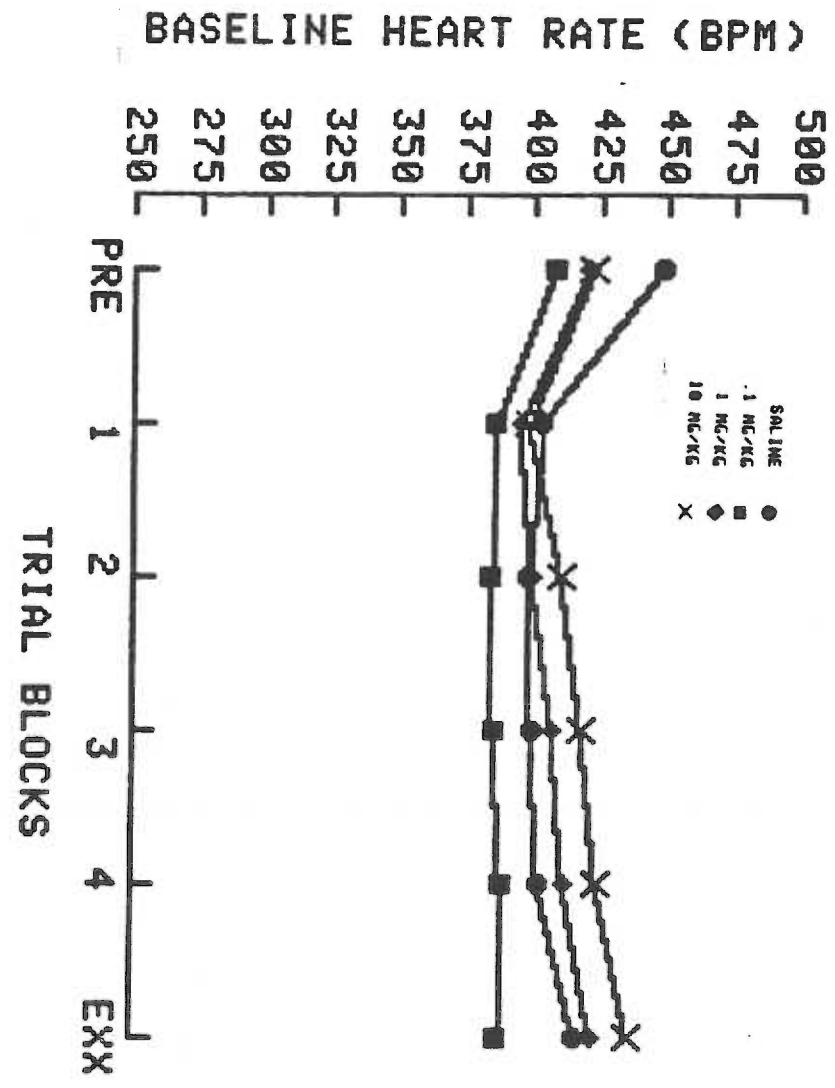

MEAN CHANGE FROM BASELINE (BPM)
60
50
40
30
20
10
0
-10
-20
-30
-40
1-5
6-10
11-15
16-20
TRIALS
SALINE
.1 MG/KG
1 MG/KG
10 MG/KG

Figure 11. Mean pre-CS heart rates of the saline group and the naloxone groups plotted for preconditioning (PRE), successive blocks of five conditioning trials and for the extinction (EXX) phase of Experiment 2.


BASELINE HEART RATE (BPM)
250
275
300
325
350
375
400
425
450
475
500
PRE
1
2
3
4
EXX
TRIAL BLOCKS
SALINE
.1 MG/KG
1 MG/KG
10 MG/KG

Discussion: Experiment 2

Naloxone, when administered at doses of .10 and 10-mg/kg, decreased the magnitude of the HR ORs. A previous experiment has shown that naloxone had little effect on ORs of rabbits to auditory stimuli (e.g. Gallagher et al., 1981) If morphine acted at an opiate receptor in attenuating HR ORs in Experiment 1, it is not clear why naloxone, which should block any endogenous activity at opiate receptors, would also reduce ORs. An additional finding of Experiment 2 was that the effect of naloxone on ORs was not dose dependent, suggesting that its attenuation of HR ORs may not have been due to its activity at an opiate receptor. None of the doses of naloxone affected HR CRs or HR URs. This finding can be interpreted in a number of ways. First, it may be that endogenous opiates were not released during conditioning and, as a result, there was no opiate effect for naloxone to reverse. This seems unlikely since, in the rat, endogenous opiates are released in response to restraint stress (Amir and Amit, 1979) as well as in response to repeated presentation of shock (Chance, White, Krynock and Rosencrans, 1978). Secondly, endogenous opiates may have been released, but they may not have participated in the classical conditioning process. A third possibility is that endogenous opiates influenced the

expression of the HR CR, but that this effect was not due to their activity at the mu receptor, and therefore was not affected by the administration of naloxone. Naloxone, which binds preferentially to the mu receptor, may not antagonize the effects of endogenous ligands that are acting at different receptors.

The results of Experiment 2 contrast with those of Gallagher et al. (1981) who have shown that naloxone injections directly into the central nucleus of the amygdala, which contains both mu and delta receptors (Goodman and Snyder, 1982), enhanced HR CRs in the rabbit. Gallagher et al. (1981) suggested that the release of endogenous opiates in the central nucleus of the amygdala attenuates the HR CR. An alternative explanation of Gallagher et al.'s (1981) results would be that locally high concentrations (they injected 2.5 nmoles bilaterally into the amygdala) of naloxone exert a non-specific activating influence on the learned response.

Heart rate URs were accelerative in each of the naloxone groups and in the saline group, and remained stable throughout the 20 acquisition trials. If the release of endogenous opiates had influenced the UR by acting at an opiate receptor (i.e. mu receptor), then naloxone, which blocks opiate receptors, should have reversed the effect. Thus, if endogenous opiate activity normally reduces the

magnitude of the HR UR by a mu receptor mechanism, then naloxone should result in an enhanced HR UR. The failure of naloxone to have any effect on HR URs implies that the endogenous opiates have little influence on HR URs. This outcome was not unexpected because a number of previous experiments have failed to demonstrate that naloxone alters reactivity to shock (Goldstein et al., 1976; Smith and McKearney, 1977).

Naloxone had very little effect on baseline HR. The .1-mg/kg dose of naloxone did lower baseline HR during extinction. This result is difficult to interpret since neither the 1-mg/kg nor the 10-mg/kg doses had an effect on baseline HR. If anything, one might expect naloxone to produce tachycardia if it were antagonising the effects of endogenous opiates.

EXPERIMENT 3

Rationale

The 10-mg/kg dose of morphine in Experiment 1 decreased the magnitude of the HR ORs, of the CRs, of the URs and suppressed baseline HR. By contrast, the 5-mg/kg dose decreased the HR ORs, the URs, and baseline HR, but had no effect on the HR CRs. The different effects of the two doses on the CRs and URs raised the possibility that the the effect of morphine on the HR CR was not entirely due to its analgesic activity. Nevertheless, it seems reasonable

to conclude that the adverse effect of 10-mg/kg of morphine was primarily due to an analgesic mechanism. At the same time however, morphine could have interfered with the HR CR as the result of a general effect on cardiovascular function. A morphine produced modification of cardiac function could also help explain the OR and UR decreases and shift in baseline HR shown by morphine groups.

A distinction between morphine's learning and/or performance effects on the HR CR cannot be made given the design of the first experiment. One way to study this distinction would be to condition a group under morphine, and then inject naloxone to block morphine's effects. In Experiment 3, morphine was given prior to conditioning as was the case in Experiment 1. For one group, naloxone was then administered after a number of conditioning trials, and a number of non-reinforced test trials were then given to determine if a latent HR CR appeared, or if the CR remained depressed. The test phase of the experiment had three possible outcomes: 1. Naloxone could uncover a latent test-phase CR in the group that received morphine prior to conditioning. This result would imply that morphine exerted a major effect on the performance of the HR CR and not on the learning of the CR. 2. Naloxone could fail to reverse morphine's attenuation of the HR CR; this would indicate that morphine had little effect on performance and that the

drug had a primary effect on learning. 3. Naloxone could have produced CRs that were larger than those exhibited under morphine, but smaller than HR CRs of a control group that received saline before conditioning. This outcome of partial recovery would imply that morphine affected both learning and performance.

A second feature of Experiment 3 involved an assessment of the effects of morphine on an already established HR CR. This was accomplished by giving conditioning trials to a group injected with saline. Subsequently the group was divided into three subgroups: one subgroup was given saline and labelled S-S; a second subgroup was given naloxone and labelled S-N; the third group was given morphine and labelled S-M. All subgroups were given non-reinforced trials under the new drug treatment. If morphine affects the performance of the HR CR then the HR CR of the S-M group should be attenuated immediately after the morphine injection.

Method

Subjects

Forty-nine male rats of the Sprague-Dawley strain (Charles River), weighing between 275 and 325 g were employed as subjects in Experiment 3. Rats were maintained under the same feeding and lighting schedules described for Experiment 1.

Apparatus

Restraint and stimulus parameters for Experiment 3 were identical to those employed in Experiment 1. Heart rate was recorded directly onto magnetic disks by means of the Apple // computer lab control system described for Experiment 1. Difference scores for HR were calculated in an identical manner as that described for Experiment 1.

Procedure

Initially, there were two groups of rats: A morphine group (N= 21) that received 10 mg/kg of morphine and a saline group (N=28) that received an equivalent volume of saline. Each group was injected with the appropriate agent subcutaneously in the neck, 15 min prior to the CS-alone phase (phase 1) of the experiment. After the CS-alone phase, each group received 20 reinforced conditioning trials in which a CS+ was paired with a .5 sec shock at a 10-sec CS-US interval, and 20 trials in which a CS- was presented for 10.5-sec without shock.

At the completion of the conditioning phase, the morphine and saline groups were removed from the sound isolation chamber and given a second subcutaneous injection. The morphine group was divided into two subgroups: one subgroup, labelled M-S (N=10), received saline (1 ml/kg) and the other, labelled M-N (N=11), received naloxone (1mg/kg). The main saline group (N=28) was divided into 3 subgroups:

one subgroup, designated S-S (N=10), received an injection of saline, the second subgroup, designated S-M (N=10) received morphine (10 mg/kg), and a third subgroup, designated S-N (N=8) received naloxone (1mg/kg).

Fifteen min after the second drug injections, all groups were given four presentations each of the CS+ and the CS- without shock. These test trials allowed an assessment to be made of the effects of various drug combinations on established HR CRs in the absence of further conditioning. The non-reinforced test trial paradigm eliminated the possibility that the appearance of HR CRs in the morphine-naloxone (M-N) groups could be due to new learning.

In the next phase, the reconditioning phase of the experiment, 10 additional discrimination trials were given; there were five trials with the CS+ being paired with the US and five trials with the CS- being given alone. This phase was included to examine the possibility of any change in the HR CRs of various groups as a consequence of further conditioning under the new drug condition. In the final phase of the experiment, each group was given five trials with the CS+ paired with the US and five US alone trials. This phase of was included in order to assess the effects of the HR CR on the subsequent HR UR. The US-alone trials provided direct information on this possibility.

In each phase, trial types were presented in a quasi-random order. Heart rate responses depicted in the figures below were averaged over the five measurement intervals of each CS. Measurement interval was included as a factor in each of the statistical analyses reported below, and any significant effects involving measurement interval and the groups factor are described in the results section. Frequency of the CS was included as a factor in initial statistical analyses and was not statistically significant.

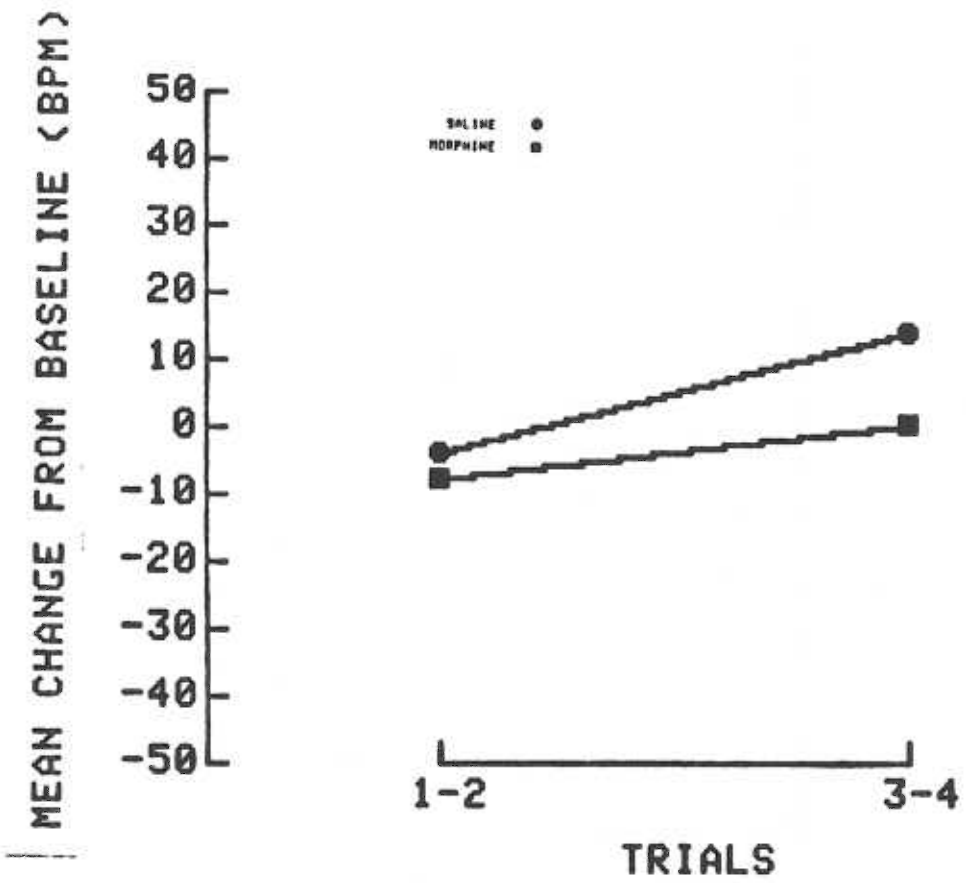
Results

As was true of Experiment 1, HR was averaged over measurement intervals for purposes of plotting the data in figures, but was included as a factor in all relevant ANOVs. In general, the HR CRs increased in magnitude over measurement intervals being larger at the end than at the beginning of the CS+, just as in Experiment 1.

Orienting Responses

Heart rate responses of the morphine and saline groups to preconditioning presentations of the CSs averaged over both CSs, and over two-trial blocks are shown in Figure 12. The responses to the two CSs were averaged after an analysis of variance showed that there were no significant

Figure 12. Mean pre-CS minus CS heart rate responses of the saline and the morphine groups averaged over type of CS in successive blocks of two pre-conditioning trials.


MEAN CHANGE FROM BASELINE (BPM)
50
40
30
20
10
0
-10
-20
-30
-40
-50
SALINE
MORPHINE
1-2
3-4
TRIALS

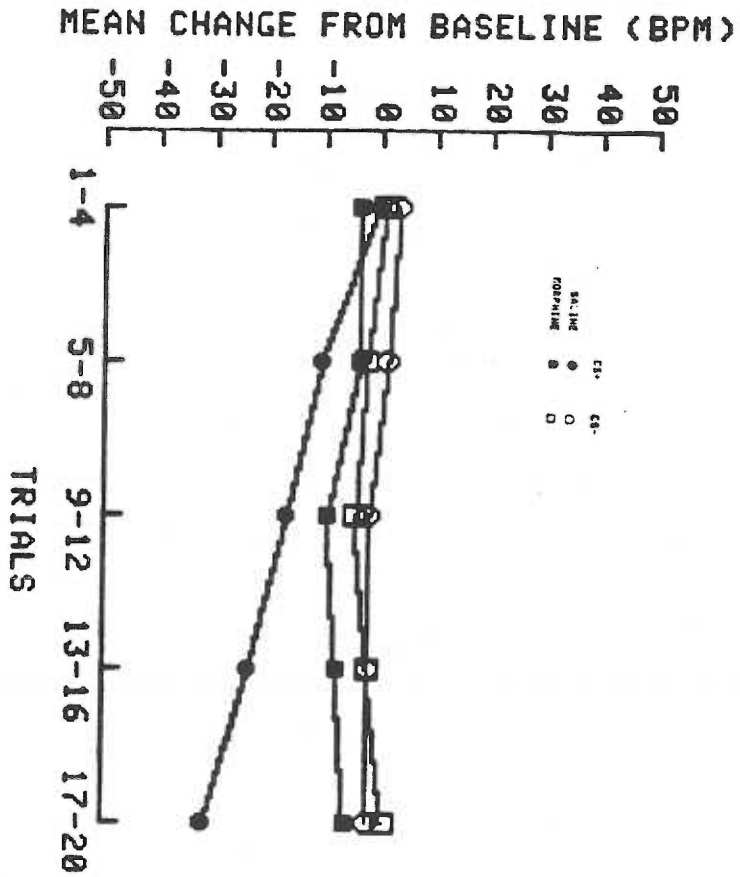
effects relating to the frequency of the CS. Separate ANOVs also revealed that the subgroups within each main treatment condition were not reliably different from each other allowing a single morphine group and a single saline group to be formed.

Figure 12 shows that the HR ORs of both groups were similar and decelerative in direction during the first trial block. In the second block of CS-alone trials, ORs of the saline group became accelerative, while in contrast, HR of the morphine group changed very little from baseline. A 2 x 2 x 2 x 5 (groups x trial blocks x type of CS x measurement intervals) ANOV revealed a significant main effect due to groups, $F(1, 47) = 15.26$, $p < .01$, and a significant groups x trial blocks x measurement interval interaction, $F(4, 180) = 2.98$, $p < .05$. Follow-up 1-way ANOVs at each trial block revealed that HR ORs of the saline group were more accelerative than those of the morphine group on the second trial block, during the first ($p < .01$), the second ($p < .01$) and third ($p < .05$) measurement intervals.

Conditioned Heart Rate Responses

Conditioning. Heart rate responses to the CS+ and the CS- during the conditioning phase of the experiment are depicted for the morphine and saline groups in Figure 13. Each data point represents a four-trial average of the HR difference scores averaged over measurement intervals. The

Figure 13. Mean pre-CS minus CS heart rate responses of the saline and morphine groups in successive blocks of four conditioning trials.


MEAN CHANGE FROM BASELINE (BPM)
-50
-40
-30
-20
-10
0
10
20
30
40
50
1-4
5-8
9-12
13-16
17-20
TRIALS
SALINE
FOSFMIME
CS+
CS-

data were averaged over the subgroups within the morphine and saline conditions after separate ANOVs showed that there was only one significant effect involving the subgroup dummy variables. This effect was a group x trial blocks interaction within the saline condition, and was due to small but reliable group differences in the second trial block. There were no other significant group differences in any of the remaining trial blocks. It can be seen in Figure 13 that the HR CR to the CS+ was a deceleration for the saline group and that the CR developed gradually over the 20 conditioning trials. The responses of the saline group to the CS- were generally small magnitude decelerations or accelerations. By contrast, the morphine group showed a relatively small HR deceleration to the CS+ that was only slightly different from its responses to the CS-.

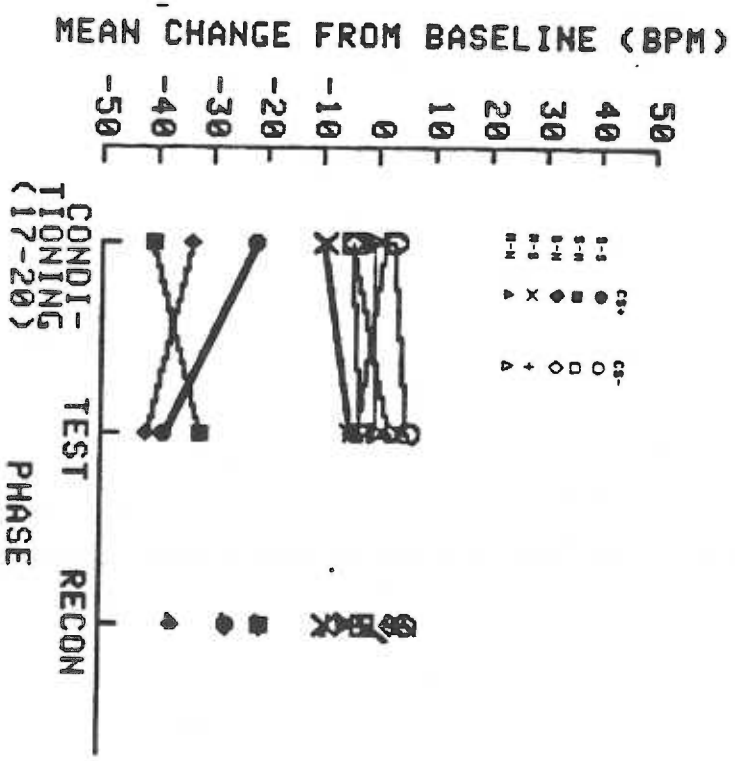
A 2 x 5 x 2 x 5 (groups x trial blocks x type of CS x measurement interval) ANOV on the combined saline and combined morphine group, yielded significant effects due to groups, $F(1, 47) = 5.06$, $p < .01$, trial blocks, $F(4, 188) = 14.75$, $p < .01$, and type of CS, $F(1, 47) = 18.33$. There were a number of significant interactions involving: groups x type of CS, $F(1, 47) = 18.33$, $p < .01$,, groups x trial blocks x type of CS, $F(4, 188) = 6.65$, $p < .01$.

The significant group x trial blocks x type of CS

interaction was probed by separate group x trial blocks ANOVs on CS+ and CS- responses. The ANOV on CS- responses failed to yield any significant effects. The ANOV on CS+ responses revealed a significant groups effect, $F(1, 47) = 12.82$, $p < .01$. a significant trial blocks effect, $F(4, 188) = 14.66$, $p < .01$, and a significant group x trial blocks interaction, $F(4, 188) = 14.66$, $p < .01$. Follow-up 1-way ANOVs indicated that the responses of the two groups to the CS+ differed significantly on trial block 3 ($p < .05$), trial block 4 ($p < .01$) and trial block 5 ($p < .01$).

Conditioning and Test Phase The HR responses to the CSs of each subgroup within the saline and morphine conditions averaged over the last four conditioning trials (17-20), over the four test trials, and over the five reconditioning trials are shown in Figure 14. In general, the HR responses of the M-S and M-N groups during the test phase were quite similar to their responses during the final block of conditioning. The M-S and the M-N groups continued to respond to the CS+ and the CS- with very small HR changes and showed only modest evidence of discriminative conditioning. Thus, there was no indication that naloxone reversed the effects of morphine allowing a major HR CR to appear. The M-N and M-S groups during the final block of conditioning trials and the test phase were compared in a 2

Figure 14. Mean pre-CS minus CS heart rate changes of each group for the final four-trial block of conditioning, the test phase, and the reconditioning phase.


MEAN CHANGE FROM BASELINE (BPM)
-50
-40
-30
-20
-10
0
10
20
30
40
50
CONDI-
TIONING
(17-20)
TEST
RECON
PHASE

x 2 x 2 x five (groups x phase x type of CS x measurement intervals) ANOV. There was a significant effects of group, $F(2, 25) = 1.37$, $p > .05$, which was due to the slightly larger responses of the M-S group over the M-N group. There was also a significant type of CS x measurement interval interaction, $F(4, 100) = 5.34$, $p < .01$, which indicated that reliable conditioning occurred in the morphine groups.

Turning to the saline subgroups, it may be seen that the S-S and S-N groups showed an increase in HR CR magnitude from the end of conditioning to the test trials, whereas the S-M group given morphine showed a decrease. Responding to the CS- was similar in the three groups. A 3 x 2 x 2 x five (group x phase x type of CS x measurement intervals) ANOV on the S-S, the S-M and the S-N groups revealed a significant groups x phase x type of CS interaction. This interaction was probed by using separate groups x phase ANOVs for CS+ and CS-. No significant effects were found for CS-. The ANOV on CS+ responses revealed a significant groups x phase interaction, $F(2, 25) = 4.69$, $p < .05$, indicating that there were group differences in responses to the CS+.

During reconditioning, the M-N and M-S groups continued to respond with small HR changes to both CS+ and CS-. A 2 x 2 (groups x type of CS) ANOV revealed a significant effect due to type of CS, $F(1, 19) = 5.19$, $p < .05$, indicating that reliable conditioning was still

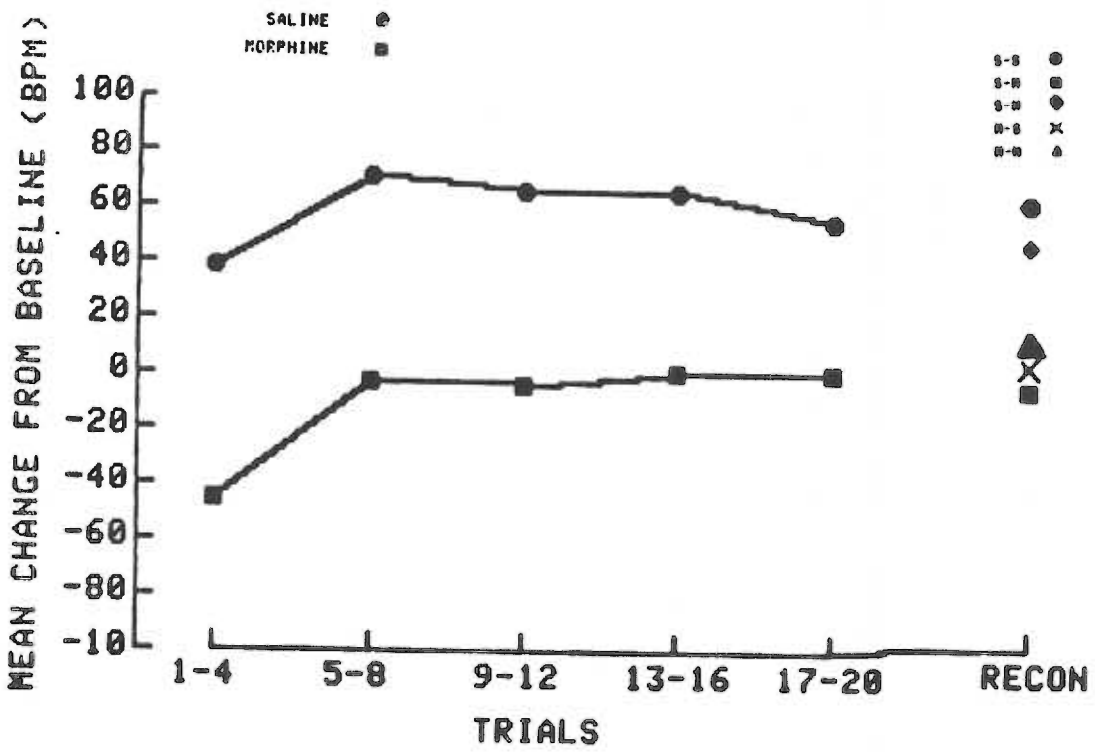
present in the M-S and M-N groups during reconditioning. The responses of the S-M group that received morphine prior to the test phase continued to be slightly smaller than those of the S-S and S-N groups, although not significantly so.

Unconditioned Responses

The HR URs of each group averaged over the three post-shock measurement intervals during the various phases of the experiment are depicted in Figure 15. During conditioning, shown on the left, the URs were averaged over blocks of four trials and over the subgroup dimension. The right of the figure shows HR URs averaged over the five reconditioning trials for each subgroup.

Conditioning Phase. Consistent with what was observed in Experiment 1, the URs of the morphine groups were originally substantial decelerations (first trial block) after which there was almost zero change to shock. In sharp contrast, the saline group showed consistent and large magnitude HR accelerations to shock. Because the second injection condition was a dummy variable at this point in the experiment, the two morphine groups (M-N and M-S) were combined into a single group, as were the three saline groups. Separate ANOVs showed that there were no significant effects due to the dummy variable before the

Figure 15. Mean pre-CS minus post shock heart rate changes of the saline and morphine groups plotted over successive blocks of four conditioning trials, and for a five-trial block of reconditioning (RECON) trials.


SALINE
MORPHINE
MEAN CHANGE FROM BASELINE (BPM)
100
80
60
40
20
0
-20
-40
-60
-80
-10
1-4
5-8
9-12
13-16
17-20
RECON
TRIALS

groups were combined.

Heart rate URs of the combined-saline group were compared with the HR URs of the combined-morphine group by means of a 2 x 5 (groups x trial blocks) ANOV. There was a significant groups effect and a significant groups x trial blocks interaction, $F(4, 188) = 5.53$, $p < .01$. Follow-up 1-way ANOVs showed that HR URs of the groups were significantly different at each trial block ($p < .01$).

Examination of each of the first four conditioning trials indicated that HR URs of each of the three saline groups consisted of low magnitude accelerations in the first and third measurement intervals, and a moderate deceleration in the second measurement interval. Unconditioned responses of the saline groups became accelerative on trials 2, 3 and 4. Heart rate URs of the morphine groups were decelerative through out the first 4 trials.

Reconditioning

It will be recalled that the reconditioning trials shown to the right in Figure 15 took place place after the second injection had been administered and they provided the first opportuninty to evaluate the effects of the second injection on HR URs. It may be seen that the HR URs of the M-N group and the M-S groups continued to be similarly depressed, even though the M-N group had received naloxone at that point. The morphine given to the S-M group dropped

their UR to the level of the M-N and the M-S groups.

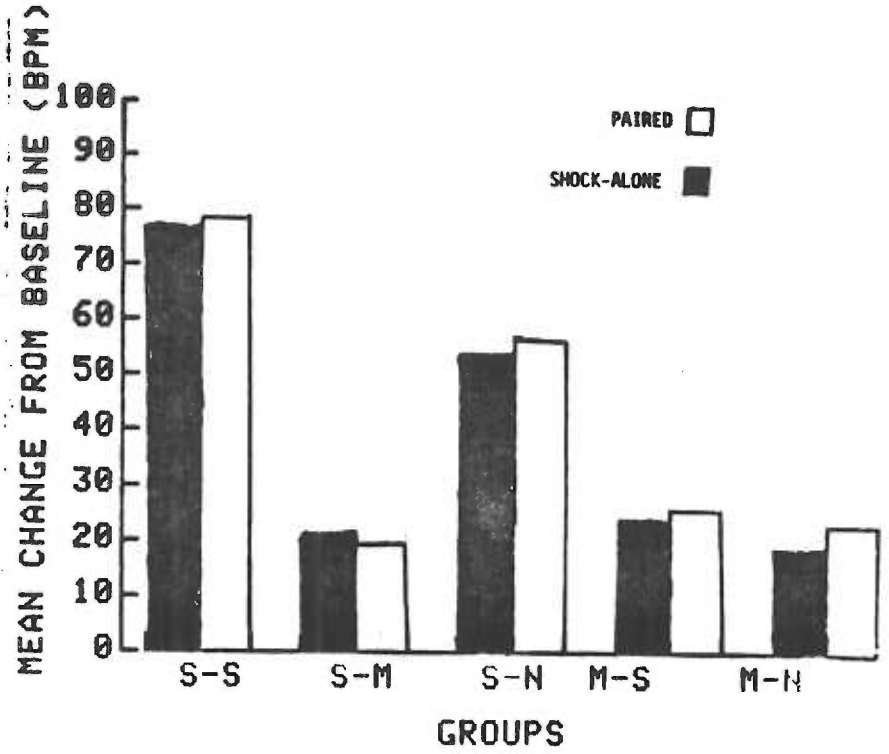
A one-way (groups) ANOV was carried out on HR URs of all five groups during the reconditioning phase. There was a significant main effect $F(4, 44) = 15.25$, $p < .01$, and a Neuman-Keuls analysis indicated that reconditioning phase HR URs of the M-S, the M-N and the S-M groups were significantly smaller than HR URs of the S-S and the S-N groups ($p < .01$).

The major results from the reconditioning phase of the experiment are that naloxone failed to reverse the effect of morphine on HR CRs and on HR URs of the M-N group. Morphine had only a relatively minor adverse effect on the expression of an established CR of the S-M group, did markedly alter the magnitude and form of the post shock HR URs. Naloxone had no effects on an established HR CR or on post-shock URs of the S-N group.

Shock Alone Phase. Post-shock URs averaged over trials and measurement intervals are shown in Figure 16. As this figure shows, URs of the M-S, the M-N and the S-M groups remained depressed relative to URs of the S-S and S-N groups. It was apparent that the occurrence of a decelerative CR had no effect on the magnitude of the UR.

A 5 x 2 (groups x trial-type) ANOV of phase 5 URs revealed that there was was a significant groups effect, $F(4, 44) = 12.82$, $p < .01$. A Neuman-Keuls test showed

Figure 16. Mean pre-CS minus post shock responses of each group plotted for paired and shock alone trials.


MEAN CHANGE FROM BASELINE (BPM)
100
90
80
70
60
50
40
30
20
10
0
PAIRED
SHOCK-ALONE
S-S
S-M
S-N
M-S
M-N
GROUPS

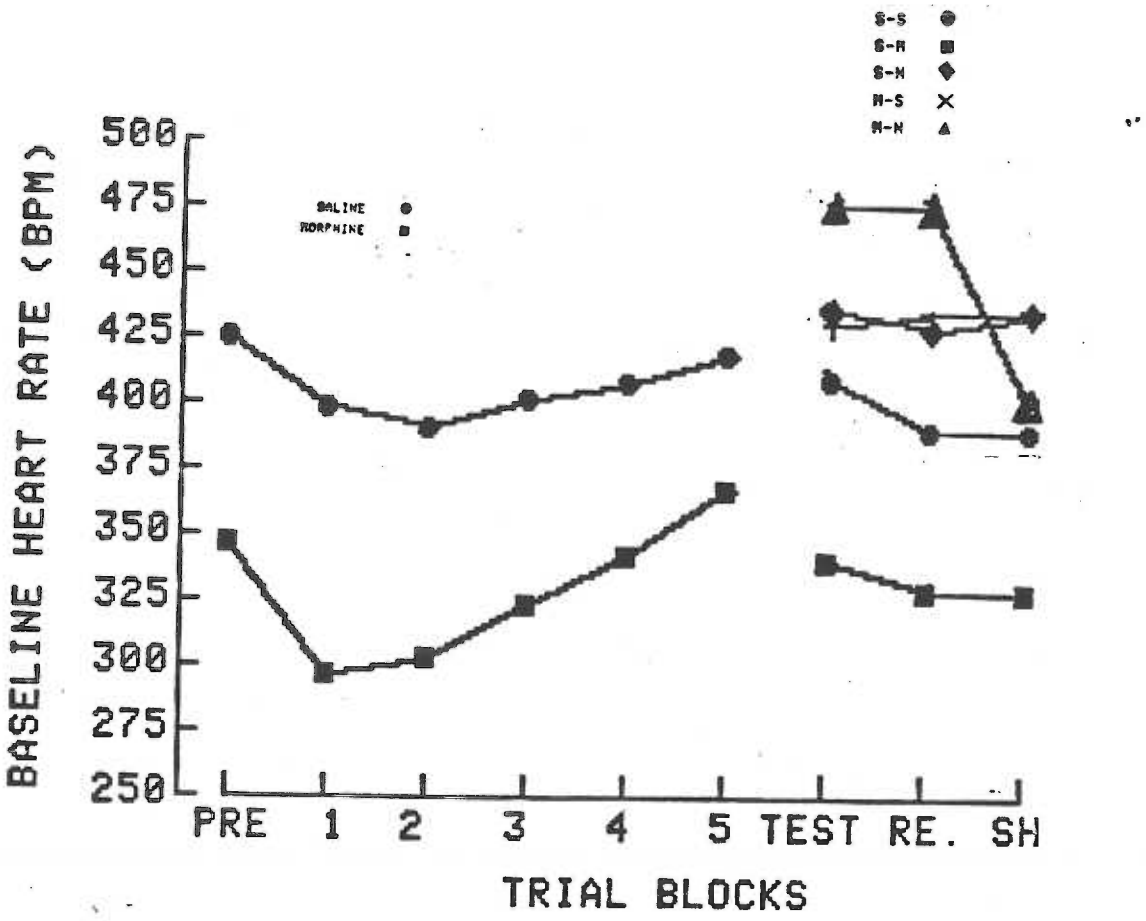
that URs of the S-M, the M-S and the M-N groups were significantly ($p < .05$) smaller in magnitude than URs of the S-S group.

Baseline Heart Rate

Baseline HRs of each group averaged over the four pre-conditioning trials, over successive blocks of four conditioning trials, and over the trials within the test, reconditioning and US-alone phases are shown in Figure 17. As was true of experiment 1, baseline HRs of the morphine groups (M-S and M-N) were depressed during pre-conditioning and at the beginning of the conditioning phases. Baseline HR of the morphine groups recovered toward control levels during the final three blocks of conditioning. In the test phase and reconditioning phase, after an injection of naloxone was given, baseline HR of the M-N group exhibited a sharp increase relative to baseline HR of the M-S group that received an injection of saline.

A 2 x 9 (groups x phase) ANOV on the morphine groups yielded a significant effect due to phase, $F(8, 152) = 50.76$, $p < .01$, and a significant group x phase interaction, $F(8, 152) = 2.18$, $p < .05$. Separate 1-way ANOVs established that baseline HR of the M-N group was significantly higher than baseline HR of the M-S group during the test ($p < .05$) and reconditioning phases ($p < .05$).

Figure 17. Mean pre-CS heart rate of the saline and the morphine groups for preconditioning and conditioning trial blocks (Left). Mean pre-CS heart rate of each subgroup for the test, the reconditioning (RE) and the shock (SH) phases of Experiment 3.


S-S
S-M
S-N
M-S
M-N
SALINE
MORPHINE
BASELINE HEART RATE (BPM)
500
475
450
425
400
375
350
325
300
275
250
PRE
1
2
3
4
5
TEST
RE.
SH
TRIAL BLOCKS

Baseline HRs of the S-S and the S-M saline-groups were similar to one another during the preconditioning and conditioning phases of the experiment with the baseline HR of the S-N group being slightly but consistently higher. Figure 17 suggests that baseline HR of the S-N group was elevated, relative to baseline HRs of the S-S and the S-M groups, during the test phase, the reconditioning phase and the shock phase. Baseline HR of the S-S group remained stable throughout conditioning, but decreased somewhat during the final 3 phases of the experiment. Baseline HR of the S-M group decreased dramatically after morphine was administered.

A 3 x 9 (groups x trial blocks) ANOV on baseline HR of the saline groups (i.e.S-S, S-M and S-N)indicated that there were significant main effects due to groups, $F(2, 25) = 12.29$, $p < .01$ and trial blocks $F(8, 200) = 10.20$, $p < .01$. There was also a significant groups x trial blocks interaction, $F(16, 200) = 7.12$, $p < .01$. Separate 1-way ANOVs at each trial block were employed to probe the significant group x trial block interaction. There was a significant difference in baseline HR , $F(2, 25) = 4.83$, $p < .05$, among the saline groups during the first trial block of conditioning. A Neuman-Keuls comparison of saline-group means revealed that the baseline HR of the S-N group was significantly higher than the baseline HRs of the

two other saline groups during the first trial block. The saline groups also differed during the first conditioning trial block $F(2, 25) = 4.16$, $p < .05$, and during the final conditioning trial block of conditioning, $F(2, 25) = 7.49$, $p < .01$. Neuman-Keuls tests demonstrated that baseline HR was higher in the S-N group, than in the other saline groups ($p < .05$) during the first and last conditioning trial blocks. Saline-group baseline HRs were also significantly different during the test phase, $F(2, 25) = 26.41$, $p < .01$, and a Neuman-Keuls test indicated that baseline HR of the S-M group was significantly lower than baseline HR of the S-S and the S-N groups ($p < .01$). Baseline HR of the saline groups differed during the reconditioning, $F(2, 25) = 22.46$, $p < .01$, and the shock phases of the experiment, $F(2,25) = 20.17$, $p < .01$. A Neuman-Keuls test of reconditioning baseline HR showed that baseline HR of the S-M group was significantly lower than baseline HR of the S-S and the S-N groups ($p < .01$), and baseline HR of the S-S group was significantly lower than baseline HR of the S-N group ($p < .05$). During the shock alone phase baseline HR of the S-M group was significantly lower than baseline HR of the S-N group ($p < .01$).

Experiment 3 : Discussion

In Experiment 3, naloxone failed to reverse the

adverse effects of morphine on the HR CR and UR, although it did appear to block morphine-produced suppression of baseline HR. These differential effects of naloxone suggest that morphine's reduction of baseline HR and its attenuation of the HR UR and CR were mediated by different receptor sub-types. Regardless of the mechanism, the absence of a normal HR UR to shock when the M-N group was given naloxone, suggested that the effects of morphine were not blocked, as the results of a large number of studies suggest they should have been (Dewey and Harris, 1975). A fourth experiment was carried out in order to determine whether 1 mg/kg of naloxone was effective in blocking the analgesic effects of 10 mg/kg of morphine. The findings of Experiment 3 are discussed more fully below, following a description of the design and outcome of Experiment 4.

EXPERIMENT 4

The purpose of Experiment 4 was to assess the effects of naloxone on morphine-induced analgesia using a standardized tail-flick test. The tail-flick response to radiant heat is a spinal reflex that has been found to be inhibited by mu receptor agonists such as morphine, and to be returned to normal by naloxone (Dewey and Martin, 1975). The injection-test interval and the dose parameters employed

in the fourth experiment were chosen to correspond to those employed in Experiment 3.

Method

Subjects

Eleven male rats of the Sprague-Dawley strain, weighing between 280 and 350 g, were used as subjects in Experiment 4. Rats were maintained under conditions that were identical to those described for Experiments 1, 2 and 3.

Apparatus

Rats were restrained in the plastic holders described in Experiments 1 and 2. The tail flick apparatus consisted of a wooden platform that was painted black to help control temperature variation. The platform contained a wooden slot into which the rat's tail was placed during testing. A Sylvania, 250 Watt, 125 Volt, resistant infrared lamp, located 15 cm above the rat's tail provided the source of radiant heat. The heat lamp was not focussed on a given spot on the rat's tail; however, the distal 6 cm to 7 cm of the rat's tail was blackened with polygraph ink in order to increase the amount of energy absorbed by this part of the tail. A Hunter photosensitive relay automatically turned off the heat lamp and a Hunter clock counter when a rat removed its tail from the wooden slot. Pilot work

indicated that the response latency of non-injected control rats averaged 7 to 8 sec which is comparable to latencies usually reported under similar conditions (Dewey and Harris, 1975).

Procedure

Two rats were tested in a squad and two squads were tested on a typical day. Rats were removed from their home cages, restrained in the plastic holders and their tails were dabbed with polygraph ink. Animals were then allowed 15 min to adapt to restraint. The rat's tail was then placed in the wooden slot below the heat lamp, and after 1 min, the heat lamp and clock counter were turned on. After a rat made a response its tail was placed back in the wooden slot and another trial was given 1 min later. If a rat failed to make a response within 20 seconds the heat lamp was turned off and the animal was given a latency of 20 seconds for that trial. A total of five trials were given in a block. If a rat made a response during the 1-min intertrial interval its tail was immediately placed in the wooden slot and an additional 1 min was allowed to elapse before the heat lamp was turned on.

There were two groups of subjects: a morphine group and a saline group. Both groups were first given two blocks of five trials. The morphine group was then given a subcutaneous injection of morphine (10 mg/kg). The saline

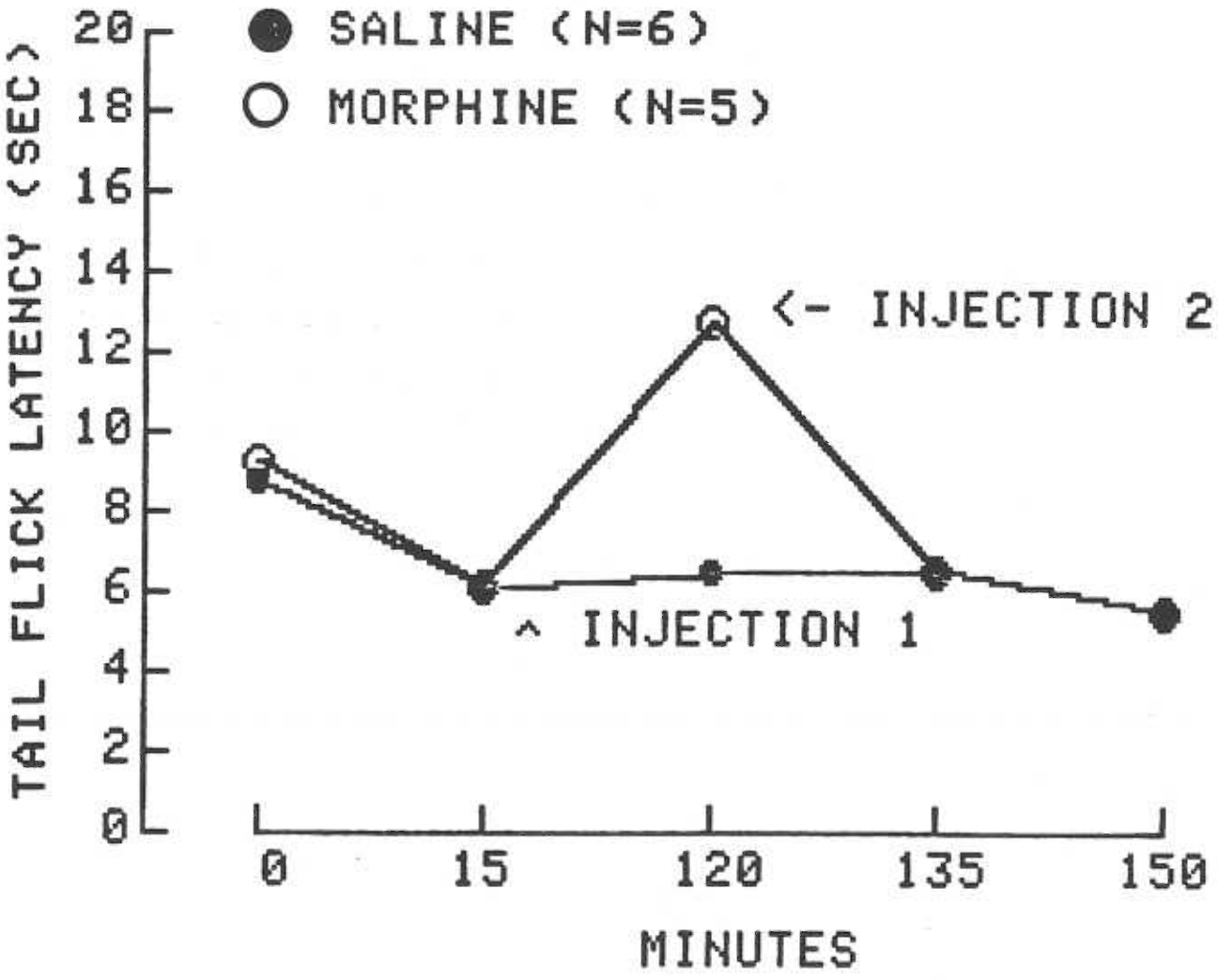
group was given 1-ml/kg of saline. Two hours later, each group was given five additional trials, and both groups were then immediately injected with 1 mg/kg of naloxone. After a 15-min drug-absorption interval, each group received two additional blocks of five trials each. The timing of these last two sets of trials under naloxone was comparable to the times that the HR URs were measured under naloxone in Experiment 3.

Results

Mean tail flick latencies for each group averaged over each trial block are shown in Figure 18. This figure indicates that the latencies of the two groups were quite similar at all trial blocks, except the third, where the latency of the morphine group was greater than the latency of the saline group. The third trial block was given 2 hr after the morphine injection, but prior to the naloxone injection; there were no differences between the two groups on trial blocks 4 and 5 that were given after the naloxone injection.

A 2 x 5 (drug treatment x trial blocks) ANOV was employed to test the reliability of these observations. There were significant main effects due to drug, $F(1, 9) = 5.89$, $p < .05$ and trial blocks, $F(4,36) = 13.07$, $p < .01$. There was also a significant interaction between drug

Figure 18. Mean tail flick latencies of the saline and morphine groups in succesive blocks of five trials.


SALINE (N=6)
MORPHINE (N=5)
TAIL FLICK LATENCY (SEC)
20
18
16
14
12
10
8
6
4
2
0
<- INJECTION 2
^ INJECTION 1
0
15
120
135
150
MINUTES

treatment and trial blocks, $F(4,36) = 5.06$, $p < .01$. A follow-up test confirmed that the only significant group difference occurred at trial block 3 ($p < .01$).

Discussion

The results of the fourth experiment indicate that morphine (10 mg/kg) produced analgesia 120 minutes after it was injected and suggest that the same dose of morphine should have produced analgesia in Experiments 1 and 3. The results of Experiment 4 also imply that naloxone probably reversed the analgesic effects of morphine when it was injected prior to the test phase of Experiment 3. The tail flick response is a spinal reflex that is particularly sensitive to the naloxone reversible analgesic effects of mu receptor agonists. The time and dose parameters in Experiment 4 corresponded to those that were used in the third experiment. Thus, it seems reasonable to conclude that naloxone was effective in antagonising any mu receptor activity of morphine during the test phase of Experiment 3. In the second experiment, the effects of morphine on HR URs were not reversed by naloxone which suggests that the attenuation of the HR UR by morphine is not mediated by the mu-receptor.

Discussion: Experiment 3 and Experiment 4

The major findings of Experiments 3 and 4 are summarized below: The HR CR of the morphine group was impaired relative to the CR of the saline group throughout conditioning. The morphine-induced CR decrement exhibited by the M-N group was not reversed when naloxone was injected prior to the test phase. Morphine had only a minor effect on the established HR CR of the S-M sub-group during the test and reconditioning phases of Experiment 3. The HR UR of the morphine group was attenuated relative to the UR of the saline group, and the adverse effect of morphine on the HR UR of the M-N sub-group was not reversed by naloxone. Baseline HR of the morphine group was significantly lower than baseline HR of the saline group during the conditioning phase of the experiment. Naloxone produced a sharp rise in the baseline HR of the M-N group when it was injected prior to the test phase. Baseline HR of the M-S subgroup also increased during conditioning, and remained at control levels during the test and reconditioning phases. Morphine lowered the test phase, the reconditioning phase and the shock phase baseline HR of the S-M group. Experiment 4 demonstrated that the analgesic effects of morphine (10 mg/kg), as measured by the tail-flick test, were reversible by naloxone (1 mg/kg) 120 min after morphine was injected.

The primary aim of Experiment 3 was to distinguish between the learning and performance effects of morphine on the HR CR of the restrained rat. Outcomes involving the M-N and the S-M subgroups provide the bulk of information on the learning vs. performance question and are the primary focus of the discussion that follows.

The fact that morphine failed to have a major influence on the test-phase CRs of the S-M group suggested that morphine had no major effect on performance. The finding that naloxone failed to uncover a latent CR in the M-N group supports the idea that morphine exerted a minor effect on performance and a major effect on learning. The present results are consistent with previous work that has shown that the central injection of levorphanol impairs the development of the HR CR of the rabbit, and are also consistent with the finding that a peripheral injection of morphine impairs the devlopment of pole jump avoidance responses of rats (Banurjee, 1971).

An alternative explanation of the failure of naloxone to reverse the morphine produced CR decrement was that the dose of naloxone was inadequate and as a result, naloxone failed to reverse <u>any</u> of morphine's effects. This seems unlikely because the 1-mg/kg dose of naloxone reversed the analgesic effects of morphine in Experiment 4 and bradycardic effects of morphine in Experiment 3. The

morphine-induced reduction in baseline HR and morphine's suppression of the tail-flick response may be primarily mediated by the mu-receptor activity of the drug (Dewey and Harris, 1975; Holaday and Ward, 1982). The fact that naloxone reversed the effects of morphine on baseline HR in Experiment 3 and the tail-flick response in Experiment 4 suggests that other mu receptor effects of morphine should have been reversed by naloxone. The results of Experiment 3 raise the possibility that the adverse effect of morphine on the HR CR was not due to a mu receptor mediated performance decrement.

This finding leaves open the possibility that morphine produced a performance decrement that was mediated by the delta or kappa receptors. If this were the case, then one would have expected a major decrement in the CR of the S-M group after morphine was injected. The fact that established CRs of the S-M group were only slightly altered after the injection of morphine indicated that morphine exerted little influence on the performance of the CR. Taken together, the finding that naloxone failed to reverse the morphine produced CR decrement, and that morphine did not have a major effect on an established HR CR strongly suggest that morphine interfered with learning and had a relatively minor effect on performance of the HR CR.

The minor effect of morphine on the established HR

CR of the S-M group contrasts with the recent findings of Mauk et al. (1982a,b) and Lavond et al. (1982) who have shown that peripheral or central injection of mu-receptor agonists impaired established aversively conditioned CRs. Mauk et al. (1982) have demonstrated that a classically conditioned nictitating membrane response is abolished within 8 min of an intravenous injection of morphine (5 mg/kg). Similarly, Lavond et al. (1982a) have shown that the intra-cisternal injection of morphiceptin impairs an established decelerative HR CR within minutes of injection. Species differences in sensitivity to drug effects, differences in conditioning paradigm, route of drug administration and response systems might account for some of the discrepancies beteen the present findings and those of Mauk et al. (1982a) and Lavond et al (1982). Nevertheless, it is surprising, in light of the previous findings of Mauk et al. (1982a), that morphine failed to exert any major effect on an established HR CR in the present experiment.

As in Experiment 1, morphine had a dramatic effect on the HR UR. In the first trial block, the morphine groups responded to shock with large decreases in HR. On later trials, the magnitude of decelerative responses to shock decreased and the response t shock seemed to habituate. This finding supports the idea that HR URs under morphine

were "OR-like" reactions to intense tactile or vibratory stimulation generated by the US. Morphine, while producing analgesia, does not interfere with other forms of tactile sensation (Jaffe and Martin, 1980). Accelerative HR URs, which are characteristic responses of rats to electrical shock (e.g. Fitzgerald and Tyler, 1970), were almost completely absent in the morphine group throughout conditioning.

Two important aspects of the UR data obtained from the reconditioning phase should be mentioned. First, naloxone failed to reverse the suppressive effects of morphine on HR URs of the M-N group, in that HR URs of the M-N group were no different from those of the M-S group given saline. The possibility that this result occurred because the dose of naloxone was inadequate or that the injection test interval was inappropriate seems unlikely because naloxone reversed the analgesic effects of morphine in Experiment 4. The fact that naloxone reversed the analgesic effect of morphine, but did not reverse morphine's suppressive effects on the HR UR could mean that the HR UR to shock may not be a reliable index of pain sensitivity. Thus, the action of morphine on the HR UR did not appear to be directly tied to its analgesic effects.

Morphine may have acted at delta or kappa receptors to interfere with the performance of the HR UR in a way that

was not related to analgesia. Results from Experiment 4 provide strong support for the view that the dose of naloxone employed in Experiment 3 blocked activity at the mu receptor. Naloxone also reversed the morphine-induced decrease in baseline HR which is also mediated by the mu receptor (Holaday and Ward, 1982). Thus, the results of the third and the fourth experiments support the notion that morphine's effect on HR URs were not primarily mediated by the mu receptor.

The primary reason for including the shock-alone phase in the third experiment was to determine if the occurrence of the HR CR had any effect on the subsequent UR. It was argued that HR URs were similar for the 5 mg/kg and 10 mg/kg groups in Experiment 1. However, it was possible that differences in HR URs between the two groups were masked by the occurrence of a large decelerative HR CR in the five mg/kg group. If the occurrence of a CR had an effect on the subsequent UR, then URs on trials in which shock was presented alone should differ from URs on trials in which shock was preceded by the CS+ and a CR occurred. It was found that the magnitude of the HR UR did not depend upon whether it was preceded by the CS+. Thus, the occurrence of a decelerative CR before the presentation of the shock had no effect on the HR UR.

Morphine lowered baseline HRs of the M-N and the M-S

groups. This finding is consistent with the work of others who have shown that morphine produces bradycardia in both awake (Holaday and Ward, 1982), and anesthetized rats (Feldberg and Wei, 1978). During the test phase, baseline HR of the M-S group was not different from baseline HRs of the S-S and the S-N groups. The steady rise in baseline HR of the M-S and the M-N groups during original conditioning was similar to the increase in baseline HRs of the 5-mg/kg and 10-mg/kg groups in Experiment 1. It could be argued that the gradual rise in baseline HR was a direct reflection of decreasing CNS levels of morphine as a function of time after drug injection. This interpretation seems unlikely because baseline HRs of the M-S group had risen to control levels within 3 hours of morphine injection. Mullis et al., (1979) have shown that the half-life of morphine in the CNS of the rat is five hours. It seems reasonable to conclude that baseline HR returned to normal even though morphine concentrations were still relatively high. One might argue that baseline HR returned to control levels because central morphine concentration fell below some threshold level. If this were the case, then one would have expected baseline HRs of the 5 mg/kg and 10 mg/kg groups to have risen at different rates during Experiment 1.

It is possible that the profound bradycardia produced by morphine triggered a compensatory cardiovascular

response resulting in a gradual increase in HR of both the M-N and the M-S. Thus, the sharp increase in baseline HR of the M-N group, observed after naloxone was injected, could have represented an unmasking of a compensatory response by naloxone blockade of the opiate receptor.

SUMMARY AND GENERAL CONCLUSIONS

In Experiment 1, three doses of morphine (.25, 5 and 10 mg/kg) were given prior to discriminative Pavlovian HR conditioning. The HR CR of the 10-mg/kg group was reduced relative to the HR CRs of the other groups. Heart rate CRs of the .25 and 5-mg/kg groups were no different from the HR CRs of the saline group. The HR ORs of each of the groups receiving morphine were attenuated in comparison with the HR OR of the saline group. Baseline HRs of the 5-mg/kg and 10-mg/kg groups were significantly lower than the baseline HRs of the saline and the .25-mg/kg groups. The HR URs of the 5 and 10-mg/kg groups were decelerative during the first five conditioning trials and were low magnitude accelerations during later conditioning trials. By contrast, HR URs of the .25-mg/kg group and of the saline group were accelerative throughout most of conditioning. A number of rats in the saline group exhibited decelerative HR URs on the first conditioning trial that were replaced by accelerative URs on later trials.

In Experiment 2 groups received either saline or naloxone (.1, 1, and 10 mg/kg) prior to Pavlovian HR conditioning. Heart rate ORs of the naloxone groups were attenuated relative to the HR OR of the saline group. Baseline HR of the .1-mg/kg group was significantly lower than the baseline HRs of the remaining groups during the preconditioning and extinction phases of Experiment 2. There were no additional significant effects involving naloxone treatment.

The results of Experiment 2 indicate that mu receptor activity of endogenous opiates plays only a minor role in the development of or performance of HR CRs and HR URs. Previous work has shown that endogenous opiates are released in response to restraint and repeated electric shock (Amir and Amit, 1979; Chance et al., 1978). Thus, it seems unlikely that the conditioning paradigm employed in the present study failed to evoke the release of endogenous opiates. Naloxone, which is primarily a mu receptor agonist, may not have reversed the possible delta or kappa mediated cardiovascular effects of endogenous opiates. Alternatively, endogenous opiates may not exert a major influence on CV activity in the conditioning situation employed here.

The purpose of Experiment 3 was to assess the effects of morphine on learning and performance of the HR

CR. An additional aim of Experiment 3 was to examine the effects of morphine and naloxone on an established HR CR. Morphine (10 mg/kg) or saline (1 ml/kg) injections were given to two groups of rats prior to Pavlovian HR conditioning. After 40 discrimination trials, the saline group was split into three sub-groups and given an additional injection: one group (S-S) received an injection of saline; a second group (S-M) received an injection of morphine (10 mg/kg); and a third group (S-N) was given an injection of naloxone (1 mg/kg). The morphine group was split into two subgroups after discrimination training and each was given a second injection: one group (M-S) received an injection of saline (1 ml/kg); and the other group (M-N) was given an injection of naloxone (1 mg/kg). All groups were then given a series of non-reinforced test trials that were followed by additional conditioning trials.

The HR CR of the morphine group was attenuated relative to the CR of the saline group throughout the conditioning phase of Experiment 3. The HR UR and baseline HR of the morphine group were also significantly different from the HR UR and baseline HR of the saline group during conditioning. The test-phase HR CR of the S-M group was only slightly different from the test phase CRs of the S-S and the S-N groups. The test phase HR CR of the S-S was slightly larger than the HR CR exhibited by the S-S group

during the final block of conditioning. The test phase HR CR of the M-N group was not different from the test phase HR CR of the M-S group, and the test phase HR CRs of both morphine groups appeared to be attenuated relative to the HR CRs of the saline groups. Baseline HR of the combined morphine group was lower than baseline HR of the saline group throughout conditioning. The test phase baseline HR of the M-N group rose sharply after naloxone was injected. Baseline HR of the M-S group was similar to the baseline HR of the saline groups during the test phase. The HR UR of the morphine group was smaller in magnitude than the HR UR of the saline group throughout the conditioning phase of Experiment 3; naloxone failed to reverse morphine's suppression of the reconditioning phase HR UR of the M-N group.

The present research suggests that morphine interferes with learning and not performance of the declerative HR CR of the retrained rat. The adverse effect of morphine on the HR CR did not appear to be related to a morphine-induced reduction in baseline HR, or to morphine-produced changes in the HR UR to shock.

Naloxone did not reverse morphine-induced suppression of the HR UR; the same dose of naloxone was found to reverse the analgesic effects of morphine. The failure of naloxone to reverse the effects of morphine on the UR suggests that

morphine exerted an effect on the performance of the HR UR that was not directly related to its analgesic effects.

The conditioning paradigm employed here provided a useful way for examining the learning and performance effects of morphine, a mu receptor agonist. The recent development of irreversible mu and delta receptor antagonists (Rice, Jacobson, Burke, Bajwa, Streaty and Klee, 1983) may allow a more precise characterization of the behavioral functions of each opiate receptor sub-type. The new mu and delta receptor antagonists could be used in the present conditioning paradigm in order to examine the role that each receptor sub-type plays in the learning and performance of the HR CR.

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