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Differential Effects on Feeding Behavior in Satiated Rats by Activation of Selected Opioid Receptor Fields

A Thesis

in

The Department

of

Psychology

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

May 1988

ISBN 0-315-44886-5

ABSTRACT

Differential Effects on Feeding Behavior in Satiated Rats by Activation of Selected Opioid Receptor Fields

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Concordia University, 1988

Opioids in the ventral tegmental area are intrinsically rewarding. It was of interest to examine the ability of opioid microinjections into this region to elicit a natural reward such as feeding. The effects of morphine and the endogenous opioid peptide fragment dynorphin$_{1-13}$ on ingestive behavior were compared among several brain regions associated with a variety of opioid-mediated effects. Feeding and other behaviors were observed and recorded for a period of 15 minutes following opioid administration into the ventral tegmental area, the nucleus accumbens, the paraventricular nucleus of the hypothalamus, the substantia nigra - pars reticulata, or the periaqueductal gray area of freely moving, food satiated rats. Morphine and dynorphin$_{1-13}$ each elicited dose-dependent feeding within a short time following microinjection into the ventral tegmental area or the nucleus accumbens. Moreover, a 50,000-fold difference in potency was observed between the two ligands in their ability to produce feeding. Whereas the ED_{50} for morphine to elicit feeding was in the low nanomolar range, the ED_{50} for dynorphin$_{1-13}$ was in the low femtomolar range. This difference is consistent with the relative binding affinities of morphine and dynorphin$_{1-13}$ at kappa receptors. Highest feeding scores arose from injection of dynorphin$_{1-13}$ into the ventral tegmental area; this effect was naloxone-reversible, confirming that it was opioid-mediated. In the paraventricular nucleus and the substantia nigra, dynorphin$_{1-13}$ but not morphine produced

feeding. Feeding did not occur in response to microinjections of either drug into the periaqueductal gray. Differential effects of the two ligands on the initiation and maintenance of feeding depended on brain site. In the ventral tegmental area both opioids increased mean feeding bout durations, whereas in the nucleus accumbens dynorphin$_{1-13}$ increased the number of feeding bouts. Drinking behavior was typically preprandial and was not dose-related at any brain site. Grooming was enhanced only in response to dynorphin$_{1-13}$ in the substantia nigra. These findings are discussed in terms of a possible dissociation of the roles of different opioid receptor subtypes in naturally rewarding behavior. Methodological considerations in the evaluation of behavioral responses to intracerebral drug microinjections are also addressed.

ACKNOWLEDGEMENTS

Deepest appreciation is expressed to Michael Bozarth, my mentor, my teacher, and my friend, for his sound scientific advice and guidance, his kind support and encouragement, and his enduring patience and sense of humour.

Gratitude is extended to Roy Wise for his advice and very helpful criticisms, and for permitting me to attend his lab meetings and use some of his facilities. I would also like to thank Jane Stewart for helpfully providing much needed information on more than one occasion. Thanks also to Avram Goldstein, Blake Gosnell, Peter Kalivas, and Paul Wood for sending reprint packages that were of enormous assistance.

The value of my family's love, encouragement, and pride is beyond expression. I am also grateful to my Dad for helping me amass the courage to take the decisive steps in my chosen direction. From my Mum, I especially cherish the lighter moments such as her outrage that my thesis had to be defended.

The affection and encouragement of my friends has meant more than I could ever tell them. Special thanks to Aileen Murray, John Mitchell, Valarie Bozarth, Carlos Aragon, David Barer, Sylvia Nalbandian, Michael Anstead, Lynne-Marie Holland, Rosemary and Keith Patterson, and Trish and Rob Maynard, for the many ways of their true friendship.

Finally, I would like to acknowledge my rats, who made this research not only scientifically revealing but also a lot of fun.

Talking of Pleasure, this moment I was
writing with one hand and with the other
holding to my Mouth a Nectarine --
good God how fine.

John Keats.

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GENERAL INTRODUCTION

Opioids have been implicated in analgesia and sedation (Jaffe & Martin, 1980; Pert & Yaksh, 1975), locomotor activity (Joyce & Iversen, 1979; Pert, DeWald, Liao, & Sivit, 1979), thermoregulation (Eikelboom & Stewart, 1979; Vezina & Stewart, 1985), sympathetic nervous system functioning (Kiritsy-Roy, Appel, Bobbitt, & Van Loon, 1986; Randich & Callahan, 1987), and behaviors motivated by rewarding stimuli, including feeding (see review by Stewart, de Wit, & Eikelboom, 1984) and responding for electrical brain stimulation (Broekkamp, Phillips, & Cools, 1979). Discrete populations of central endogenous opioids occur throughout the brain (for a brief review, see Khatchaturian, Lewis, Schafer, & Watson, 1985). The behavioral functions of some of these opioid systems have been anatomically dissociated (Bozarth & Wise, 1984; Broekkamp, Van den Bogaard, Heyneh, Rops, Cools, & Van Rossum, 1976; Jenck, Gratton, & Wise, 1986), but further research is required to identify all the central sites where opioids contribute to different behaviors.

The influence of opioids on feeding behavior has been well documented (Brown & Holtzman, 1979; Cooper, 1980; Sanger, 1983; Sanger, McCarthy, & Metcalfe, 1981; Woods & Liebowitz, 1985). Although central opioid systems have been shown to play an important role in opioid-mediated feeding, relatively few laboratories have attempted to identify the specific brain areas that may participate in opioid regulation of ingestive behavior. Early findings that lesions of discrete hypothalamic nuclei produced dramatic alterations in feeding behavior (Anand & Brobeck, 1951; Hetherington & Ranson, 1942) led to a research focus on hypothalamic regions by most investigators interested in the central mechanisms of feeding (Grandison & Guidotti, 1977; Grossman,

1960; Hoebel & Teitelbaum, 1962; Leibowitz, 1975, 1978; Luiten, ter Horst, & Steffens, 1987; McLean & Hoebel, 1983; Roberts, 1969; Tepperman, Hirst, & Gowdey, 1981a, 1981b; Valenstein, Cox, & Kakolewski, 1970; Wise, 1974).

Feeding is a complex behavior that involves a number of sequentially organized responses (Roberts, 1969). Several motor behaviors including locomotor approach to food, sniffing, tongue protrusion and licking, biting, gnawing, mastication, and swallowing are synchronized to produce feeding. This implies a complex integration of both sensory and motor systems that is believed to take place within the central nervous system (Mogenson, 1982; Mogenson & Wu, 1982; Neill & Justice, 1981). As a complex behavior requiring approach to and interaction with environmental stimuli, feeding is generally considered to be representative of naturally motivated behavior (Miller, 1957; Mogenson, 1982; Roberts, 1969; Valenstein, 1971; Wise, 1974). A philosophical review of motivational constructs, which include reward, is beyond the scope of this presentation. The potential involvement of central reward processes in feeding behavior is relevant, however.

Strong empirical support of a role for central reward systems in feeding behavior arises from findings that rats work for electrical brain stimulation from the same electrodes in the lateral hypothalamus that elicit feeding during experimenter-delivered stimulation (Carr & Simon, 1983a; Jenck, Gratton, & Wise, 1987a; Jenck, Quirion, & Wise, 1987b; Margules & Olds, 1962; Roberts, 1980; Wise, 1974). Both brain stimulation reward and feeding produced by lateral hypothalamic stimulation arises from the activation of fibers of passage (Bielajew & Shizgal, 1982; Mogenson & Wu, 1982; Roberts, 1980; Yeomans, 1982), suggesting that the release of reward-relevant neurochemicals by the

stimulation occurs at sites in the brain distal to the stimulation. Considerable evidence suggests that endogenous opioids may be among those substrates (Broekkamp et al., 1976, 1979; Carr & Simon, 1983a, 1983b, 1984; Jenck, Gratton, & Wise, 1986, 1987a; Jenck et al., 1987b).

The endogenous opioid peptide fragment, dynorphin A_{1-13}, has been shown to produce feeding following injection into the cerebral ventricles of food satiated rats (Morley & Levine, 1981; Walker, Katz, & Akil, 1980). Dynorphin$_{1-13}$ binds with high affinity at kappa opioid receptors (Chavkin, James, & Goldstein, 1982), whereas morphine binds primarily at mu receptors but has some affinity at delta and kappa receptors as well (Magnan, Paterson, Tavani, & Kosterlitz, 1982). Although no direct association between dynorphin and reward processes has been established, kappa receptors have been found in several taste and feeding areas of the brain (Lynch, Watt, Krall, & Paden, 1985), as well as in brain regions identified with opioid reward (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987).

The present investigation was undertaken to examine the role of opioid reward systems in the elicitation of feeding. It was of interest to assess the comparative abilities of dynorphin A_{1-13} and morphine to produce feeding from reward-relevant brain areas. A comparison of feeding behavior elicited by opioids at these sites to behavioral effects at those brain regions associated with other opioid-mediated functions was expected to further elucidate the regional behavioral functions of opioids.

Involvement of Central Opioids in Reward

Opioids are known to be rewarding. Drugs of this class are self-administered peripherally by humans (Jaffe, 1980; Schuster & Thompson, 1969), and both intracranially (Bozarth, 1983) and systemically (Balster

& Lukas, 1985) by non-human animals. Rats readily learn to lever press for microinjections of morphine into cerebral ventricles (Amit, Brown, & Sklar, 1976; Belluzzi & Stein, 1977) and the ventral tegmental area (Bozarth & Wise, 1981; Van Ree & De Wied, 1980). Other investigators have reported intracranial self-administration of opioids into the nucleus accumbens (Goeders, Lane, & Smith, 1984) and the lateral hypothalamus (Olds, 1979, 1982); however these findings have not been consistently observed (Bozarth & Wise, 1982). In contrast to ventral tegmental area rats, other experimentally naive animals failed to acquire the lever-press response for morphine microinjections into the periaqueductal gray area (Bozarth & Wise, 1982, 1984). This region was shown to mediate central opioid dependence and withdrawal (Bozarth & Wise, 1984; Wei, 1981), considered by some researchers to underlie opioid addiction (Dole, 1972). The anatomical distinction between the functions of opioids in these two regions was an important demonstration that opioids in different brain regions are not necessarily involved in the same behaviors.

Morphine administered into the ventral tegmental area also produced a conditioned place preference in rats (Bozarth & Wise, 1982; Phillips & LePiane, 1980), and facilitated responding for rewarding electrical brain stimulation (Broekkamp et al., 1976, 1979). The ability of morphine in the ventral tegmental area to produce place preference was shown to be confined to a discrete area within this region (Bozarth, 1987). In addition, microinjections of the hydrophilic opioid antagonist, diallyl-nor-morphinium bromide, into the ventral tegmental area of rats produced a dose-dependent increase in responding for intravenous infusions of heroin (Britt & Wise, 1983). Compensatory increases in responding for intravenous drug following antagonist

administration are presumed to reflect an attempt by the animal to overcome the reduction in reward produced by the antagonist (Yokel & Wise, 1975, 1976). Similar injections into the striatum or nucleus accumbens failed to affect responding (Britt & Wise, 1983), further supporting the notion that opioid reward is not generalized throughout the brain and opioid receptors in the ventral tegmental area are critical for this phenomenon. In the absence of evidence to the contrary, it is presumed that pharmacological manipulations mimic neurochemical conditions that occur naturally to produce the behaviors elicited by the experimental treatment. Therefore, it may be presumed that reward is a naturally occurring phenomenon. Anatomically distinct localization of opioid reward suggests that specific opioid receptor fields and endogenous opioids at these sites may be involved in the mediation of naturally rewarding behavior.

Anatomy, Biochemistry, and Pharmacology of Central Opioids

In addition to the well-documented opioid-elicited analgesia, the effects of opium and later of opium-derived alkaloids such as morphine on mood may have engendered their use for over 2,000 years (see review by Jaffe & Martin, 1980). Despite this lengthy history, the stereospecific binding of opioid alkaloids to discrete opiate receptor populations in brain and spinal cord was identified less than 15 years ago (Kuhar, Pert, & Snyder, 1973; Pert & Snyder, 1973a, b; Simon, Hiller, & Edelman, 1973; Terenius, 1973). The findings of these investigators led in turn to the isolation of the endogenous opioid peptides (Cox, Opheim, Teschemacher, & Goldstein, 1975; Goldstein, 1976; Hughes, 1975; Hughes, Smith, Kosterlitz, Fothergill, Morgan, & Morris, 1975; Lord, Waterfield, Hughes, & Kosterlitz, 1977; Teschemacher, Opheim, Cox, & Goldstein, 1975), and were accompanied by the proposal

and identification of putative endogenous opioid receptors (Chavkin & Goldstein, 1981a, b; James, Chavkin, & Goldstein, 1982a, b; Duka, Schubert, Wuster, Stoiber, & Herz, 1981; Lord et al., 1977; Mansour, Lewis, Khachaturian, Akil, & Watson, 1986; Martin, 1967; Martin, Eades, Thompson, Huppler, & Gilbert, 1976).

Anatomy

Peptide Distributions.

Considerable overlap exists in the distribution in brain of the naturally occurring opioid peptides; however regional differences in the presence of specific endogenous opioid peptide-containing cell bodies and terminal regions have been reliably observed, and several discrete peptide-containing systems have been demarcated (Fallon, Leslie & Cone, 1985; Larsson, Childers & Snyder, 1979; Vincent, Hokfelt, Christensson, & Terenius, 1982a, b; Watson, Khachaturian, Akil, Coy, & Goldstein, 1982). The central distribution of dynorphin A (Goldstein, Tachibana, Lowney, Hunkapiller, & Hood, 1979) was of particular interest in view of its potent feeding-eliciting properties following intracerebroventricular administration (Morley & Levine, 1981; Walker, Katz, & Akil, 1980).

Immunoreactive dynorphin-containing terminals have been detected in several rat brain regions, including the substantia nigra - pars reticulata, the periaqueductal gray region, the nucleus of the solitary tract, the median eminence and posterior lobe of the pituitary, all trigeminal sensory nuclei, the magnocellular nuclei of the hypothalamus, the lateral, central and cortical amygdaloid nuclei, the dentate gyrus of the hippocampus, and most regions containing dopamine terminals including the striatum, globus pallidus, ventral pallidum and nucleus accumbens, as well as a diffuse cortical network (Vincent et al.,

1982b). These investigators postulated that dynorphin in the nucleus of the solitary tract may play a role in regulating afferents from baro- and chemo-receptors terminating in this area, suggesting an involvement of endogenous dynorphin in taste mechanisms. A similar suggestion was offered by Lynch, Watt, Krall, & Paden (1985) following a kappa receptor binding investigation. Dynorphin-containing perikarya were found mainly in the magnocellular and parvocellular nuclei of the hypothalamus, the striatum, the central amygdaloid nucleus, the stria terminalis, the periaqueductal gray, and the central amygdaloid nucleus (Vincent et al., 1982b; Watson et al., 1982).

Enkephalin-containing terminals share most regions with dynorphin, with the notable exception of the substantia nigra - pars reticulata. Dense enkephalinergic innervation was detected in the substantia nigra - pars compacta, just dorsal to the pars reticulata, however (Watson et al., 1982). Beta-endorphin-containing cells appear to be confined to two main regions, the arcuate nucleus of the hypothalamus and the nucleus of the solitary tract. Fibers and terminals extend to most regions where the other endogenous opioids are found, including the paraventricular nucleus and other hypothalamic nuclei, the periaqueductal gray, the striatum, the substantia nigra - pars compacta, and the ventral tegmental area (Bloom, Battenberg, Rossier, Ling, & Guillemin, 1978; see summary by Khachaturian, Lewis, Schafer, & Watson, 1985). This distribution was found to be distinct from reported enkephalin distributions, however (Larsson, Childers, & Snyder, 1979).

Another important finding by Vincent and colleagues was the presence of a dynorphin-containing pathway originating in the striatum and terminating in the substantia nigra - pars reticulata (Vincent et al., 1982a). Subsequently, using a combined immunofluorescence and

retrograde tracing technique, Fallon et al. (1985) detected dynorphin B-containing pathways extending both from the striatum to the substantia nigra – pars reticulata and from the hypothalamus and central amygdaloid nucleus to the ventral tegmental area and to more caudal structures. This investigation was the first to detect any dynorphin in the ventral tegmental area. The reasons for this may be two-fold. First, the ventral tegmental area is not anatomically well-defined. Except for investigations specifically involving dopamine cell bodies in this region it frequently is included, undifferentiated, as part of the "midbrain" or "mesencephalon" category. Second, these measurements are qualitative rather than quantitative, showing relative and not absolute intensities. The intense dynorphin-immunoreactive staining in the adjacent substantia nigra - pars reticulata may have detracted from the much fainter reactivity in the ventral tegmental area. A similar situation applies for this region in the identification of dynorphin-appropriate receptors.

Receptor Distributions and Potential Corresponding Natural Ligands.

Both dynorphins A and B are reported to be extremely potent (Goldstein et al., 1979), with high affinity for the kappa-opioid receptor (Chavkin & Goldstein, 1981a, 1981b; Chavkin, James, & Goldstein, 1982; James, Chavkin, & Goldstein, 1982a, 1982b; James, Fischli, & Goldstein, 1984), and lower affinities for mu and delta receptors (James & Goldstein, 1984). The distribution of kappa receptors appears to be consistent with the reported distribution of dynorphin-containing terminals, including the nucleus accumbens, substantia nigra - pars reticulata, periaqueductal gray, and most hypothalamic areas (Cone, Weber, Barchas, & Goldstein, 1983; Lynch, Watt, Krall, & Paden, 1985; Mansour et al., 1986, Quirion, Weiss, &

Pert, 1983a). Kappa receptors have also recently been detected in the ventral tegmental area (Mansour, Khachaturian, Lewis, Akil, & Watson, 1987). The precise location of kappa receptors (i.e., on cell bodies, dendrites, axons or terminals of postsynaptic neurons) and the biochemical identification of neurons receiving dynorphin input remain to be determined.

The prototypic opioid morphine binds primarily to mu receptors but its relative binding affinities at delta and kappa receptors suggest that morphine cannot be called mu-specific (James & Goldstein, 1984; Lord et al., 1977). Because the majority of behavioral opioid research has been conducted using morphine, findings with this compound represent a standard against which the effects of other opioids are compared. An endogenous opioid selective for the mu receptor has not been determined. This leads to difficulty in making inferences concerning the endogenous opioids that may naturally mediate behaviors observed following morphine administration. Some assumptions about such natural peptide functions may be derived by integrating the information from comparative binding and physiological assays of different endogenous opioids with receptor and peptide distributions, and by comparing these with the findings of similar procedures using morphine. Similarities on these measures between morphine and naturally occurring opioids may suggest possible morphine-like behavioral functions for specific endogenous opioids.

Met- and leu-enkephalin bind with highest affinity to delta receptors. Both peptides have been reported to demonstrate some binding at mu receptors as well, however. Met-enkephalin was found to have low to moderate affinity at mu receptors, whereas leu-enkephalin was observed to be more selective for delta sites. An extremely low binding affinity was evident for both the enkephalins at kappa receptors

(Kosterlitz, Paterson, & Robson, 1981; Waterfield, Leslie, Lord, Ling, & Kosterlitz, 1979). Beta-endorphin was reported to bind with equal preference to mu and delta receptors (Hewlett & Barchas, 1983), and little or no kappa binding by this ligand has been reported. In addition, minimal binding of the enkephalins and beta-endorphin at kappa receptors further supports the suggestion that dynorphin is the endogenous ligand for this receptor (James, Chavkin, & Goldstein, 1982a).

Mu receptors are localized in several brain regions, including the ventral tegmental area, the nucleus accumbens, the substantia nigra - pars compacta, the periaqueductal gray, and the striatum (Mansour et al., 1987). Delta receptors were detected in the nucleus accumbens, substantia nigra - pars reticulata, and striatum, but not in the ventral tegmental area, periaqueductal gray, or substantia nigra - pars compacta. The naloxone-reversible behavioral (Leibowitz & Hor, 1982) and physiological (Kiritsy-Roy et al., 1987) responses to microinjection of mu and delta agonists in the paraventricular nucleus of the hypothalamus suggest that these receptors exist in that region. Interestingly however, neither mu nor delta receptors were detected in the paraventricular nucleus. In an earlier report (Mansour et al., 1986) these investigators pointed out that results achieved by their computer-enhanced imaging technique were qualitative and not quantitative. It is possible that the technique was not sensitive to a low density of mu or delta receptors in this region.

Similar to kappa receptors, morphological localization of mu and delta receptors has not been fully examined. Mu receptors in the ventral tegmental area were found on interneurons (Dilts & Kalivas, 1987) and a large proportion of the binding by enkephalins in the

nucleus accumbens (Pollard, Llorens, Bonnet, Costentin, & Schwartz, 1977a) and striatum (Pollard, Llorens-Cortes, & Schwartz, 1977b) may occur on dopamine terminals.

Biochemistry and Pharmacology

The prodynorphin precursor contains alpha-neo-endorphin and beta-neo-endorphin, as well as dynorphin-32 (consisting of both dynorphin A, a 17-amino acid residue chain with a leu-enkephalin sequence at its amine (N-) terminus, and Dynorphin B, a 13-residue chain that is linked to the carboxyl (COOH) terminus of dynorphin A by a Lys-Arg sequence) and dynorphin B-29, which includes dynorphin B and 16 further residues (James et al., 1984). Of concern to the present investigation, is dynorphin A, for which behavioral effects have been the most extensively documented.

The full dynorphin A sequence was not initially identified, and the original work with this peptide was conducted using the (1-13) sequence (Chavkin & Goldstein, 1981a, 1981b; Chavkin, James, & Goldstein, 1982; James, Chavkin, & Goldstein, 1982). The octapeptide dynorphin A_{1-8} was reported to be extensively represented in brain (Weber, Evans, & Barchas, 1982) and to exhibit a preference for the kappa receptor (Corbett, Paterson, McKnight, Magnan, & Kosterlitz, 1982). James et al. (1982b) found, however, that both the arg-7 and lys-11 residues were important for the selectivity and potency of dynorphin$_{1-13}$ at this receptor. Shorter fragments were also observed to be extremely vulnerable to degradation by peptidases. Unlike dynorphin$_{1-8}$ or $_{1-9}$, however, the potency and duration of action of the longer sequence was not enhanced by peptidase inhibitors (Corbett et al., 1982; James et al., 1982b) suggesting that inactivation by peptidases may not be the primary mechanism of dynorphin degradation. Moreover, the addition of

an amide group to the COOH terminus of dynorphin$_{1-13}$ considerably prolonged the integrity of the unbound ligand without affecting potency (Leslie & Goldstein, 1982). This is the form in which commercially available dynorphin is now offered. Dynorphin$_{1-13}$ was also reported to have an extremely low dissociation rate and a long duration of action and to be protected from enzyme attack while bound (Leslie & Goldstein, 1982). Furthermore, no difference in either binding selectivity or pharmacological potency was observed between dynorphin$_{1-13}$ and the full dynorphin$_{1-17}$ sequence (Corbett et al., 1982).

Concern has been expressed about the apparent biologic activity of a metabolite of dynorphin A. Cleavage at the NH-terminal removes the tyrosine residue from the first position of the peptide sequence, yielding des-Tyr-dynorphin (Herman & Goldstein, 1985). It has been proposed that in vivo, degradation of dynorphin A to des-Tyr-dynorphin is effected by a nonspecific, non-peptidase enzyme (Young, Walker, Houghten, & Akil, 1987). Des-Tyr-dynorphin was shown to mimic the effects of dynorphin A on spinal analgesia, hippocampal unit activity, and motor function including paralysis, and these effects were not naloxone-reversible suggesting that they occurred through a non-opioid mechanism (Herman & Goldstein, 1985; Stevens & Yaksh, 1986; Walker, Moises, Coy, Baldrighi, & Akil, 1982). Subsequent examination of tissue in the spinal cord preparation revealed cell damage (Caudle & Isaac, 1986; Stevens, Weinger, & Yaksh, 1987). In support of an opioid-mediated physiological role for dynorphin A, the highly selective kappa agonist, U50,488H (Lahti, VonVoigtlander, & Basruhn, 1982) mimicked the effects of dynorphin$_{1-13}$ on DAGO inhibition of electrically stimulated C-fiber responses in the spinal cord (Dickenson & Knox, 1987). The reported non-opioid responses and cell damage followed intrathecal or

intracerebroventricular administration of extremely high doses (20 to 100 nanomoles) dynorphin A and up to 30 nmoles of des-Tyr-dynorphin. Whether behaviors elicited by lower doses of dynorphin A are opioid or non-opioid in nature remains to be determined.

Opioid Antagonist Inhibition of Ingestive Behavior

Food Deprivation

The finding that naloxone suppressed food and water ingestion in food or water deprived animals (Holtzman, 1974, 1975, 1979) led to the suggestion that endogenous opioids may be important in mediating natural ingestive behavior. Food and sometimes water deprivation represents the classical approach to examining the ability of opioid antagonists to attenuate feeding and drinking (Brown & Holtzman, 1979; Holtzman, 1974, 1975; Lowy & Yim, 1981; Sanger, McCarthy, & Metcalfe, 1981; Stapleton, Ostrowski, Merriman, Lind, & Reid, 1979).

Rodents are mainly nocturnal feeders (Armstrong, 1980), and most experiments are conducted during daylight hours, when these animals typically eat very little. In the laboratory, spontaneous daytime feeding is frequently produced by depriving animals of food for usually 4 to 24 hours before testing. Mild, 12-hour deprivation was shown to produce daytime food intake that was indistinguishable from nocturnal ingestion, and naloxone inhibited feeding identically in rats in both conditions (Jalowiec, Panksepp, Zolovick, Najam, & Herman, 1981). In contrast, these investigators observed that the reduction by naloxone of food consumption was far less pronounced in nondeprived rats during the daytime, when control level feeding was extremely low. Indeed, the effect did not appear for at least 4 hours following drug administration, compared to significant decreases in intake by the first measurement, at 30 minutes, in the other conditions. These findings

supported the suggestion that endogenous opioids probably play a natural role in feeding behavior following a period of fasting.

Various adaptations of the original deprivation/opioid antagonist technique have revealed further interesting characteristics of the possible role of opioids in feeding. For instance, a cross-species analysis demonstrated that naloxone and the anorectic agents fenfluramine and diethylproprion were each effective in reducing feeding in mildly deprived rats, rabbits, and cats. The decrease in intake by naloxone was less marked than that produced by the other two drugs; however no additional behavioral effects were noted, whereas at the effective anorectic doses of both fenfluramine and diethylproprion gross behavioral alterations occurred that undoubtedly interfered with feeding (McCarthy, Dettmar, Lynn, & Sanger, 1981).

Naloxone attenuation of feeding behavior has been demonstrated in rats (Jalowiec et al., 1981), mice (Kavaliers & Hirst, 1985; Tannenbaum & Pivorun, 1984), and several other species, including cats (Foster, Morrison, Dean, Hill, & Frenk, 1981), sheep (Baile, Keim, Della-Fera, & McLaughlin, 1981), squirrel monkeys (Herman & Holtzman, 1984; Locke, Brown, & Holtzman, 1982), pigeons (Deviche & Wohland, 1984), and invertebrates (Kavaliers, Hirst, & Teskey, 1984). This effect apparently does not extend to golden hamsters, however. It has been suggested that this animal's apparent lack of an opiate-dependent feeding system may be related to its natural tendency to hibernate (Lowy & Yim, 1982, 1983); however other hibernating species show appropriate ingestive responses to opioid manipulations (Kavaliers & Hirst, 1986; Tannenbaum & Pivorun, 1984). The differences observed in the golden hamster may instead reflect its lack of circadian variations in ingestive behavior and its failure to increase intake following food

deprivation (Borer, Rowland, Mirow, Borer, & Kelch, 1979; Silverman & Zucker, 1976; Zucker & Stephan, 1973), that in other species appear to involve endogenous opioids. The golden hamster appears to be atypical. The otherwise cross-species concordance of the effects of opioid manipulations on feeding suggests that studies using rats probably generalize overall to most other species, including humans.

The reduction in food intake by naloxone may arise from alterations in feeding patterns. In 6-hour food deprived rats, both the rate and duration of feeding were reduced by naloxone, and first and final feeding bouts were terminated earlier than controls. Each of these factors contributed to a net reduction in total intake. Interestingly, however, the latency to initiate the first eating bout was also reduced by naloxone, and the number of bouts was not significantly affected (Kirkham & Blundell, 1984). Similar effects were observed in an operant paradigm. In rats trained to bar-press for food, the first meal size and duration and first postmeal intervals, but not meal frequency, were reduced by naloxone (McLaughlin & Baile, 1984). In contrast, naloxone and naltrexone failed to reduce intake in schedule-fed rats. Operant responding for food in deprived rats was also unaffected, although spontaneous feeding by these rats was attenuated by opioid antagonists (Sanger & McCarthy, 1982a, 1982b). Similarly, in a timed food presentation paradigm naloxone failed to reduce the latency to initiate eating on any trial by food deprived rats, but total food intake for the session was decreased (Wise & Raptis, 1986). It appears that conditioned behaviors associated with the initiation of feeding may be less responsive to opioid inhibition. The frequency of approaches to food was unaffected by antagonist treatment in all cases. With the exception of Sanger and McCarthy's (1982) observations, total food

intake was consistently reduced. This suggests that opioid antagonists either act on satiety mechanisms or interact with subjeotive responses to properties of the food such as palatability.

Palatability and Satiety

The attenuation of food intake by naloxone in food deprived rats suggests that endogenous opioids are naturally involved in eating following a period of fasting. The ability of opioid antagonists to reduce feeding under conditions other than food deprivation may provide information as to the possible physiological mechanisms involved in feeding behavior. The majority of feeding-eliciting treatments are naloxone-sensitive. Opioid antagonists reduced feeding produced by tail pinch (Lowy, Maickel, & Yim, 1980; Morley & Levine, 1980; Rowland & Antelman, 1976) or cold swim stress (Vaswani, Tejwani, & Mousa, 1983), 2-deoxy-d-glucose (Bodnar, Kelly, Brutus, & Glusman, 1978; Lowy, Starkey, & Yim, 1981; Sewell & Jawaharlal, 1980), electrical stimulation of the lateral hypothalamus (Carr & Simon, 1983a, 1983b; Jenck et al., 1986, 1987), and presentation of highly palatable foods (Cooper, Jackson, Morgan, & Carter, 1985b). Insulin-elicited hyperphagia was reported to be naloxone-sensitive by some researchers (Levine & Morley, 1981; Rowland & Bartness, 1982) but not by others (Lowy et al., 1980, 1981).

The effect of opioid antagonists on intake of highly palatable foods has received considerable attention. In the context that such food is considered to be rewarding (Dum, Gramsch, & Herz, 1983; Morgan, 1974; Rogers & Blundell, 1980) research in this area is of interest to the present investigation. Naloxone has been observed to be maximally effective in paradigms where apparent palatability is an independent variable (Apfelbaum & Mandenoff, 1981; LeMagnen, Marfaing-Jallat,

Miceli, & Devos, 1980; Levine, Murray, Kneip, Grace, & Morley, 1982; Wu, Lind, Stapleton, & Reid, 1981). In fact, naloxone was shown to exert the greatest suppression of intake at saccharin concentrations for which rats had demonstrated highest preference (Cooper & Turkish, 1983; Lynch & Libby, 1983; Turkish & Cooper, 1983). Vigorous feeding responses were reported among non-deprived animals presented with powdered lab chow mixed with sweetened condensed milk. Naloxone reduced feeding in a dose-dependent manner (Cooper et al., 1985b). Naloxone attenuation of sweetened milk consumption was also consistent among different strains of rats (Cooper, Barber, & Barbour-McMullen, 1985a).

The inhibitory influence of opioid antagonists on ingestion has been interpreted as reflecting a satiety mechanism (Cooper, 1980; Wise & Raptis, 1986). Eating of novel food was more easily disrupted by naloxone than ingestion of familiar food (File, 1980). It was suggested that emotional factors may enhance sensitivity to naloxone (see review by Cooper, 1983b). Findings of other investigations have indicated that opioid antagonism may produce a reduction in the reward properties of food (Cooper, 1983a, 1983b; Frenk & Rogers, 1979; Jalowiec et al., 1981; Siviy, Calcagnetti, & Reid, 1982). It has been suggested that fat-containing foods tend to be the most palatable (Romsos, Gosnell, Morley, & Levine, 1987), and that opioid modulation of food intake may be specifically related to palatability (Morley, Mitchell, & Levine, 1986). In support of this, rats fed on high fat diets ate more than rats given standard, high carbohydrate, or high protein diets (Vaswani et al., 1983). Rats eating food with high fat content also ate more than the other groups following food deprivation or cold swim stress, and showed the highest sensitivity to naloxone (Vaswani et al., 1983).

Studies of naloxone treatment in both obese and normal humans have

revealed that although food intake was significantly decreased, the subjects' perception of satiety was unaltered (Cohen, Cohen, & Pickar, 1985; Thompson, Welle, & Lilavivict, 1983; Trenchard & Silverstone, 1983). Moreover, although naloxone reduced intake during binge eating among bulimic patients the putative satiety agent, cholecystokinin (CCK) was ineffective (Morley et al., 1986). Administration of the mixed opioid (kappa) agonist/(mu) antagonist, butorphanol, increased food intake among normal subjects without affecting their perception of hunger (Morley et al., 1985a). This finding is further evidence in favour of kappa-mediated feeding that is independent of satiety. Taken together, these results provide correlative support for a possible relationship between opioid-mediated feeding and natural reward processes.

Drinking

Although feeding and drinking are both appropriately categorized as ingestive behaviors, these activities show differential responses to opioid manipulation. Whereas investigations of antagonist inhibition of eating support a strong role for endogenous opioids in the mediation of feeding behavior, the sensitivity of drinking to this treatment appears to be limited. For instance, naloxone and naltrexone decreased water consumption in water-deprived rats, with a maximum obtainable reduction of approximately 50% (Brown, Blank, & Holtzman, 1980; Maickel, Braude, & Zabik, 1977; Stapleton et al., 1979).

Similar to findings with food, latency to initiate drinking following deprivation was not affected by naloxone, but behavior slowed compared to controls after an initial period of fluid intake (Siviy et al., 1982). Sensitivity of drinking to opioid antagonism appears to depend on the conditions eliciting the behavior. The response to

naloxone of drinking elicited by the hypovolemic agents salbutamol and polyethylene glycol was similar to deprivation-induced drinking (Rowland, 1982; Wallace et al., 1984). In contrast, drinking induced by administration of hypertonic saline (Brown et al., 1980; Brown & Holtzman, 1981b; Rowland, 1982; Wallace, Willis, & Singer, 1984) or angiotensin (Brown & Holtzman, 1981b; Rowland, 1982) was inhibited in a more pronounced fashion by naloxone. Similar to studies with food, schedule-induced polydipsia (drinking that accompanies the intake of small portions of intermittently delivered food in food-deprived rats) was reported to be entirely resistant to naloxone (Brown & Holtzman, 1981b; Wallace et al., 1984). It appears that although endogenous opioids may participate in the mediation of drinking behavior, this role is limited and other, non-opioid mechanisms may be primarily responsible for the regulation of drinking behavior.

Opioid Agonist Enhancement of Ingestive Behavior

Systemic injections of morphine in food-satiated rats were observed to increase both feeding and drinking in a dose-dependent manner. A delayed onset of feeding at 10 mg/kg, i.p., most likely reflected an initial sedative effect of the drug. No delays were apparent at lower doses, and prolonged feeding followed the initiation of eating at the high dose. Drinking was clearly postprandial and increases were proportional to the increases in food consumption (Sanger, 1983; Sanger & McCarthy, 1981). In contrast, food intake by food-deprived animals was decreased by morphine in a dose-dependent manner (Sanger & McCarthy, 1980). The evidence from opioid antagonist studies in food-deprived animals suggests that endogenous opioids probably contribute importantly to feeding following deprivation. A decrease in feeding by morphine following food deprivation suggests first, that a further facilitation

by opioid agonists of an apparently opioid-mediated performance that may be already near maximum is perhaps unlikely and second, that food deprivation may alter the sensitivity of animals to other effects of exogenously administered opioids, such as sedation (see Sanger, 1983). It is also apparent that examination of the ability of opioid agonists to increase feeding is more revealing under conditions when spontaneous feeding is typically low. Consequently, most investigations of opioid agonist-elicited feeding are conducted during the light portion of the light/dark cycle with rats that have not been food-deprived (e.g., Cooper et al., 1985b; Jalowiec et al., 1981).

Central Opioid Mediation of Feeding Behavior

Endogenous opioids occur both centrally and peripherally. Morphine and other opioid agonists administered systemically reliably elicit feeding in non-deprived rats. The sites of opioid action in producing feeding are not revealed by this technique, however. Comparison of effects of systemically administered naloxone on ingestion to those of its quaternary analog, which does not readily enter the central nervous system, indicated that opioid regulation of feeding (Jones & Richter, 1981) and drinking (Brown & Holtzman, 1981a) occurs centrally.

Intracerebroventricular administration of the endogenous opioid peptides beta-endorphin (McKay, Kenney, Edens, Williams, & Woods, 1981) and dynorphin$_{1-13}$ (Morley & Levine, 1981; Walker, Katz, & Akil, 1980) were reported to produce marked elevations in food intake in food-satiated rats. These investigations supported an involvement of central opioid systems, but they were unable to identify those brain regions that may participate in opioid mediation of feeding.

The classical findings that hypothalamic lesions, depending on their location within this structure, dramatically increased or

decreased feeding behavior (Anand & Brobeck, 1951; Hetherington & Ranson, 1942) led to considerable research and the traditional acceptance of the hypothalamus as the "feeding center" of the brain (see reviews by Hoebel & Teitelbaum, 1962; Wise, 1974). Most investigations of feeding by site-specific centrally administered opioids and other drugs naturally have focussed attention on this region (e.g., Grandison & Guidotti, 1977; Liebowitz & Hor, 1982; McLean & Hoebel, 1983; Tepperman & Hirst, 1982; Tepperman, Hirst, & Gowdey, 1981; Thornhill & Saunders, 1984; Woods & Liebowitz, 1985).

Beta-endorphin, levorphanol, or morphine, microinjected into the ventromedial nucleus of the hypothalamus (VMH), were reported by some investigators (Grandison & Guidotti, 1977; Tepperman & Hirst, 1982) but not by others (Gosnell, Morley, & Levine, 1986; Woods & Leibowitz, 1985) to stimulate feeding behavior in non-deprived rats. Gosnell et al. (1986) did observe dose-dependent feeding following dynorphin injection into this region, however. Feeding occurred following microinjection of beta-endorphin (Woods & Leibowitz, 1985), D-Ala^2, Met^5-enkephalin (DALA: McLean & Hoebel, 1983), dynorphin$_{1-13}$ (Gosnell et al., 1986), or morphine (Leibowitz & Hor, 1982) into the paraventricular nucleus of the hypothalamus. Animals were consistently reported to be sedated following opioid administration, however, and feeding was reported by all investigators not to commence for at least 30 minutes to 1 hour after injection. This issue will be addressed in the following section on long-duration paradigms.

Few investigators have addressed the potential contribution of opioids in other brain regions to feeding behavior. Electrolytic lesions of either the globus pallidus or caudate resulted in reduced responsiveness to the daytime feeding-enhancing properties of

the putative kappa agonist ketocyclazocine. The inhibitory effects of naloxone on nocturnal food intake were unaffected by the lesions, however (Gosnell, Morley, & Levine, 1984). It was suggested that the lesions had reduced animals' sensitivity to the orexigenic effects of ketocyclazocine. The higher doses used of this drug produced sedation in both lesioned animals and sham-lesioned controls, who recovered more rapidly. A further examination of the data indicates that the lesions may have removed part of the natural competition with the sedative effects of ketocyclazocine. This explanation would be more consistent with the naloxone data in the same investigation. Other studies have determined that the central amygdaloid nucleus may be involved in central opioid-mediated feeding (Gosnell, 1988) but that the medial hippocampus is not (Gosnell, Morley, Levine, Kneip, Frick, & Elde, 1984). Only recently have investigations begun of the potential role of brain regions associated with opioid reward in eliciting feeding (e.g., Mucha & Iversen, 1986).

Opioid-Mediated Behavior and Long-Duration Paradigms

Feeding studies traditionally involve measurement of the weight of food consumed over the course of up to several hours following treatment. This procedure provides useful information concerning the time course of a drug or other condition (e.g., stress), particularly when drug administration is systemic or intracerebroventricular. Problems may arise with this technique, however, when the objective is to identify the specific brain sites where drug action may be responsible for the behavioral outcome. Regardless of the potentially different pharmacokinetic characteristics of different compounds, the longer the duration of a test session following a specifically targetted intracerebral injection, the greater the probability will be that the

drug will diffuse to other brain regions where it may have biologic activity. In addition, close proximity of an injection site to a ventricle further increases the likelihood of diffusion to distal brain areas.

Opioid peptides, including dynorphin, are extremely vulnerable to hydrolysis by peptidases (see Goldstein, 1984). Co-administration of peptidase inhibitors such as thiorphan in a "cocktail" mixture may delay the breakdown of the injected peptide. This treatment does not prolong the action of dynorphin (Corbett et al., 1982), but it will inhibit the degradation of all endogenous opioid peptides at the injection site. Depending on whether neuronal activity of peptide-containing terminals in that region is clonic or phasic peptidase inhibitors may also enhance the synaptic availability of all local endogenous opioids, possibly obscuring the specific effect of the injected peptide.

One approach to this problem is to use high concentrations of the injected peptide to ensure sufficient undegraded ligand. When extremely high doses of a ligand are used, however, the natural function of the ligand in the brain region of interest is obscured. This leads to several problems in the interpretation of the findings. First, most opioid ligands bind to all opioid receptor subtypes with varying selectivity (see reviews by Goldstein, 1984; Paterson, Robson, & Kosterlitz, 1984). Local injection of a high concentration of a ligand markedly enhances the probability of its binding to and perhaps activating other opioid systems as well as the system of interest. Thus, the same difficulty occurs as when peptidase inhibitors are used. Second, this procedure may also initially produce unintended effects (For instance, severe locomotor debilitation was observed following nanomolar doses of intracerebroventricular dynorphin$_{1-13}$ or the

selective mu agonist Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$: DAGO; see Gosnell, Levine, & Morley, 1986.) that may either completely mask or delay the onset of the behavior of interest for up to several hours. The question then arises as to whether subsequent behavior was elicited directly by remaining drug or if the initial drug-induced suppression has been followed by a rebound behavioral recovery. Also unresolved is the possibility of diffusion of the drug over time to another brain site that may be responsible for the behavior observed.

Both drug diffusion and rebound recovery are plausible alternative explanations for the findings of investigations of the effects on feeding of beta-endorphin (Liebowitz & Hor, 1982) or morphine (Woods & Liebowitz, 1985) microinjected into the paraventricular nucleus of the hypothalamus. This structure surrounds a ventricle. Considerable diffusion through ventricular fluid could precede the protracted first latency to initiate feeding (between 30 minutes to 1 hour for beta-endorphin and 1 hour for morphine). In addition, morphine injected into the paraventricular nucleus has been observed to elicit sympathetic effects that mimic the peripheral response to stress (Kiritsy-Roy, Appel, Bobbitt, & Van Loon, 1986; Randich & Callahan, 1986). Opioid mediation of stressor-provoked feeding has received empirical support (Antelman & Rowland, 1981; Bertiere, Mame Sy, Baigts, Mandenoff, & Apfelbaum, 1984; Lowy, Maickel, & Yim, 1980; Morley & Levine, 1980a, 1980b).

Animals permitted to choose their own levels of opioid stimulation rarely self-administer high cumulative amounts of drug but seem to maintain relatively constant drug levels throughout a test session. For instance, the response patterns of rats lever pressing for ventral tegmental area infusions of morphine demonstrated that after an initial

"loading" of drug to an accumulated total of about 6 nmoles, regular self-administration intervals were quite consistent over time at approximately 7.5 nmoles/hour (Bozarth & Wise, 1981; M. Bozarth, personal communication, 1987). This also appears to be true when other rewarding stimuli are available in addition to opioids. A similar regular intake pattern was observed among rats permitted concurrent access to intravenous heroin and lateral hypothalamic electrical brain stimulation reward (BSR: Bozarth, Gerber & Wise, 1980). The current threshold for BSR was dramatically reduced. In addition, increases in the doses of heroin per infusion produced concomitant increases in responding for BSR and decreases in responding for drug self-administration. A further 10-fold increase in the heroin dose resulted in a notable increase in the interval between infusions. Concurrently, delays between drug self-administration and increases in responding for BSR became apparent, although a regular temporal relationship was evident between responses for drug and electrical stimulation (Bozarth et al., 1980).

Taken together, opioid self-administration findings indicate that although the rewarding impact of opioids is clear, drug levels above a certain amount may not add significantly more reward. Furthermore, the optimal rewarding level of opioids may occur at doses much lower than those frequently employed in studies of opioid-mediated behavior when the drug is experimenter-delivered. This is consistent with Chavkin & Goldstein's (1984) suggestion that only 10% of functional opioid receptors need be occupied to achieve a maximal behavioral effect. Moreover, it appears at least for opioids that the maximum desired effect may be of relatively brief duration. The levels of drug chosen by animals during self-administration might serve as an

indication of the optimal dose for experimenter-delivered drug in behavioral analysis.

Depending on the behavior of interest and the rate of degradation of a given ligand, the classic paradigms may not be sufficiently sensitive to detect the effects of a particular drug. If the action of a ligand on receptors in a specific brain region contributes directly to a behavior, the dose-related effect should be observable within a relatively brief time following microinjection into that region. Considering also the vulnerability of opioid ligands to rapid hydrolysis by peptidases and the time-related probability of drug diffusion to other brain regions, either a different approach to drug administration or an alternative behavioral measurement to the classical approaches may be required. Low-level constant infusion of drug is a potential option; however two major difficulties are also presented. First, the problem of drug diffusion over time still remains, and constant infusion may result in an accumulation of drug both at the injection site and at distal regions. Second, this technique presents methodological difficulties that have been neither systematically addressed nor refined, and injection volume rate and accumulation may be unreliable. Finally, in order to choose appropriate injection parameters it would be imperative to consider the different kinetic properties of each opioid ligand, most of which are not yet fully determined.

The foregoing suggests that in anatomically specific studies of the effects of opioids on feeding the most preferable alternative to high dose/long duration paradigms where food is weighed appears to be the administration of a low dose of drug and a different measurement of feeding behavior. In recent investigations, a correspondence has been found between food intake and the duration of feeding behavior (Jackson

& Cooper, 1986; Kirkham & Blundell, 1984; Sanger, 1983). Direct, constant behavioral observation for a short period following microinjection of a low drug concentration provides a reliable index of feeding and other behaviors, and minimizes the problems of interpretation associated with overdosing and with diffusion and degradation of the injected drug. In addition, it may be possible to detect behavioral effects of the drug that are likely to be overlooked in other procedures.

Summary

Opioids have been strongly implicated in centrally mediated feeding behavior. This is supported by findings that opioid antagonists inhibit spontaneous feeding under conditions such as food deprivation, that typically enhance consumption, suggesting that endogenous opioids may play a natural role in the regulation of food intake. Conversely, feeding can be elicited in food-satiated animals by opioid agonists administered either systemically or centrally. Furthermore, hypothalamic pools of endogenous opioids are released during feeding (Dum, Gramsch, & Herz, 1983), certain morphine-sensitive neurons are activated during operant responding for food (Nakano, Oomura, Lenard, Nishimo, Aou, Yamamoto, & Aoyagi, 1986), and alterations in basal levels of opioid peptides in some brain regions have been correlated with periods of food deprivation (Gambert et al., 1980; Vaswani & Tejwani, 1983). Given the prolonged latency to onset of feeding following opioid injection into some hypothalamic areas and the observation that the treatments frequently produced sedation or even catalepsy, however, the nature of the regional influence of opioids on consumption remains unclear.

Alterations in the patterns of feeding behavior have been observed

in animal studies following the administration of opioid agonists or antagonists, in such a manner as to suggest that the reward value of the food may have been qualitatively modified by the treatment. Similarly, obese humans treated with naloxone were found to reduce food intake without any changes in their reported perceptions of satiety. In addition, the consumption of highly palatable substances, demonstrated to be rewarding, was antagonized by naloxone. These findings, among other evidence, led to the speculation that opioid reward systems may participate in feeding behavior.

Opioids microinjected into the ventral tegmental area are rewarding, and some evidence indicates that activation of opioid receptors in the nucleus accumbens may also be rewarding. Nucleus accumbens involvement in opioid reward processes may be less robust than the ventral tegmental area, however. It was of interest to compare the effects of opioid microinjections into these areas with opioid-mediated feeding in the paraventricular hypothalamus, where opioids are known to elicit delayed eating. Comparison with possible feeding elicited from the opioid-rich substantia nigra, associated with brain stimulation reward but where opioid reward has not been examined was also desirable. The periaqueductal gray region, linked with opioid-induced analgesia, tolerance, and inhibition of stimulation-induced feeding was expected to represent a negative site for opioid-elicited feeding. It was anticipated that these investigations would facilitate the empirical evaluation of the impact of central opioid reward systems in feeding relative to feeding elicited from opioids in other selected brain regions.

Discrete populations of endogenous opioids are extensively distributed throughout the brain. Opioid reward has been established by

central administration of morphine or, to a far lesser extent, the met-enkephalin analogue, DALA (D-Ala^2-Met-enkephalin: Pert, Pert, Chang, & Fong, 1976). Robust feeding behavior has consistently been observed following either peripheral or intracerebroventricular injection of the putative endogenous kappa receptor agonist, dynorphin, or of any of several synthetic kappa-agonistic drugs. The role of kappa agonists in reward has not been established; however kappa receptors have been detected in all areas traditionally associated with taste and feeding, and recently kappa as well as mu binding was discovered in the ventral tegmental area.

In contrast to the well-documented behavioral effects of morphine, the effects of the putative endogenous mu- and delta-agonists have been less extensively studied and therefore are less conclusive. Morphine is known to bind preferentially to mu receptors, but it is also an agonist at delta and, at much higher concentrations, at kappa receptors. Not unimportantly, morphine was the prototype for classifying the endogenous ligands as opioid or not, and it also traditionally serves as the standard against which the effects of other opioid agonists are compared. For these reasons, it was decided to use this compound as the standard for the present investigation.

A number of problems are associated with long duration paradigms, in which food intake is measured in grams consumed over time, that may hinder the interpretability of the data obtained. Observations of sedation and locomotor or behavioral disruption following site-specific drug administration suggest a possibility of overdose. Protracted latencies to the onset of eating further introduce the question as to whether the activation of regional opioid receptors by residual drug is responsible for feeding, or if drug diffusion to other brain areas may

have elicited the delayed behavioral response. If opioid receptors at the site of injection indeed participate in feeding, the behavior should be apparent within a relatively short time following drug administration. A correspondence between time spent eating and total consumption has been reliably observed, suggesting that the former may be a valid measure of feeding in an observation paradigm. The doses of drugs selected for intracerebral microinjection should include a wide range of concentrations, and should reflect 1) some relationship, at least at the midpoint of the range, to the typical hourly self-administered dose of morphine, and 2) recognition of different molecular weights and an adjustment for differences in the reported pharmacological potencies between drugs being compared.

The present investigation was intended to explore the ability of microinjections of morphine or dynorphin$_{1-13}$ into selected brain regions to elicit feeding within a short period of time following drug administration. The observed behavioral responses were expected to reflect any direct relationship between activation of opioid receptors at the targetted site and feeding behavior.

GENERAL METHOD

Subjects: Experimentally naive, male, Long-Evans rats (Charles River, Wilmington, MA), weighing 355 to 430 g at time of surgery were acclimatized to the colony room for at least one week prior to surgery. Animals were individually housed in stainless steel cages having open mesh fronts and floors. Free access to standard laboratory rat chow and tap water was permitted at all times. The colony room was climate-controlled with a 12-hour light/dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.). In addition to the food in the food hopper, a few fresh pellets were scattered daily on the floor of each rat's home cage to reduce potential novelty effects associated with similar food availability in the experimental apparatus, described below.

Surgery: Rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) and were given atropine sulfate, (0.3 mg/kg, s.c.) and Penicillin G (30,000 I.U., i.m.) as soon as the anesthetic had taken effect. Animals were secured in a Kopf stereotaxic apparatus and a 22-gauge stainless steel guide cannula (Plastic Products Co., Roanoke, VA) was permanently implanted in the ventral tegmental area with the tip 0.5 mm above the targetted injection site. The upper incisor bar was fixed at 5.0 mm above the interaural line, and the stereotaxic co-ordinates used (Pellegrino, Pellegrino, & Cushman, 1979) were as follows. For Experiments 1 and 2 the cannulae were placed 3.8 mm posterior to bregma, 0.6 mm lateral to the midline, and 7.9 mm ventral to dura. For Experiment 3 the cannulae were angled 20° laterally, and co-ordinates were adjusted to 3.8 mm posterior to bregma, 3.6 mm lateral to the midline, and 8.2 mm ventral to dura. At the end of surgery a 28-gauge stainless steel obturator, previously matched and cut flush with the guide cannula tip, was inserted into the guide cannula. Animals were

handled daily during the week following surgery and were habituated to the observation chamber and to the presence of the experimenter for approximately 10-minute periods on at least two separate days prior to testing.

Apparatus: An electrolytic microinfusion transducer system (EMIT: Bozarth & Wise, 1980), with a constant current of 150 μA, DC, for 28 seconds, delivered 0.5 μl solution through a 28-gauge stainless steel injector that extended 0.5 mm beyond the cannula tip. The behavioral observation chamber, measuring 26 x 37 x 38 cm, was constructed of wood with one side Plexiglas. The floor of the chamber was covered with standard lab chow pellets weighing approximately 4 to 5 g each (Prolab: Agway Inc., Syracuse, NY). During the 15 minutes immediately following drug injection, numerically coded ongoing behavior was recorded by digital entry to a microcomputer. An in-house program automatically timed, compiled, and tabulated the results at the end of each observation period. All testing was conducted during the light portion of the light/dark cycle.

Drugs: Morphine sulfate (Department of Health & Welfare, Canada) and dynorphin$_{1-13}$ (Sigma Chemical Co., St. Louis, MO) were each dissolved in Ringer's solution. Naloxone hydrochloride (Experiment 3) was dissolved in 0.9% saline.

Histology: Rats were deeply anesthetized with chloral hydrate and were perfused intracardially with 0.9% saline and then with 10% formalin. Brains were stored in 10% formalin for at least 24 hours, blocked, and sliced on a coronal plane at -25°C in 40 μm sections. Slices were positioned on gelatin-coated slides, then stained with Cresyl violet, and were viewed at 10x magnification for verification of cannula placements.

EXPERIMENT 1

The first step in this investigation was to examine the ability of microinjections of opioids into the ventral tegmental area to elicit feeding in food satiated rats within a short time following drug administration. Morphine in this region is rewarding. Endogenous dynorphin has been found in the ventral tegmental area, and it produces feeding when it is injected into the cerebral ventricles. A possible role for ventral tegmental dynorphin in feeding has not been previously established. Because morphine and the opioid peptide fragment dynorphin$_{1-13}$ bind primarily to different opioid receptor subtypes, it was of interest to compare the effects of these two ligands on feeding behavior.

Method

Twelve rats with unilateral guide cannulae aimed at the ventral tegmental area as described in the General Method served as subjects. Each rat was connected to the injection apparatus and was placed in the observation box for a 5-minute habituation period. The stainless steel obturator was cleaned with 70% ethanol and allowed to air-dry until the end of the session. Microinjection of either morphine sulfate (0, 1, 3, 10 or 30 nmoles) or dynorphin$_{1-13}$ (0, 0.003, 0.03, 0.3, 1, 3 or 30 pmoles) was performed in the freely moving animal by activating the electrical current for 28 seconds. Feeding, grooming, contacting or moving food, activity, inactivity, sniffing, and pauses between behaviors were observed and were recorded on microcomputer as they occurred during the 15 minutes immediately following injection. Criteria for feeding were as described by Roberts (1980): " ... biting off morsels from pellets that were often held in the forepaws, followed by mastication and swallowing." All three of these elements were

required for a behavior to qualify as eating. Simple snout or oral contact with food was scored as "food contact." Transporting a food pellet from one part of the chamber to another was scored as "moving food."

The injector remained in place until the end of the test session, so that the rat remained undisturbed both during and following drug administration. At the termination of the test period, the rat was disconnected from the injection apparatus, and the stainless steel obturator was replaced in the guide cannula. The injector was then checked for drug flow. The flow test involved turning on the current and watching for the appearance of a fluid bubble at the tip of the injection cannula. If tissue or other organic debris in the cannula had interfered with drug delivery, the drug flow was either sluggish and delayed, or it was blocked and no fluid bubble appeared at all. If this occurred the data for the session were omitted and the animal was retested at the same dose on another day. The only other circumstance leading to retesting a rat at any drug dose was the occurrence of an unusual event such as a sudden noise that caused the rat to freeze during the session. Drug doses were delivered in random order to minimize possible behavioral conditioning effects (see Vezina & Stewart, 1984). Animals received all doses of either morphine or dynorphin$_{1-13}$ before being retested with Ringer's solution and then switched to the alternate drug. The Ringer's test that preceded the dose regimen of either drug was used as the rat's baseline measure for that drug. The interval between injections was typically 2 days.

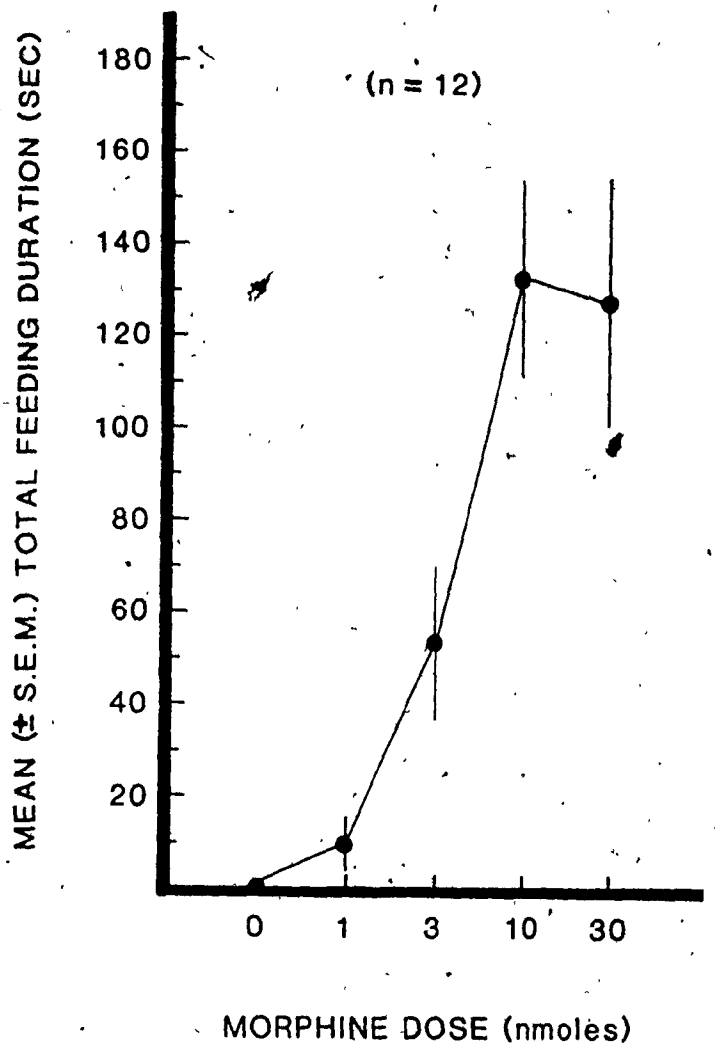

Figure 1. Total feeding durations for rats receiving unilateral microinjections of morphine into the ventral tegmental area. Dose order was random and the intertest interval was at least 48 hours. Vertical bars represent the standard error of the mean for each drug dose.

Results and Discussion

One-way analysis of variance (ANOVA: Kirk, 1982; Winer, 1971) for repeated measures of the total feeding duration scores for each drug revealed that both morphine [$F(4,44) = 12.93$, $p < 0.001$; see Figure 1], and dynorphin$_{1-13}$ [$F(5,50) = 8.39$, $p < 0.001$; see Figure 2] elicited

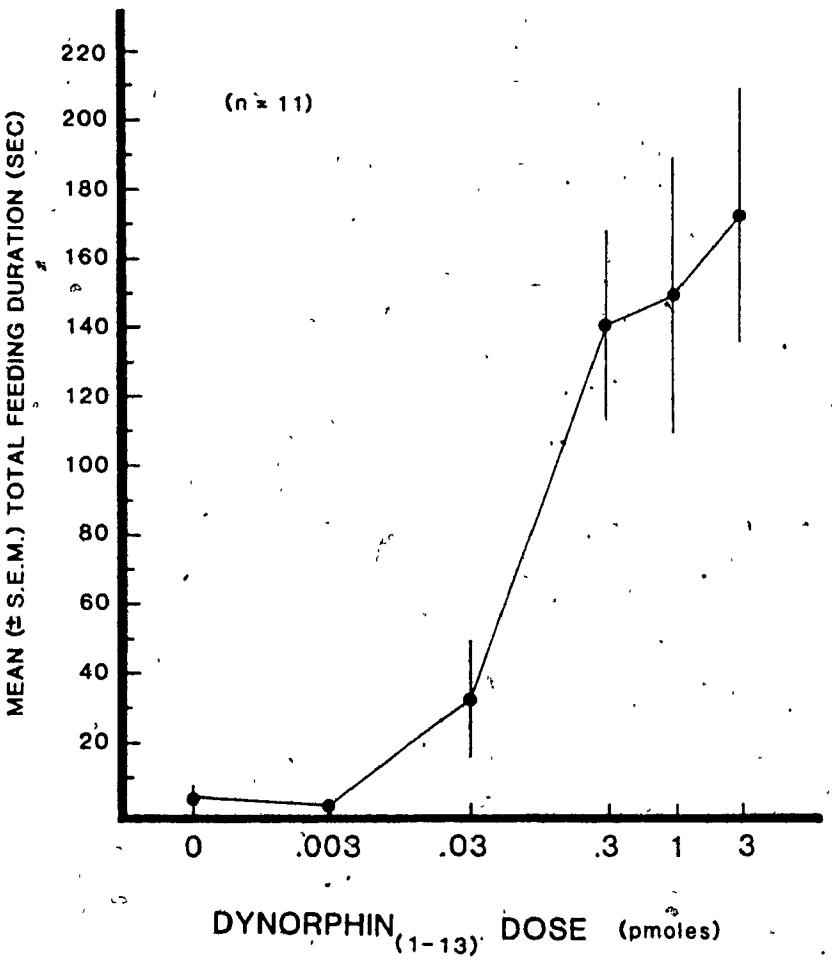

Figure 2. Total feeding durations for animals receiving unilateral microinjections of dynorphin$_{1-13}$ into the ventral tegmental area. Dose order was random and the intertest interval was at least 48 hours. Vertical bars represent the standard error of the mean for each drug dose.

eating among non-deprived rats within 15 minutes following injection into the ventral tegmental area. Multiple *t*-tests were used to determine significant changes from vehicle control injections. For this procedure, to hold the the α-level constant across the series of *t*-tests, the nominal α-level was divided by the number of comparisons (Fisher, 1935; Lindman, 1974). As illustrated in Figures 1 and 2, the effects of both drugs were dose-dependent.

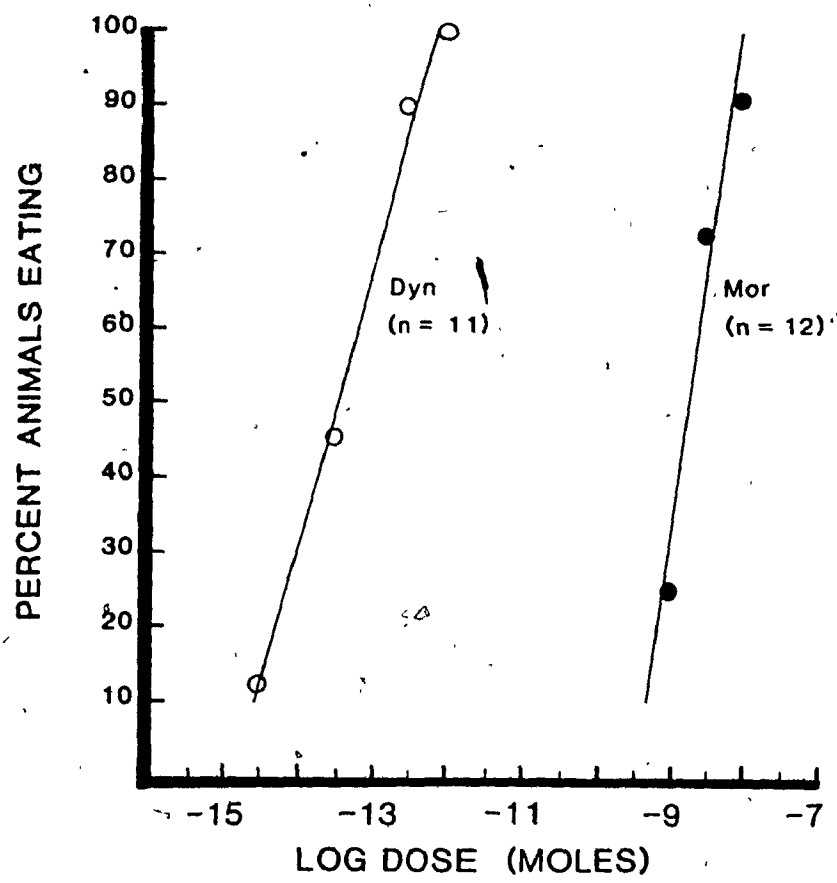

Figure 3. Quantal dose-response for morphine and dynorphin$_{1-13}$, showing the percentage of animals eating at each dose of either drug. Calculated ED_{50}'s were: morphine, 2 nmoles; dynorphin$_{1-13}$, 40 fmoles. This represents a potency difference of 50,000-fold between the two drugs.

The percentage of animals eating > 20 seconds following each dose of morphine and dynorphin$_{1-13}$ followed parallel linear progressions for each drug (see Figure 3). A comparison of the ED_{50} values (Litchfield & Wilcoxon, 1949; Tallarida & Murray, 1981) derived from the quantal dose-response analysis revealed that dynorphin$_{1-13}$ was 50,000 times more potent than morphine in eliciting eating when injected into the ventral tegmental area. Whereas the ED_{50} for morphine was 2 nmoles, the ED_{50} for dynorphin$_{1-13}$ was 40 fmoles.

The highest dose of dynorphin$_{1-13}$ (30 pmoles) produced what appeared to be a pronounced sedation in the first seven animals tested. Mean total feeding durations decreased from 172.9 ±37.0 seconds to 49.9 ±30.2 seconds, and the percentage of animals eating was reduced from 100% to 28.6%. This dose was discontinued for the remaining animals and was not included in the analysis.

Microinjections of morphine or dynorphin$_{1-13}$ into the ventral tegmental area were effective in eliciting dose-dependent feeding in food satiated rats within 15 minutes following drug administration. The short latency to onset (typically 5 to 10 minutes after injections) is in marked contrast to other studies reporting that feeding begins an hour or more after opioid injections into other brain regions (Tepperman, Hirst, & Gowdey, 1981a; Woods & Liebowitz, 1985). The occurrence of dose-dependent feeding soon after the central injections suggests that the behavioral response was produced by a local drug action and was not the result of drug diffusion to some distal brain site. In addition, it was demonstrated that opioids microinjected into the ventral tegmental area of food satiated rats during the daytime elicited feeding of the same food that comprised the animals' normal daily diet. Highly palatable substances were not required to induce approach to and consumption of the food. This experimental method avoided food-associated novelty (Barnett, 1956) and palatability factors (Cooper, 198[illegible] 1983b) that may influence baseline consumption and may also interact or interfere with the drug effect.

Previous work has implicated kappa receptors in the modulation of feeding behavior. Both intracerebroventricular administration of dynorphin$_{1-13}$ (Katz, 1980; Levine, Morley, Gosnell, Billington, & Bartness, 1985; Morley & Levine, 1981; Morley, Levine, Grace, & Kneip,

1982) and systemic injection of the kappa-preferring synthetic ligand, U50,488H (Jackson & Cooper, 1986), produced eating in food-satiated rats. Dynorphin$_{1-13}$ is an extremely potent opioid peptide with strong actions on kappa receptors (Chavkin, James, & Goldstein, 1982; Corbett, Paterson, McKnight, Magnan, & Kosterlitz, 1982; Schulz, Wuster, & Herz, 1982), although it also binds at mu and delta receptors (Goldstein & James, 1984). The present finding that this peptide is 50,000 times more potent than morphine in eliciting feeding is consistent with the apparent relative binding affinity of these two compounds at kappa receptors (James & Goldstein, 1984). The large potency difference is also in agreement with the proposed role of kappa receptors in the modulation of feeding behavior.

EXPERIMENT 2

Experiment 1 demonstrated that microinjection of opioids into the ventral tegmental area was sufficient to produce eating in satiated rats and that standard lab chow was adequately palatable for the effect to be observed. A number of other studies have shown a concordance between observed eating duration and weight of food consumed (Jackson & Cooper, 1986; Kirkham & Blundell, 1984; Sanger, 1983), suggesting that the former is a reliable measure of feeding. High doses of enkephalin analogues, however, have been reported to produce "morphine-like behaviors," including stereotypic gnawing of the cage bars and forepaws, following microinjection into the ventral tegmental area (Joyce, Koob, Strecker, Iversen, & Bloom, 1981). Although stereotypic behavior was not observed at any drug dose used in Experiment 1, it was important to confirm empirically that the apparent feeding behavior in the observation paradigm had reflected actual consumption of the food and not merely a gnawing response. Experiment 2 was intended as a test of

the validity of the observation approach; a full dose regimen of morphine was chosen to examine this issue. The more traditional technique, measuring the weight of food consumed during a longer period of time than that employed in the observation paradigm, was used for this investigation.

Method

Five of the rats used in Experiment 1 received, in random order and typically at 2-day intervals, 0.3, 1, 3, 10 and 30 nmoles morphine in the ventral tegmental area with the microinjection technique described in Experiment 1. The injector remained in place for 180 seconds following administration to allow absorption of the drug into the target tissue. Rats were then disconnected from the injection unit, obturators were replaced in the guide cannulae, and the animals were placed in separate wooden chambers (26 x 37 x 38 cm) with stainless steel grid floors and equipped with water bottles. Pre-weighed dishes of standard lab chow pellets were removed and reweighed at one hour intervals for 3 hours. Care was taken at each of these times to collect and include in the weighing any food crumbs that had fallen through the grid flooring. The pre- and post-test differences in food weights represented the amount eaten. Occasional water spillage on removal from the chambers at the end of the 3 hours prevented an accurate measurement of drinking.

Results and Discussion

One-way analysis of variance for repeated measures revealed a significant dose-dependent effect of morphine in increasing food intake [$F(4,16) = 7.02$, $p < 0.01$; see Figure 4]. Comparison with the eating by three uninjected animals (mean = 2.8 ± 0.5 g), also from Experiment 1 and tested at the same time as morphine animals, indicated a significant difference in consumption by group [$F(1,6) = 11.23$, $p < 0.05$], and for

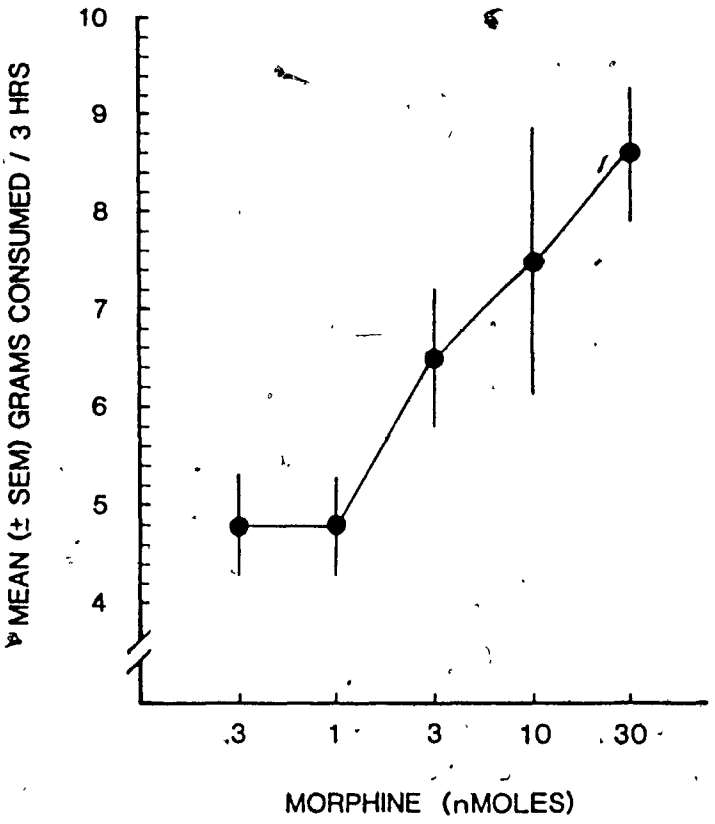

Figure 4. Total food consumed (grams) during 3 hours following unilateral microinjections of morphine into the ventral tegmental area. Vertical bars represent the standard error of the mean for each dose.

group by dose [$F(3,18) = 4.49$, $p < 0.05$].

These findings were consistent with the observation that the apparent feeding in response to opioid injection in the first experiment had reflected actual eating and not a nonspecific oral behavior. In addition, the validity of the brief-duration observation technique in examining the immediate influence of central opioid microinjections was supported by the similar findings of the more conventional approach.

EXPERIMENT 3

In Experiment 1, the highest dose of dynorphin$_{1-13}$ (i.e., 30 pmoles) was discontinued due to an apparent sedative effect of the drug at this dose. When the guide cannula directed toward the ventral tegmental area is placed on a vertical plane as in the first experiment, it passes through the periaqueductal gray region. Opioids in that region have been associated with sedation (Pert, DeWald, Liao, & Sivit, 1979; Tissot, 1980), catatonia (Thorn-Gray, Levitt, Hill, & Ward, 1981), analgesia (Jenck, Schmitt & Karli, 1983; Pert, DeWald, Liao, & Sivit, 1979; Pert & Yaksh, 1975; Sharpe, Garnett, & Cicero, 1974), and physiological dependence (Bozarth & Wise, 1984; Wei, 1981). It is possible that the apparent sedation produced by 30 pmoles dynorphin$_{1-13}$ was a consequence of diffusion of the drug up the cannula shaft to the periaqueductal gray. If guide cannulae are implanted in the ventral tegmental area on a sufficiently wide lateral angle, the cannula shafts do not pass through the periaqueductal gray. Indeed, this surgical procedure avoided naloxone-precipitated opioid withdrawal symptoms that were anatomically localized to the periaqueductal gray (Bozarth & Wise, 1984). If the sedation observed in the first experiment was due to dorsal diffusion up the cannula exterior resulting in activation of opioid receptors in the periaqueductal gray, then angling the cannula to avoid that area should likewise eliminate the pronounced sedative effect of 30 pmoles dynorphin$_{1-13}$. Eating in response to this dose might be further enhanced beyond that observed at the next lower dose. In addition, the possibility of nonspecific physico-chemical effects of the injections as an explanation for the observed effects was examined by opioid antagonist administration together with the lowest dose of dynorphin$_{1-13}$ that produced feeding in 100% of the rats. Finally, it

was of interest to assess the effects of injections of dynorphin$_{1-13}$ into the ventral tegmental area on drinking behavior. It was possible that water availability during the session may be required to contribute to an increase in grooming by this ligand. Although both drinking and grooming were reported to be enhanced by central opioid microinjection (Aloyo, Spruijt, Zwiers, & Gispen, 1983; Morley & Levine, 1981; Walker et al., 1980), no significant effect on grooming by either morphine or dynorphin$_{1-13}$ was observed in Experiment 1.

Method

Nine male, Long-Evans rats were implanted unilaterally with stainless steel guide cannulae aimed, on a 20° angle, at the ventral tegmental area (see General Method). Procedures and apparatus were identical to those employed in Experiment 1, except that a water bottle was attached to the exterior of the observation chamber with the spout extending into the chamber. Dynorphin$_{1-13}$ (0, 0.003, 0.03, 0.1, 0.3, 1, 3 and 30 pmoles) microinjections were delivered in random dose order, and the interval between tests was typically 2 days. In order to eliminate the possibility of nonspecific effects of the central injection as an explanation for the observed results, following this regimen animals received naloxone injection (1.0 or 3.0 mg/kg, i.p., 3 days apart) 10 minutes prior to ventral tegmental area administration of 0.3 pmoles dynorphin$_{1-13}$, the lowest dose that had produced eating in 100% of the rats. Behavior was monitored and recorded as described earlier.

Results and Discussion

Consistent with the findings of Experiment 1, one-way analysis of variance for repeated measures showed a significant dose-dependent effect for dynorphin$_{1-13}$ in producing feeding [$\underline{F}(7,56) = 7.93$, $\underline{p} <$

0.001; see Figure 5]. Eating scores were higher at all doses than in Experiment 1. In response to 30 pmoles dynorphin$_{1-13}$, however, feeding decreased from the maximum duration scores observed at 3 pmoles (from 266.9 ± 49.7 seconds to 165.1 ± 24.4 seconds). The apparent sedation and reduction in feeding also occurred in the first experiment among rats that had received 30 pmoles dynorphin$_{1-13}$. This finding suggests that it is unlikely that the sedative influence of this dose was attributable to direct dorsal diffusion of the drug to the periaqueductal gray. Perhaps the most parsimonious explanation is that the apparent sedation may have arisen from recruitment, within the ventral tegmental area, of a separate opioid receptor subtype population for which dynorphin$_{1-13}$ has a lower affinity than it has for those receptors involved in the feeding response. Unfortunately, current knowledge renders this argument untenable. The only opioid receptor population, apart from kappa, identified to date in the ventral tegmental area is mu (Mansour et al., 1987). Morphine, a primarily mu receptor agonist, did not produce sedation in this study. Moreover, mu activation in the ventral tegmental area has been found to enhance the release of dopamine in the nucleus accumbens (Latimer, Duffy, & Kalivas, 1987), which produces locomotor activity (Kelley, Stinus, & Iversen, 1980) and therefore is inconsistent with sedation. Chavkin & Goldstein (1984) have suggested that only 10% of functional opioid receptors may need to be occupied to achieve maximum effect. It is possible that a much higher proportion of drug-occupied receptors may lead to an interference with the behavior, in this case, eating. It must also be considered that a dose of dynorphin$_{1-13}$ as low as 30 pmoles may be sufficient to produce non-opioid effects as described by Stevens, Weinger, and Yaksh (1987). This issue requires further investigation before a clear explanation can be

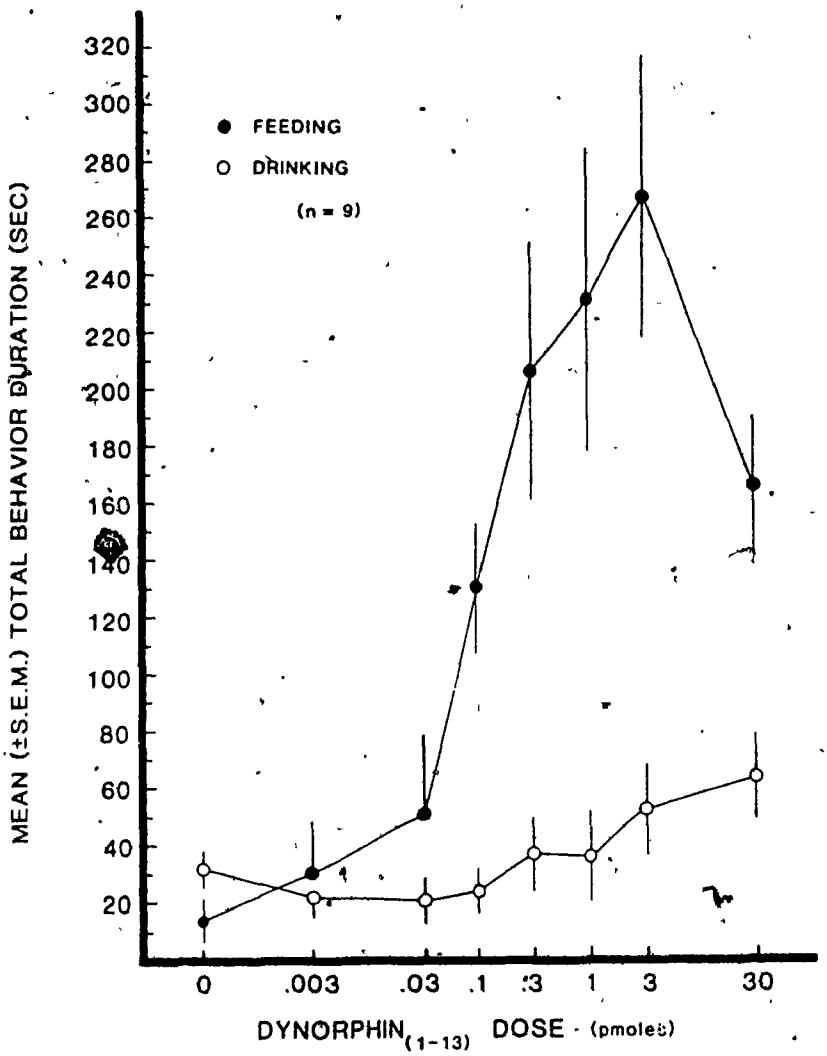

Figure 5. Total feeding and drinking durations following microinjections of dynorphin$_{1-13}$ into the ventral tegmental area. Unilateral cannulae were angled 20° to avoid the periaqueductal gray. Vertical bars represent the standard error of the mean for each dose of dynorphin$_{1-13}$.

determined. In terms of other behaviors, dynorphin$_{1-13}$ had no significant effect on either drinking [$F(7,56) = 2.12$, $p > 0.05$], or grooming [$F(7,56) = 1.11$, $p > 0.05$]. Neither of these behaviors was sequentially associated with feeding.

Naloxone reduced total eating duration in a dose-dependent fashion [$F(2,16) = 9.95$, $p < 0.01$; see Figure 6]. In addition, the percentages of rats eating > 20 seconds at 0, 1 and 3 mg/kg naloxone with 0.3 pmoles

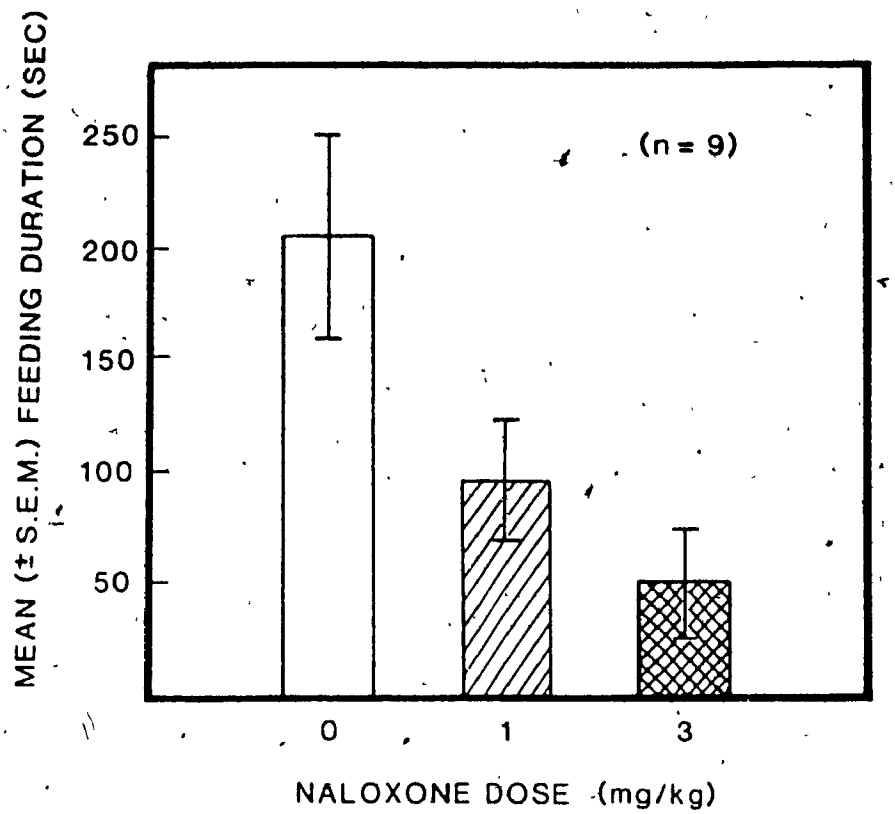

Figure 6. Effect of naloxone on total feeding durations. Naloxone HCl was administered i.p. 10 min prior to unilateral VTA microinjection of 0.3 pmoles dynorphin$_{1-13}$. Tests with naloxone were separated by at least 3 days. Vertical bars represent the standard error of the mean for each treatment.

dynorphin$_{1-13}$ were 100%, 78% and 33%, respectively. These data demonstrated that the enhancement of feeding by dynorphin$_{1-13}$ administration into the ventral tegmental area could be attenuated by a peripherally administered opioid antagonist. The lipophilicity of naloxone renders this drug unsuitable for site-specific central injection (Misra, Pontani, Vadlamani, & Mule, 1976), and the hydrophilic quaternary analogue, although preferable, is not readily available. Naloxone binds preferentially to mu receptors, but it also binds at delta and kappa receptors (see James & Goldstein, 1984). Unfortunately,

a specific kappa antagonist is not yet available; the K_i for binding at mu receptors by the putative kappa antagonist, Mr-2266, is only about twice that at kappa receptors (Paterson et al., 1984). The possibility remains that the enhancement of feeding behavior by opioids microinjected into the ventral tegmental area may require the integrity of other, perhaps distal, spontaneously active opioid systems that would be affected by peripheral naloxone treatment but not necessarily by dynorphin$_{1-13}$ administered into the ventral tegmental area. This possibility has not been examined, however. It is likely, in the absence of evidence to the contrary, that the inhibition by naloxone of dynorphin$_{1-13}$-elicited feeding in the present experiment is attributable to antagonist action at kappa receptors in the ventral tegmental area.

EXPERIMENT 4

The first three experiments demonstrated that the ventral tegmental area is an important site for opioid-elicited feeding. Although dose-dependent feeding was produced by both morphine and dynorphin$_{1-13}$, the latter was 50,000 times more potent than morphine. Other brain regions, including specific hypothalamic nuclei (Woods & Liebowitz, 1985), the globus pallidus, central amygdala, and striatum (Gosnell, Morley, & Levine, 1984, 1986) and the nucleus accumbens (Mucha & Iversen, 1986), have also been demonstrated to support opioid-mediated feeding. Among these and other central areas, a number of specific sites were of particular interest.

The paraventricular nucleus of the hypothalamus has been identified as a region involved in both norepinephrine- and opioid-mediated feeding (Liebowitz & Hor, 1982; Stanley, Lanthier, & Liebowitz, 1984; Woods & Leibowitz, 1985). This nucleus is adjacent to the lateral hypothalamic area, where electrical stimulation produces feeding (see Wise, 1974)

and where dynorphin-containing cell bodies have been localized (Vincent et al., 1982b). In addition, kappa receptor binding has been reported in this region (Lynch et al., 1985).

Intracranial opioid self-administration into the nucleus accumbens has been reported (Goeders, Lane, & Smith, 1984; Olds, 1982). In addition, the dopamine link between the ventral tegmental area and this region made the nucleus accumbens an interesting area to observe.

Both the substantia nigra - pars reticulata and the periaqueductal gray contain dynorphin terminals (Vincent et al., 1982a, 1982b), and both regions are proximal to the ventral tegmental area. In addition, injections of morphine into the periaqueductal gray were reported to inhibit feeding produced by electrical stimulation of the lateral hypothalamus (Jenck et al., 1986). These areas were included in the present study as being not only interesting in themselves, but they also served as lateral and dorsal controls, respectively, for potential diffusion of drug from injection sites in the ventral tegmental area (see Bozarth, 1983).

In addition to the primary feeding data, observations of other behaviors were continued during this study. Opioids in the ventral tegmental area failed to produce increases in drinking or grooming, in contrast to other studies using intracerebroventricular opioid injections (Aloyo et al., 1983; Katz, 1980; Morley & Levine, 1981; Walker et al., 1980). It was possible that one or more of the other brain regions examined in the present investigation would yield differences on these measures in response to morphine or dynorphin$_{1-13}$.

Method

The observation chamber was the same as used in Experiments 1 and 3. Water was available in addition to food during testing, as in

Experiment 3. Surgeries, post-surgical care and pre- and post-surgical handling and habituation to the apparatus were as described in the General Method section.

Rats were implanted with unilateral guide cannulae aimed at one of the following brain regions: ventral tegmental area, nucleus accumbens, paraventricular nucleus of the hypothalamus, substantia nigra - pars reticulata, or periaqueductal gray. The surgical co-ordinates for cannula placements in each of the five brain areas are shown in Table 1.

TABLE 1

Surgical co-ordinates for guide cannulae.

Brain Area	Angle*	A / P	Lateral	Ventral
VTA	20°	- 3.8	3.60	- 8.23
ACC	5°	+ 3.4	2.14	- 6.83
PVN	0°	+ 0.8	0.40	- 7.60
SNR	0°	- 3.8	2.1--2.5	- 8.3--7.9
PAG	0°	- 3.8	0.60	- 5.30

*The cannulae directed toward the VTA and ACC were angled to avoid the PAG and ventricles, respectively.
The upper incisor bar was set at +5 mm (DeGroot position: Pellegrino et al., 1979) and co-ordinates are expressed in millimeters. Anterior-posterior co-ordinates were measured from bregma, lateral from the midsagittal suture, and ventral from dura. Cannulae were implanted unilaterally.
Abbreviations: VTA, ventral tegmental area; ACC, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SNR, substantia nigra - pars reticulata; PAG, periaqueductal gray.

Actual cannula placements are illustrated in Figure 7. For testing, rats in each group were subdivided into two groups and received either morphine or dynorphin$_{1-13}$ in random dose order. When testing at all doses of the first drug was completed, animals were tested twice with

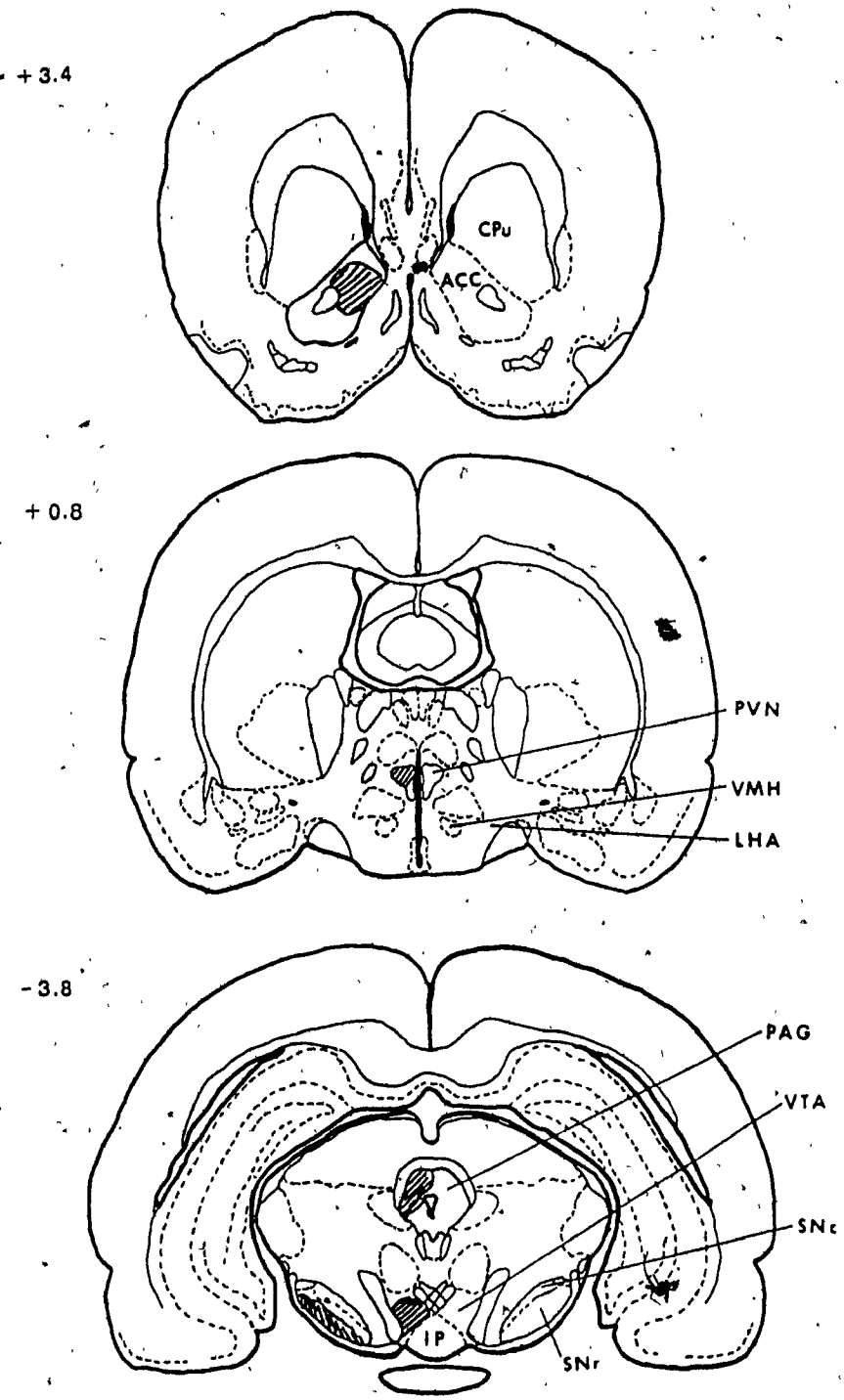

Figure 7. Schematic representation of cannula placements. Co-ordinates for each brain site appear in Table 1. **Abbreviations:** Upper panel -- ACC, nucleus accumbens; CPU, caudate-putamen. Middle panel -- LH, lateral hypothalamic area; PVN, paraventricular nucleus of the hypothalamus; VMH, ventromedial nucleus of the hypothalamus. Lower panel -- IP, interpeduncular nucleus; PAG, periaqueductal gray; SNC, substantia nigra - pars compacta; SNR, substantia nigra - pars reticulata; VTA, ventral tegmental area.

vehicle and were switched to the alternate drug. Rats were then tested with the full dose regimen, again in random dose order, of the second drug. The periaqueductal gray placement was of particular concern because of its involvement in physiological dependence (Bozarth & Wise, 1984; Wei, 1981). For this reason, dose order was planned for these rats so that a high dose was always followed 2 or 3 days later by a low dose. This procedure was intended to minimize the possibility that animals might eat as a consequence of drug-induced relief from potential withdrawal effects, rather than in response to a direct influence of periaqueductal gray opioid mechanisms on feeding behavior.

Results and Discussion

Feeding

Total Feeding Duration

Mean total feeding duration scores by cannula placement for morphine and dynorphin$_{1-13}$ are illustrated in Figures 8 and 9, respectively. Consistent with the findings of Experiments 1 to 3, microinjections of morphine [$F(4,32) = 8.99$, $p < 0.001$] or dynorphin$_{1-13}$ [$F(5,40) = 12.99$, $p < 0.001$] into the ventral tegmental area produced dose-dependent feeding. A comparison of the peak responses elicited by morphine (30 nmoles) and by dynorphin$_{1-13}$ (0.3 pmoles) at this site revealed that dynorphin$_{1-13}$ produced significantly higher maximum feeding duration scores than morphine in the same animals [means and S.E.M.'s = 205.5 $\pm$ 26.3 seconds vs. 129.5 $\pm$ 24.4 seconds, $t(8) = 2.47$, $p < 0.05$]. These results also replicated the finding in Experiment 1 of an approximately 50,000-fold potency difference between morphine and dynorphin$_{1-13}$ in the ventral tegmental area.

The nucleus accumbens was the only other brain site at which both morphine [$F(4,28) = 3.68$, $p < 0.025$] and dynorphin$_{1-13}$ [$F(5,40) = 6.91$,

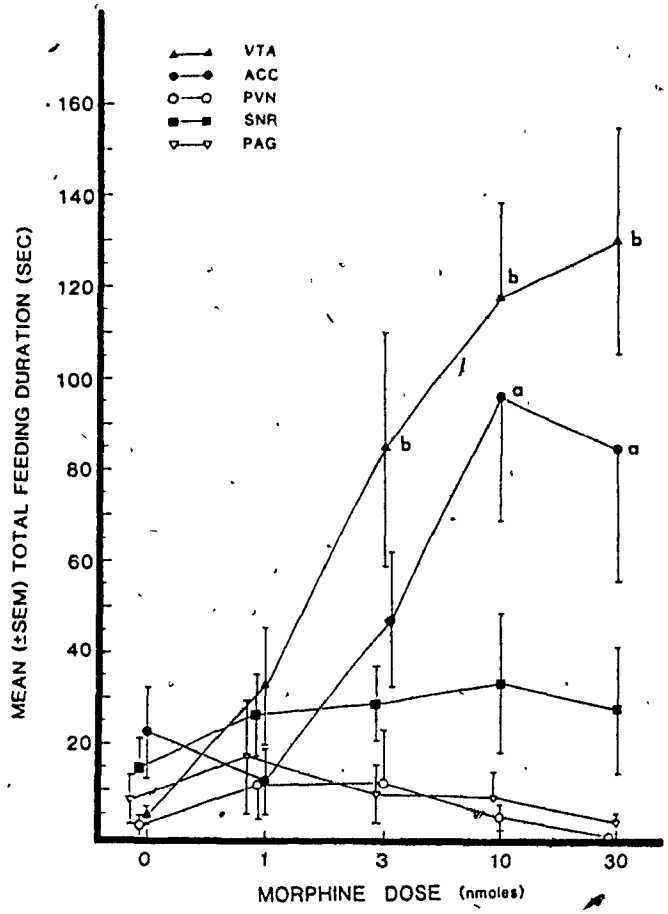

Figure 8. Total feeding durations following unilateral microinjections of morphine into different brain regions. (a = $p<0.05$; b = $p<0.01$ relative to vehicle mean by placement). Vertical bars represent the standard error of the mean at each drug dose.
Abbreviations: VTA, ventral tegmental area; ACC, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SNR, substantia nigra, pars reticulata; PAG, periaqueductal gray.

$p < 0.001$] elicited feeding. In contrast to the effects observed in the ventral tegmental area, there was no significant difference in the nucleus accumbens between the two drugs in producing feeding at their peak effective doses [10 nmoles morphine, mean = 96.0 ± 27.5 seconds vs. 0.1 pmoles dynorphin$_{1-13}$, mean = 103.5 ± 28.2 seconds; $t(7) = 0.19$, $p > 0.05$]. Total feeding durations for morphine in the ventral tegmental

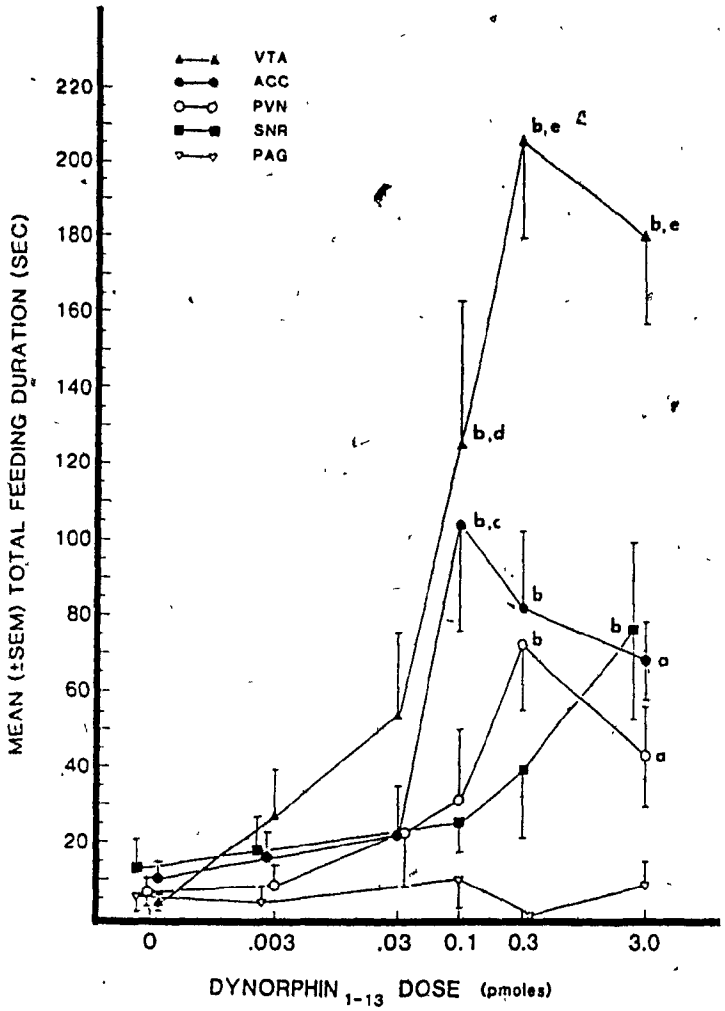

Figure 9. Total feeding durations following unilateral microinjections of dynorphin$_{1-13}$ into different brain regions. (a = $p<0.05$, b = $p<0.01$, relative to control mean by group; Dunnett's test for comparisons with a control mean). (c = $p<0.05$, d = $p<0.01$ relative to the same dose in the paraventricular nucleus and substantia nigra, pars reticulata; e = $p<0.01$ relative to all other placements at the same dose; Dunn's procedure for comparisons among means). Vertical bars represent the standard error of the mean for each group at each dose. Testing at a higher dose of dynorphin$_{1-13}$ in the substantia nigra, pars reticulata resulted in sedation.
Abbreviations: VTA, ventral tegmental area; ACC, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SNR, substantia nigra - pars reticulata; PAG, periaqueductal gray.

area and nucleus accumbens were not significantly different [$F(1,15)$ = 2.15, $p > 0.05$] and the shapes of the dose-response curves were similar; however mean total feeding scores following morphine in the nucleus

accumbens were consistently below those for the ventral tegmental area (see Figure 8).

Dynorphin$_{1-13}$ produced a significant difference in feeding between the ventral tegmental area and nucleus accumbens [$F(1,16) = 15.85$, $p < 0.005$] and by dose [$F(5,80) = 18.40$, $p < 0.001$]. A significant site x dose interaction was also evident [$F(5,80) = 4.00$, $p < 0.005$]. This reflects the differences in peak effects, defined here as the maximum mean total feeding durations for each group, and their corresponding doses for dynorphin$_{1-13}$ at the two brain sites (ventral tegmental area:

TABLE 2

Peak Feeding Responses (Total Duration)

Brain	DRUG: Morphine		Dynorphin(1-13)	
Region	Dose	Tot. Sec.	Dose	Tot. Sec.
VTA	30 nmoles	129.5 (24.4)	0.3 pmoles	205.5 (26.3)
ACC	10 nmoles	96.0 (27.5)	0.1 pmoles	103.5 (28.2)
PVN	n.s.	-	0.3 pmoles	72.2 (17.6)
SNR	n.s.	-	3.0 pmoles	75.7 (23.4)
PAG	n.s.	-	n.s.	-

Feeding responses varied among cannula placements in terms of both maximum total durations and by the drug doses that produced maximum feeding. Numbers shown represent the means (±S.E.M.'s). n.s. = no significant feeding.
Abbreviations: VTA, ventral tegmental area; ACC, nucleus accumbens; PVN, paraventricular nucleus of the hypothalamus; SNR, substantia nigra, pars reticulata; PAG, periaqueductal gray.

mean = 205.5 ±26.3 seconds at 0.3 pmoles; nucleus accumbens: mean = 103.5 ±28.2 seconds at 0.1 pmoles; significance determined by Dunn's

procedure; see Figure 8). Peak mean total feeding durations and their corresponding doses by drug and brain region appear in Table 2.

In the paraventricular nucleus, morphine failed to elicit feeding within the 15 minute observation period [$\underline{F}(4,28) = 0.63$, $\underline{p} > 0.05$]; however, dynorphin$_{1-13}$ produced a small but significant increase in feeding [$\underline{F}(4,36) = 5.13$, $\underline{p} < 0.001$]. Similarly, morphine in the substantia nigra was ineffective in producing feeding [$\underline{F}(4,36) = 1.60$, $\underline{p} > 0.05$], but dynorphin$_{1-13}$ elicited dose-dependent eating [$\underline{F}(5,45) = 3.40$, $\underline{p} < 0.025$] that was significant only at 3.0 pmoles. The dose-response function suggested that at this dose of dynorphin$_{1-13}$ in the substantia nigra the feeding response was increasing. Animals were subsequently tested at 30 pmoles dynorphin$_{1-13}$ to determine whether higher doses than those effective in the other regions examined might be required to elicit a robust feeding response. A marked sedation ensued, however, indicating that the maximum obtainable response from the substantia nigra had probably occurred at 3 pmoles. Consistent with the findings of Jenck et al. (1986), neither morphine [$\underline{F}(4,24) = 0.63$, $\underline{p} > 0.05$] nor dynorphin$_{1-13}$ [$\underline{F}(4,24) = 0.82$, $\underline{p} > 0.05$] injected into the periaqueductal gray had any effect on feeding behavior.

Two-way analyses of variance for total feeding scores among placements were performed only for those brain sites where the one-way analysis of variance indicated that the drug had produced a significant effect on feeding. Two-way analysis of variance of feeding durations elicited by dynorphin$_{1-13}$ in the ventral tegmental area, nucleus accumbens, paraventricular nucleus, and substantia nigra revealed significant main effects for placements [$\underline{F}(3,34) = 16.86$, $\underline{p} < 0.001$] and dynorphin$_{1-13}$ doses [$\underline{F}(4,136) = 24.54$, $\underline{p} < 0.001$]. In addition, a significant placement by dose interaction was shown [$\underline{F}(12,136) = 4.62$, $\underline{p}$

< 0.001]. This suggested that first, dynorphin$_{1-13}$ did not produce equal feeding durations among placements, and second, the feeding dose-response functions for dynorphin$_{1-13}$ were different among brain sites. Dunn's multiple comparison procedure (Kirk, 1982) showed that dynorphin$_{1-13}$ in the ventral tegmental area produced significantly higher total feeding duration scores than at all other placements at 0.3 and 3.0 pmoles. Both the ventral tegmental area and nucleus accumbens feeding scores were higher than those of the paraventricular nucleus and substantia nigra at 0.1 pmoles dynorphin$_{1-13}$. Scores for the paraventricular nucleus and substantia nigra were similar to one another, except that the peak response in the paraventricular nucleus occurred at 0.3 pmoles dynorphin$_{1-13}$ and dropped at the higher dose, whereas maximum feeding for dynorphin$_{1-13}$ in the substantia nigra was observed at 3.0 pmoles (see Figure 9).

Percentage of Animals Eating

The percentage of animals eating in each group was computed for each dose of morphine and dynorphin$_{1-13}$. A total feeding time of 30 seconds or longer within a single session was arbitrarily chosen as the cutoff point for a rat to qualify as an eater at any drug dose. This served to eliminate the few low eating scores occurring under the vehicle control condition. The criterion was sufficiently low to permit including the lower scores arising from opioid microinjections into those brain areas from which the feeding response was significant but less robust than from the ventral tegmental area and nucleus accumbens. The quantal dose-response comparisons between morphine and dynorphin$_{1-13}$ for each brain region are illustrated in Figures 10 and 11. Comparison of these data with those depicted in Figures 8 and 9 suggests that the dose-related increase in number of animals responding contributes

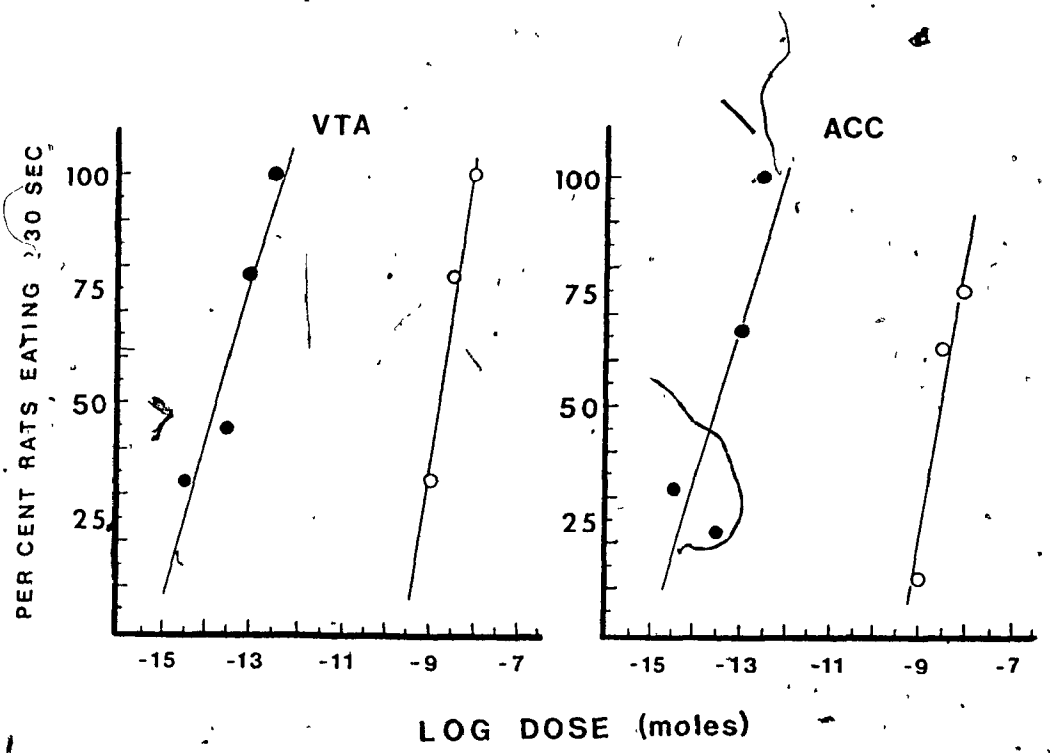

Figure 10. Quantal dose-response effect for feeding following unilateral microinjections of morphine or dynorphin$_{1-13}$ into the ventral tegmental area or the nucleus accumbens. Rats feeding for a minimum of 30 seconds during a 15-minute session were considered "eaters." The same dose range for each drug was effective in producing feeding in both brain regions, and a comparable potency difference between morphine and dynorphin$_{1-13}$ was represented at both sites. The apparent parallel linear functions are consistent with the principle that the effect of both drugs on feeding arose from activation of the same receptor population. Filled circles = dynorphin$_{1-13}$; open circles = morphine.

substantially to the magnitude of the overall dose-dependent response. Figure 10 demonstrates the relative potencies between morphine and dynorphin$_{1-13}$ in the ventral tegmental area and nucleus accumbens, brain regions where both drugs were effective in eliciting feeding. The apparent parallelness of the linear functions for the ventral tegmental area and nucleus accumbens is consistent with the suggestion that the same receptor subtype is contributing to the behavior for both drugs

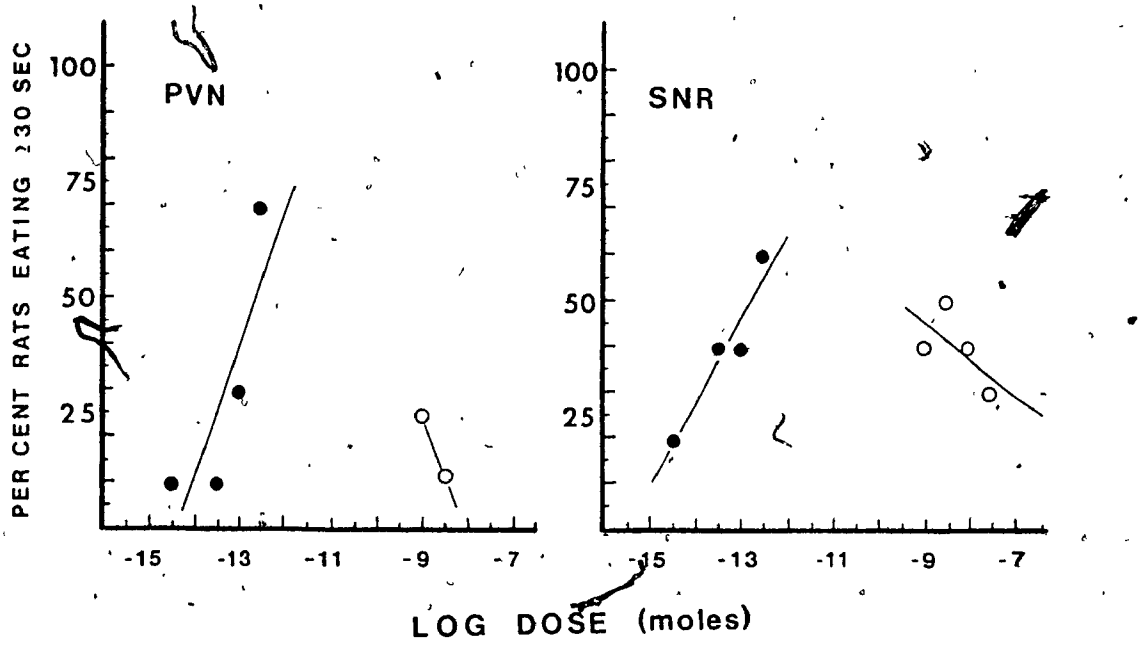

Figure 11. Quantal dose-response effect following unilateral microinjections of morphine or dynorphin$_{1-13}$ into the paraventricular nucleus of the hypothalamus or substantia nigra - pars reticulata. Rats feeding for a minimum total of 30 seconds during the 15-minute session were considered "eaters". Only dynorphin$_{1-13}$ elicited significant dose-dependent feeding at either site, and at each of these brain regions dynorphin$_{1-13}$ failed to produce feeding among 100% of the animals. Filled circles = dynorphin$_{1-13}$; open circles = morphine.

(Feldman & Quenzer, 1984; Tallarida & Murray, 1981). Figure 11 illustrates the dose-related increases in the percentage of animals eating following dynorphin$_{1-13}$ microinjections into the paraventricular nucleus and substantia nigra, in contrast to observations with morphine, which failed to produce feeding at these sites.

As might be expected, part of the significant total feeding duration scores was attributable to a dose-dependent increase in the number of animals eating in response to morphine or dynorphin$_{1-13}$. Further statistical analysis was conducted on the data for each group

where the analysis of variance of total feeding duration was significant. Simple linear regression analysis was performed by cannula placement group on the relationship between 1) the percentage of rats feeding at each dose of either dynorphin$_{1-13}$ or morphine and 2) the mean total feeding duration at the corresponding dose. The test for significance in linear regression yielded an F-statistic showing that in each case where either drug had produced a significant feeding effect, the correlations between total feeding and the percentages of rats eating at each dose were also significant. Morphine in both the ventral tegmental area [$r = 0.9894$, $F(1,3) = 139.8$, $p < 0.005$] and nucleus accumbens [$r = 0.8956$, $F(1,3) = 12.16$, $p < 0.05$] produced significant correlations on these measures, as did dynorphin$_{1-13}$ in the ventral tegmental area [$r = 0.9718$, $F(1,4) = 67.99$, $p < 0.005$], the nucleus accumbens [$r = 0.8532$, $F(1,4) = 10.70$, $p < 0.05$], the paraventricular nucleus [$r = 0.9744$, $F(1,4) = 75.09$, $p < 0.001$], and the substantia nigra [$r = 0.9405$, $F(1,4) = 30.67$, $p < 0.01$]. These findings suggested that dose-dependent increases in the percentages of rats eating had contributed importantly to the statistical significance of the overall feeding measure for each brain site. The contribution of other factors, such as dose-related changes in the duration of eating within single feeding bouts and alterations in the number of feeding bouts within a session, required further analysis.

Frequency and Duration of Feeding Bouts

During a single test session, rats frequently eat more than once. The total feeding duration score for each rat at any drug dose reflects the cumulative time that the rat ate during the session, and this time can be reflected by the number of feeding bouts multiplied by the mean of the individual bout durations. The computer program used in the

present investigation yielded the number of feeding bouts and mean bout durations per session as well as the total feeding durations. These measures were of interest in Experiment 4 because differential effects of morphine and dynorphin$_{1-13}$ on different components of total feeding behavior could be compared among brain regions. Jackson and Cooper (1986) suggested that the increase in total feeding observed following parenteral administration of putative kappa agonists is due to an increase in the number of feeding bouts within the test session rather than a prolongation of individual bout-durations. In the present study, central opioid microinjections elicited different responses on these measures depending on the brain site. This became evident when the data for "eaters" only were analyzed.

One of the basic assumptions of analysis of variance (ANOVA) for repeated measures is that there are equal cell sizes within a group. The earlier analyses therefore included the data for all animals whether they had responded or not. This requirement of ANOVA limited the power of these tests to detect possible dose-related effects of the drugs on specific behaviors that may have contributed differentially to the total feeding scores. The close correlations between the percentages of rats eating and total feeding durations suggested that the observed dose-dependent increases in feeding may have been related entirely to the proportion of animals eating at each dose within a group. It was of interest to examine the data for both the frequencies and the mean durations of feeding bouts to determine whether, among animals that ate, these measures were also affected by central opioid microinjection.

First, one-way ANOVAs were performed on the feeding bout frequency data for each drug/placement group where total feeding had previously been found significant. This yielded statistically significant effects

on bout frequency measures for morphine in both the ventral tegmental area and the nucleus accumbens, consistent with findings for both overall feeding and percentage of rats eating. The bout frequency ANOVAs were also significant for dynorphin$_{1-13}$ in the ventral tegmental area and the nucleus accumbens, but not in the paraventricular nucleus or the substantia nigra (see Table 3). Dynorphin$_{1-13}$ in the latter two brain areas produced significant effects on feeding according to the overall ANOVA, and the percentages of animals eating in these groups, although low compared to the other sites, were highly correlated with total feeding. If the increases in mean total feeding scores depended entirely on increases in the number of animals eating in the group, it would be expected that the ANOVAs on all other measures, such as bout frequency, similarly would show statistical significance. These findings suggest that further statistical evaluation may be appropriate to determine whether feeding behavioral patterns may have been influenced by the treatments.

One-way ANOVAs showed that morphine in the ventral tegmental area, but not in the nucleus accumbens, also significantly affected feeding bout durations. Dynorphin$_{1-13}$ also produced a significant dose-dependent increase in bout durations when injected into the ventral tegmental area, the nucleus accumbens, and the paraventricular nucleus, but not in the substantia nigra (see Table 3). These statistical findings suggest that following morphine injection into the ventral tegmental area rats ate more often and for longer periods of time as the dose was increased, but in the nucleus accumbens morphine affected only the number of feeding bouts. Note, however, that at most only 75% of the nucleus accumbens rats ate in response to morphine. Also according to the ANOVAs, both the frequency and duration of bouts for dynorphin$_{1-}$

$_{1-13}$ in the ventral tegmental area and in the nucleus accumbens were dose-dependent. The ANOVAs also indicate that in the paraventricular nucleus only bout duration was important, and in the substantia nigra neither measure was significant. This suggests that in the substantia nigra only the dose-dependent increase in the proportion of animals eating was important in establishing an effect of dynorphin$_{1-13}$ on feeding behavior. If we consider that the maximum percent of feeders with cannulae in the substantia nigra was only 60%, further statistical analysis is desirable.

Unfortunately, as explained earlier it was necessary to include the data for all animals, whether or not they ate, in the above analyses. A dose-related analysis of the data from responders only may not be performed using ANOVA procedures due to the equal cell size assumption. Means calculated for each treatment level therefore are reduced at lower doses by including low and zero scores. This approach is appropriate for the assessment of the effect of a treatment on a group, but it provides no information as to the ways in which this effect is achieved. For instance, when the data include a number of zero scores at lower treatment levels and few if any zero scores at higher levels, this effect alone may mask a significant influence of different treatment levels on the magnitude of the response. In the present experiment, when opioid administration produced significant feeding the number of eaters was dose-related, and at the lower drug doses several zero scores occurred. From the ANOVAs it is impossible to deduce whether different doses of a drug had produced significant differences in patterns of feeding behavior. If bout frequencies and durations were relatively constant then the drug effect must have relied entirely upon the ability of the drug to elicit the initiation of feeding. It was important to

extract and analyze the data for "eaters" only to determine whether opioids in any of the brain regions examined may have altered feeding patterns as suggested by Jackson and Cooper (1986).

The effects of morphine and dynorphin$_{1-13}$ on feeding bout frequency and mean bout duration within test sessions were evaluated by linear regression analysis, using only those scores from animals categorized as "eaters." Each of these measures was correlated independently with the animal's total feeding durations only when the first ANOVA had indicated a significant dose-dependent drug effect on total feeding duration. For morphine, there was no significant relationship between number of bouts and total feeding duration either in the ventral tegmental area or the nucleus accumbens. For dynorphin$_{1-13}$, however, the number of bouts was significantly related to total feeding for placements in the nucleus accumbens and the substantia nigra but not in the ventral tegmental area or the paraventricular nucleus (see Table 3).

In direct contrast to the bout frequency analyses, significant relationships were found between mean bout duration and total feeding duration for morphine in both the ventral tegmental area and the nucleus accumbens. For dynorphin$_{1-13}$, the bout duration relationships were also opposite to those for bout frequency. Mean bout duration was significantly correlated with total feeding for dynorphin$_{1-13}$ in the ventral tegmental area and the paraventricular nucleus, but not in the nucleus accumbens or the substantia nigra (see Table 3).

TABLE 3

Comparison of Statistics for Feeding Behavior Patterns

	Bout Frequency		Bout Duration	
	F/r	p	F/r	p
1. Morphine				
1.1. ANOVA (F)				
VTA	5.10	<.005	3.32	<.025
ACC	3.03	<.05	2.49	>.05
1.2. Correlation (r)				
VTA	0.067	>.05	0.598	<.001
ACC	-0.118	>.05	0.854	<.001
2. Dynorphin$_{1-13}$				
2.1. ANOVA (F)				
VTA	4.60	<.005	8.73	<.001
ACC	3.80	<.01	5.45	<.001
PVN	1.21	>.05	5.03	<.005
SNR	2.09	>.05	2.26	>.05
2.2. Correlation (r)				
VTA	0.208	>.05	0.520	<.001
ACC	0.639	<.001	0.272	>.05
PVN	-0.309	>.05	0.708	<.005
SNR	0.539	<.05	0.245	>.05

Note: The analyses were performed only if the Analysis of Variance for the overall main effect -- the total feeding duration -- was statistically significant. Correlations were performed between each of the measures indicated above and the total feeding durations for "eaters" only. Animals that ate for at least 30 seconds during the test session were classified as "eaters."

The ANOVAs included the data from all animals. This procedure indicated significant drug effects on bout frequency for all treatments except dynorphin$_{1-13}$ in the paraventricular nucleus and the substantia nigra (See Table 3). When the scores of non-responders were removed for the regression analysis, it was clear that the dose-dependent increases in the number of animals eating had contributed importantly to the significant ANOVA findings on bout frequency for all groups except dynorphin$_{1-13}$ in the nucleus accumbens, and that it had masked a significant effect on this measure in the substantia nigra. The maximum percentage of responders in this latter group was only 60%, yet total feeding was significant and dose-dependent. For this group it is possible that the relatively low number of eaters may have masked a significant effect of dynorphin$_{1-13}$ on feeding bout frequency.

For the mean feeding bout duration measure, the regression analysis indicated that for nucleus accumbens animals the ANOVAs had shown false positive significance for dynorphin$_{1-13}$ and false negative results for morphine. Increases in feeding bout durations were dose-related for morphine in both the ventral tegmental area and the nucleus accumbens, and for dynorphin$_{1-13}$ in the ventral tegmental area and the paraventricular nucleus.

The regression findings suggested that for placements in the nucleus accumbens, the proportion of animals responding had been a determining influence in the ANOVA findings for bout frequency and duration following morphine injection, and for bout duration following administration of dynorphin$_{1-13}$. Similarly, for animals with cannulae in the substantia nigra, the lower percentage of "eaters" led to ANOVA statistics that masked a significant bout frequency factor, revealed by

the subsequent correlational analysis. Given that the purpose of these analyses was to detect the contribution of individual elements in the feeding response patterns to the overall findings, such additional analyses of the data are important.

The relative contributions of each component of the feeding behavior measures are more readily apparent in Figures 12 to 14. Mean bout duration scores are shown in the main body of each figure with the number of "eaters" at each dose in parentheses. The mean number of feeding bouts appears in the inset. Separate values are shown for eaters only and for all animals. These numbers illustrate the contribution of percent of responders to the mean total feeding scores shown in Figures 8 and 9 as well as the influence of low or zero scores on the group means for the behavioral data presented. For instance, the data presented in Figures 12 and 13 show that when all scores (filled circles) are considered for number of bouts among ventral tegmental area rats, the greater incidence of zero scores at lower doses of either drug reduces the means for bout frequency at those doses. If the same data for eaters only are examined, however, it is clear that the effect was not dose-dependent on this measure, and that a dose-dependent increase in number of animals eating led to a statistically significant ANOVA finding for bout frequency. On examining bout duration data for the ventral tegmental area, it is clear that the magnitude of the bout duration values associated with dynorphin$_{1-13}$ compared to those for morphine constitute the important factor determining the significant difference between drugs at this placement. All animals in this group ate at the higher doses of both drugs, thus cancelling out the percentage of eaters as a factor in the comparison. In contrast, the bout frequency for nucleus accumbens animals declined somewhat among

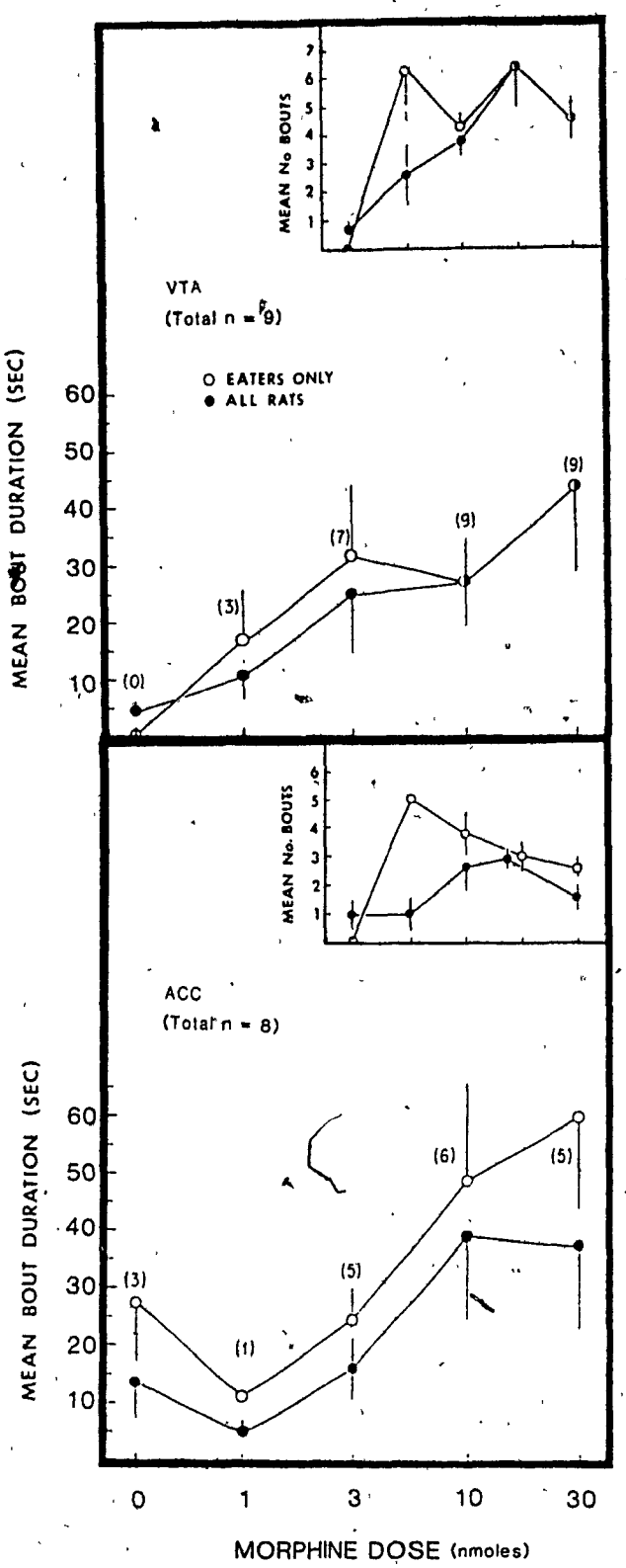

Figure 12. Mean feeding bout duration (main figure) and mean number of feeding bouts (inset) following unilateral microinjections of morphine into the ventral tegmental area (top) or nucleus accumbens (bottom). Animals feeding for a minimum total of 30 seconds during a 15-minute session were classified as "eaters." Vertical bars represent the standard error of the mean for each group at each dose.

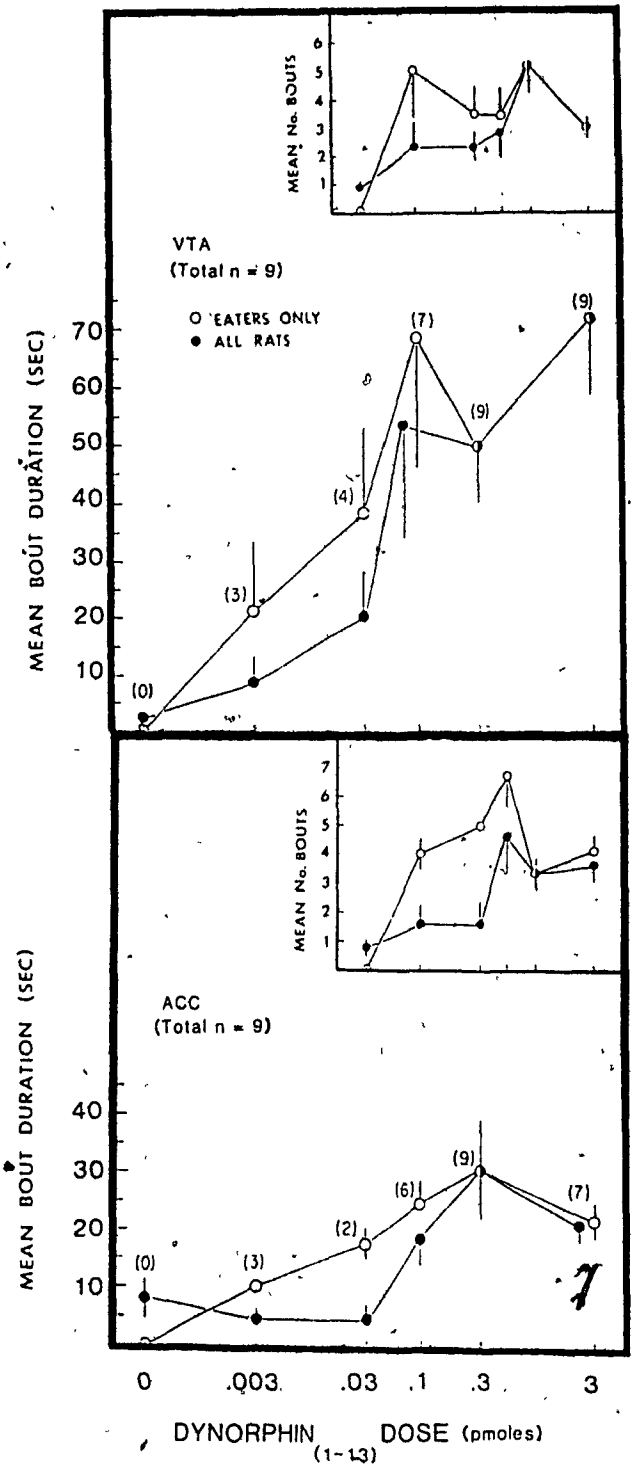

Figure 13. Mean feeding bout duration (main figure) and mean number of feeding bouts (inset) following unilateral microinjections of dynorphin$_{1-13}$ into the ventral tegmental area (top) or nucleus accumbens (bottom). Animals feeding for a minimum total of 30 seconds during a 15-minute session were classified as "eaters". Vertical bars represent the standard error of the mean for each group at each dose.

eaters with increasing doses of morphine, and the proportion of eaters failed to reach 100% at any dose. The dynorphin$_{1-13}$ data for this placement are somewhat more complex. The magnitude of the peak response for total feeding duration in the nucleus accumbens group was clearly determined by the bout frequency factor among eaters, although only two thirds of the rats ate at that dose. At the next higher dose, although 100% of the animals ate and the mean bout durations increased, the mean number of feeding bouts declined sharply. For each rat the total feeding score depends on a multiplicative relationship between number of bouts and mean bout durations. Consequently a very small reduction in frequency could dramatically affect the total score, even if durations should increase slightly, as seen at 0.3 pmoles dynorphin$_{1-13}$ for the nucleus accumbens group. In this case the reduction in bout frequency was more than 50% from the next lower dose. On examining the raw data it was apparent that this reduction was due almost entirely to a marked decrease in the frequency of feeding bouts by rats that had also eaten at 0.1 pmoles. A slight increase in bout duration and a larger increase in the number of eaters together at 0.3 pmoles dynorphin$_{1-13}$ were insufficient to neutralize the change in total feeding durations.

Figure 14 shows bout frequency and bout duration data for the paraventricular nucleus and substantia nigra, respectively. At both these sites, it is clear that the number of animals eating was important in determining the peak response, and that highest mean bout duration for paraventricular nucleus animals and highest bout frequency for substantia nigra animals corresponded to the doses of dynorphin$_{1-13}$ that had produced the greatest total feeding from these sites.

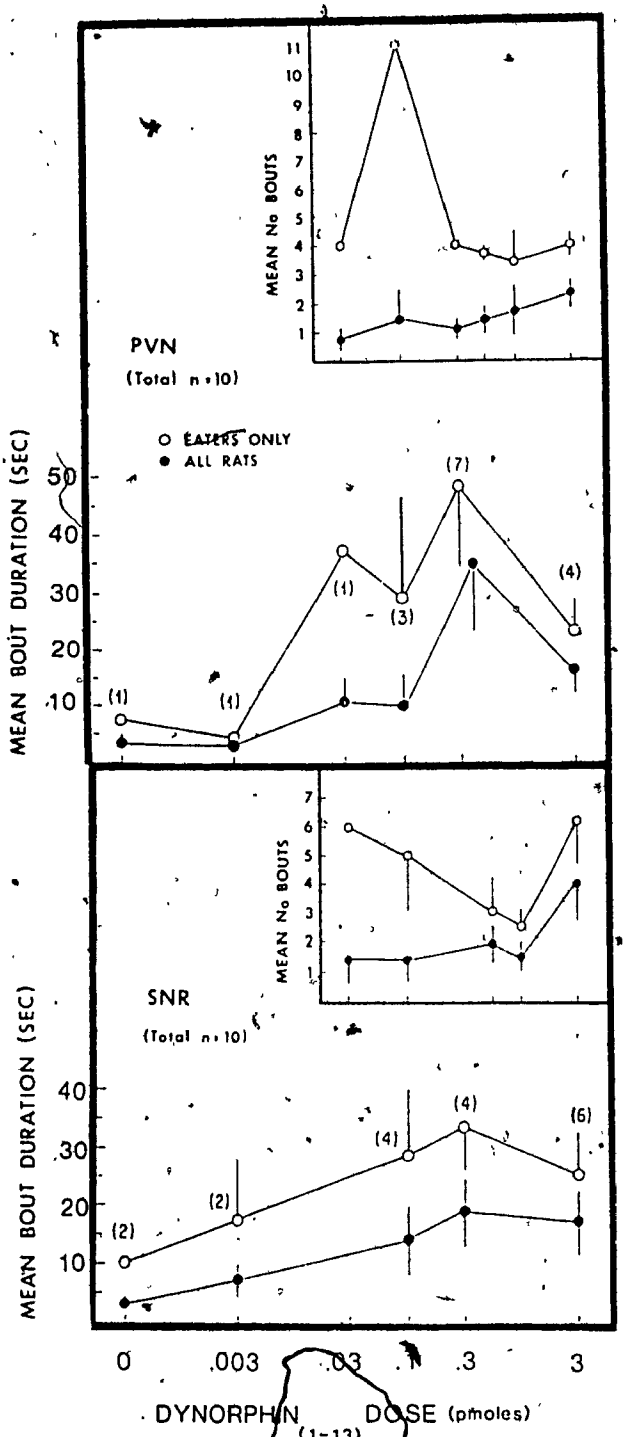

Figure 14. Mean feeding bout duration (main figure) and mean number of feeding bouts (inset) following unilateral microinjections of dynorphin$_{1-13}$ into the paraventricular nucleus of the hypothalamus (top) or the substantia nigra - pars reticulata (bottom). Rats feeding for a minimum total of 30 seconds during a 15-minute session were classified as "eaters." Vertical bars represent the standard error of the mean for each group at each dose.

Of all placements tested, the ventral tegmental area produced the most robust feeding in response to both morphine and dynorphin$_{1-13}$. The nucleus accumbens was next highest. The behavioral observations suggest that the proportion of animals responding contributed significantly to these findings. In addition, both drugs in the ventral tegmental area produced a dose-dependent feeding response by increasing feeding bout duration rather than the number of feeding bouts. In the nucleus accumbens bout durations were increased by morphine and bout frequencies were increased by dynorphin$_{1-13}$. This is consistent with observations by Jackson & Cooper (1986) following systemic administration of mu and kappa agonists. Feeding following dynorphin$_{1-13}$ in the nucleus accumbens was significantly less robust than in the ventral tegmental area, however. It is difficult to discern whether, following systemic drug administration, the effect on bout frequency of a putative kappa agonist in the nucleus accumbens might predominate.

Drinking

Drinking was not significantly affected by either drug at any of the placements examined during the 15 minutes following microinjection. One-way analysis of variance for repeated measures yielded the statistics shown in Table 4. Nonetheless, groups that ate more also tended to drink more as shown by the correlation between these measures for both morphine [$r = 0.9962$, $F(1,3) = 391.74$, $p < 0.001$] and dynorphin$_{1-13}$ [$r = 0.9583$, $F(1,3) = 33.72$, $p < 0.025$; see Figure 15]. One possible explanation for this apparent discongruity in findings is that the groups of rats that ate also appeared to explore and investigate the chamber more extensively than those groups not eating; these animals may be more likely to discover the water spout. This did not appear to be related to locomotor activity per se. For

TABLE 4

Statistical Results for Drinking Duration Scores

Placement	df	F	p
Morphine			
VTA	4,32	0.57	> 0.05
ACC	4,28	2.50	> 0.05
PVN	4,28	0.41	> 0.05
SNR	4,36	1.31	> 0.05
PAG	4,24	1.93	> 0.05
Dynorphin			
VTA	5,40	0.69	> 0.05
ACC	5,40	1.13	> 0.05
PVN	5,45	1.68	> 0.05
SNR	5,45	2.38	> 0.05
PAG	4,24	1.64	> 0.05

instance, substantia nigra animals in particular demonstrated high levels of activity in response to morphine yet failed to eat or drink significantly. Data from longer-duration paradigms have indicated that up to 3 hours may elapse between dose-dependent feeding and drinking (Sanger, 1983). In the short observation period used in the present study, postprandial drinking was not observed. In fact, nearly all drinking took place prior to eating, and rats seldom drank following feeding. Although drinking and grooming were frequently observed in alternating sequences, the two behaviors were not apparently interdependent and each also occurred alone.

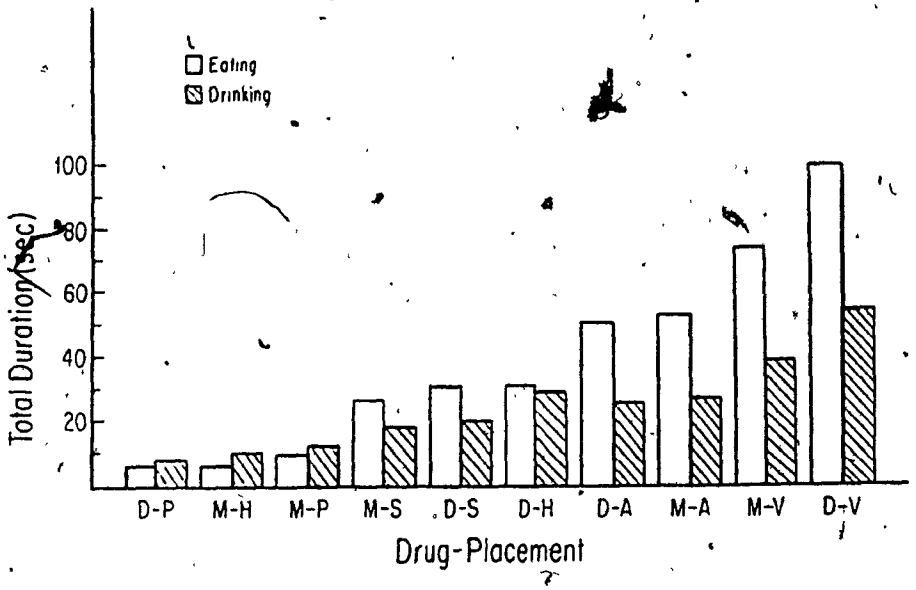

Figure 15. Mean total feeding and drinking following unilateral microinjections of morphine or dynorphin(1-13) into the ventral tegmental area, nucleus accumbens, paraventricular nucleus of the hypothalamus, substantia nigra - pars reticulata, or periaqueductal gray. Data shown are collapsed across doses: drinking was not dose-dependent for either drug at any placement examined. The data have been arranged to demonstrate the apparent linear relationship between ~~total~~ feeding and total drinking by drug/placement group. Open bars = mean total feeding; diagonal stripes = total drinking.
Abbreviations: D, dynorphin$_{1-13}$; M, morphine; P, periaqueductal gray; H, paraventricular nucleus of the hypothalamus; S, substantia nigra - pars reticulata; A, nucleus accumbens; V, ventral tegmental area.

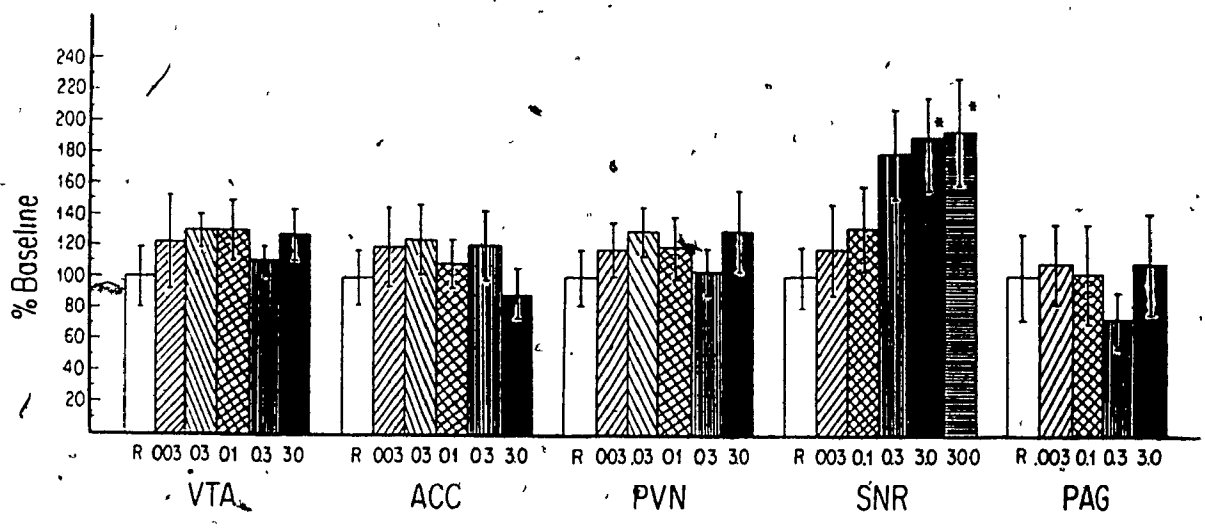

Figure 16. Total grooming behavior (percentage of vehicle baseline) following unilateral microinjections of dynorphin$_{1-13}$ into the ventral tegmental area, nucleus accumbens, paraventricular nucleus, substantia nigra - pars reticulata, or periaqueductal gray. Vertical bars represent the standard error of the mean for each placement at each dose.

Grooming

Only dynorphin$_{1-13}$ in the substantia nigra produced a significant increase in total grooming behavior [$F(5,45) = 3.02$, $p < 0.025$; see Figure 16]. Grooming following morphine in the paraventricular nucleus just missed statistical significance [$F(4,28) = 2.71$, $p = 0.05$; see Figure 17]. This appeared to arise from an initial dose-dependent decrease followed by a a return to baseline grooming levels at the highest morphine dose. This is probably a reflection both the suppression and general disorganization of behavior that was observed

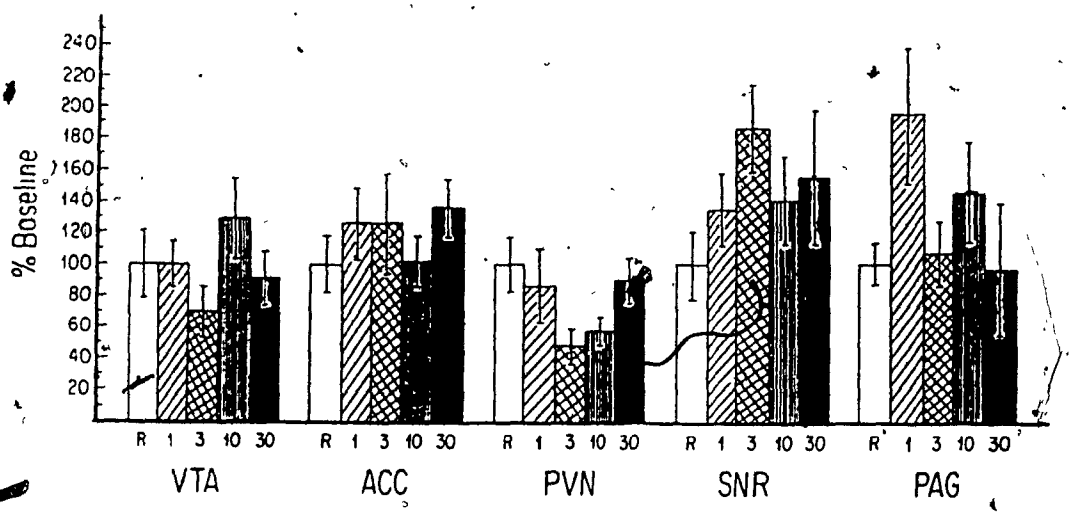

Figure 17. Total grooming behavior (percent of vehicle baseline) following unilateral microinjections of morphine into the ventral tegmental area, nucleus accumbens, paraventricular nucleus of the hypothalamus, substantia nigra - pars reticulata, or periaqueductal gray. Vertical bars represent the standard error of the mean for each placement at each dose.

following morphine injection into the paraventricular nucleus. Regardless of cannula placements, animals consistently groomed more under dynorphin$_{1-13}$ than morphine [$\chi^2(1) = 16.026$, $p < 0.005$]; however the effect of dynorphin$_{1-13}$ on grooming was significant and dose-dependent only in the substantia nigra.

Other Behaviors

A number of behaviors that were observed during the test sessions were not quantified. In both the ventral tegmental area and substantia nigra morphine produced high levels of activity that appeared to be expressed as contralateral circling. This was particularly apparent at the two higher doses of morphine in substantia nigra rats. Circling by these animals was quite tight and was confined mainly to one quadrant of the test chamber. In addition, notes made during the test sessions indicate that substantia nigra animals responded to higher doses of morphine with jerky, rapid movements. Morphine in the paraventricular nucleus also produced behavioral disturbances. At intermediate morphine doses, general behavior was suppressed. The animals appeared to be alert, however, and the effect could not be described as sedative. At 30 nmoles morphine, paraventricular nucleus animals exhibited a discontinuous, stop-start pattern of activity in which behaviors such as grooming or forward locomotion were repeatedly initiated but were not completed. Rats with placements in the nucleus accumbens were generally active during the sessions in response to both drugs. Frequently, at approximately 20 minutes after the sessions were over, however, nucleus accumbens animals appeared to be profoundly sedated following high doses. This was most noticeable following sessions when morphine was used. Periaqueductal gray rats were generally less active than the other groups, but pronounced sedation was not evident at any dose of either drug.

The pretest habituation period that preceded drug delivery for each session was originally intended to minimize handling and novelty effects. For other reasons this was an especially important procedure for nucleus accumbens and substantia nigra animals. The mechanical

disturbance produced by lowering the injection cannula elicited pronounced contralateral circling in both groups. Circling by substantia nigra rats was noticeably confined to a small area of the chamber. The turning pattern of nucleus accumbens rats was wider and tended to follow the perimeter of the chamber. The 5-minute habituation period provided sufficient time for this effect to subside completely.

An interesting observation was the apparent selectivity of food that ventral tegmental animals displayed in response to dynorphin$_{1-13}$ but not to morphine. When injected with dynorphin$_{1-13}$, ventral tegmental area rats tended to select a single pellet, frequently digging among the food to obtain it. The rat seemed always to return to eat only from that pellet regardless of the number of feeding bouts during the session. In addition, when feeding consisted of more than one bout, the initial bout durations during these sessions were typically extremely brief and preceded a single, long feeding bout that was not followed by further eating. With the exception of this group, all eaters (including the same ventral tegmental area rats when they received morphine) showing multiple feeding bouts ate from a number of different pellets in the test chamber, and bouts within a session lasted for approximately equivalent time periods.

GENERAL DISCUSSION

Unilateral microinjections of dynorphin$_{1-13}$ or morphine into the ventral tegmental area consistently elicited dose-dependent feeding in food satiated rats. This was represented by linear increases in total feeding durations, mean feeding bout durations, and the percentage of animals eating at each dose. Feeding in response to dynorphin$_{1-13}$ in the ventral tegmental area was more robust than to morphine and to either dynorphin$_{1-13}$ or morphine in any other brain region examined. A major role is implicated for naturally occurring dynorphin in the ventral tegmental area in opioid-mediated feeding behavior.

Dynorphin$_{1-13}$ in the ventral tegmental area was 50,000 times more potent than morphine in eliciting feeding. The ED_{50}'s -- the doses of each ligand required to produce eating in 50% of the animals -- were 40 femtomoles for dynorphin$_{1-13}$ and 2 nanomoles for morphine. This difference in potency is compatible with the relative binding affinities of dynorphin$_{1-13}$ and morphine at kappa receptors (James & Goldstein, 1984).

A dose of at 30 picomoles dynorphin$_{1-13}$ in the ventral tegmental area or the substantia nigra induced an apparent sedation. Cannulae in the ventral tegmental area were angled in an attempt to avoid possible diffusion of drug to the periaqueductal gray, located immediately dorsal to the ventral tegmental area and strongly associated with sedation and analgesia. This procedure failed to eliminate the sedative effect, however, suggesting that the attenuation of feeding behavior in these animals at higher dynorphin$_{1-13}$ levels may be mediated within the target site.

Neither drinking nor grooming behavior showed a dose-dependent response to microinjections of either morphine or dynorphin$_{1-13}$ in the

ventral tegmental area. Although these animals exhibited drinking for longer periods than rats in any other placement group, this is believed to reflect factors that were not quantified in the present study.

Both dynorphin$_{1-13}$ and morphine also produced dose-dependent feeding, but not drinking or grooming, when either ligand was microinjected into the nucleus accumbens of satiated rats. Dose requirements for both drugs in the nucleus accumbens to elicit feeding were in the same range as in the ventral tegmental area. The potency ratio between the effective doses of dynorphin$_{1-13}$ and morphine in producing feeding was consistent with the ventral tegmental area findings. Similar to results in ventral tegmental area rats, the quantal dose-response functions for dynorphin$_{1-13}$ and morphine in the nucleus accumbens were apparently parallel. The reductions in the percentage of feeders at the highest dose of each drug in the nucleus accumbens also appeared to be parallel. These observations are consistent with the proposal that feeding behavior elicited by both drugs in this region was probably mediated by the same receptor type.

Differences emerged in a cross-comparison of the relative magnitudes of feeding duration responses between drugs and brain regions. Dynorphin$_{1-13}$-elicited feeding was markedly greater in ventral tegmental area rats in comparison to nucleus accumbens animals. Feeding produced by morphine in the nucleus accumbens, however, was not significantly different from that observed following morphine in the ventral tegmental area or dynorphin$_{1-13}$ in the nucleus accumbens.

In the nucleus accumbens, dynorphin$_{1-13}$ and morphine also differentially influenced the patterns of feeding behavior. Among rats classified at each dose as "eaters," morphine in the nucleus accumbens increased the mean durations but not the frequency of feeding bouts,

whereas dynorphin$_{1-13}$ in this region increased the frequency but not the mean durations of feeding bouts. In contrast to ventral tegmental area rats, morphine failed to elicit feeding among 100% of nucleus accumbens animals at any dose. In the same rats however, 0.1 pmoles dynorphin$_{1-13}$ produced 100% feeders.

Dynorphin$_{1-13}$ but not morphine elicited dose-dependent feeding among rats with cannulae in the paraventricular nucleus or the substantia nigra. The maximum percentages of "eaters" in each of these groups was 70% and 60%, respectively. In substantia nigra rats dynorphin$_{1-13}$ also produced dose-dependent grooming, consistent with findings in other laboratories following intracerebroventricular injection of this peptide (Katz, 1980; Morley & Levine, 1981; Walker et al., 1980). Morphine in the paraventricular nucleus produced behavioral disruption. Drinking behavior was not elicited by opioid injection into any brain site examined. Neither drug in the periaqueductal gray area elicited feeding.

Opioid Receptors and Behavior

Taken together, the findings of this investigation suggest that feeding behavior following opioid microinjection into the ventral tegmental area, the nucleus accumbens, the paraventricular nucleus of the hypothalamus, and the substantia nigra - pars reticulata is probably mediated by an opioid agonist action at kappa receptors. Simultaneous activation of mu and possibly delta receptors at the same brain site, as would be expected with morphine, may produce effects that compete with the expression of the behavior of interest. In the ventral tegmental area and the nucleus accumbens the behavioral effect of morphine on non-kappa receptors appears to be essentially compatible with feeding. Conceivably the extent of behavioral interference by a ligand active at

more than one receptor type at any given injection site in tissue depends upon three major factors. The compatibility of behaviors affected, the pharmacological potencies of the ligand at each receptor type, and the relative importance of the different opioid systems that are activated at the injection site in eliciting different behaviors each contributes to the net behavioral expression.

The possibility of competing behaviors mediated by different opioids at a single brain site was even more strongly apparent among animals with cannulae in the substantia nigra and the paraventricular nucleus. Feeding responses occurred only in response to dynorphin$_{1-13}$, and responses were of significantly briefer total durations than among ventral tegmental area rats. Fewer than 100% of substantia nigra or paraventricular nucleus animals qualified as feeders at any dose of dynorphin$_{1-13}$. In the substantia nigra dynorphin$_{1-13}$ produced a significant increase in grooming. Morphine in this region also increased grooming, but an apparent dose-related effect at the two lower doses was not observed at higher doses. These rats showed markedly enhanced locomotor activity in response to morphine but not to dynorphin$_{1-13}$. Presumably this behavior interfered with grooming at 10 and 30 nmoles morphine. It is almost certain that the observed feeding and grooming on one hand, and locomotion on the other, were mediated by different opioid receptors. Moreover, it appears that if both or all opioid receptor types in the substantia nigra - pars reticulata are activated at the same time, motor activity, which was enhanced by mu and/or delta but not kappa activation, predominates. The true behavioral significance of the high concentrations of endogenous dynorphin in this region remains undetermined. A major role for this system in feeding behavior is unlikely.

As demonstrated in Experiment 4, dynorphin$_{1-13}$ but not morphine elicited a small dose-dependent feeding response among paraventricular nucleus rats. In contrast, morphine in the paraventricular nucleus produced what appeared to be erratic, disorganized, and discontinuous behavior. Activities such as grooming and forward locomotion were typically initiated but were not completed. These effects may have been associated with the involvement of opioids in the paraventricular nucleus in the stress response (Kiritsy-Roy et al., 1987; Luiten, ter Horst, & Steffen, 1987; Randich & Callahan, 1987), which appears to be independent of dynorphin in this region. Although endogenous dynorphin in the paraventricular nucleus may contribute to feeding behavior, the weak response to dynorphin$_{1-13}$ microinjection suggests that this effect is relatively unimportant when compared to areas such as the ventral tegmental area. When mu receptors are activated in the paraventricular nucleus and the substantia nigra - pars reticulata, the immediate behavioral effect is not compatible with feeding as mu-mediated behavior appears to be in the ventral tegmental area and nucleus accumbens.

The substantia nigra and paraventricular nucleus each show both dense kappa receptor binding and intense dynorphin immunofluorescence (Cone et al., 1983; Lynch et al., 1985; Mansour et al., 1986; Vincent et al., 1982a, 1982b). The paradigm employed in the present investigation was not sensitive to the principal behaviors that may be mediated by these dynorphin systems, despite the constant observation inherent in the tests. It is possible that endogenous dynorphin in both these regions serves a modulatory function that may be detected only by using additional pharmacological manipulations in concert with dynorphin microinjection. Alternatively, environmental stimuli other than those present during the feeding tests may be required for the robust

expression of behaviors mediated by dynorphin in the substantia nigra or paraventricular nucleus of the hypothalamus.

Hypothalamic Mu/Delta Agonists and Feeding

The endogenous opioid peptide beta-endorphin acts on mu and delta receptors, and it is found in hypothalamic nuclei. In the present study, morphine microinjected into the paraventricular nucleus produced behavioral effects that were inconsistent with feeding. The observation that beta-endorphin levels in the hypothalamus were reduced as a function of either food deprivation (Gambert, Garthwaite, Pontzer, & Hagen, 1980; Vaswani & Tejwani, 1986) or stress (Millan, Przewlocki, Jerlicz, Gramsch, Hollt, & Herz, 1981) led to the assumption that beta-endorphin utilization in this region contributes to deprivation- and stress-elicited enhancement of feeding. In further support of this notion was the finding that among non-deprived rats given highly palatable food, hypothalamic pools of beta-endorphin were mobilized during feeding (Dum, Gramsch, & Herz, 1983). It was suggested that hypothalamic beta-endorphin facilitates feeding by enhancing the reward value of the food. These latter data would have been more convincing had the control rats eaten the ordinary lab chow that was presented to them, and if a difference in beta-endorphin levels had then been found. The control rats failed to eat at all however, leaving unchallenged the possibility that beta-endorphin release may occur during all feeding independent of the strength of reward associated with the food. If this is true, another function for hypothalamic beta-endorphin in feeding is implied. The effect of morphine in the paraventricular nucleus in the present study suggests that beta-endorphin in this region probably does not produce feeding, and that data purporting to support such feeding may have been misinterpreted.

Genetically obese (fa/fa) Zucker rats show elevated pituitary levels of beta-endorphin relative to their lean littermates; this was believed to contribute to the hyperphagia typical of the fa/fa strain (Margules, Moisset, Lewis, Shibuya, & Pert, 1978). Administration of beta-endorphin and other putative mu-agonists into the paraventricular nucleus was reported to produce feeding in food-satiated rats (Leibowitz & Hor, 1982; McLean & Hoebel, 1983; Woods & Leibowitz, 1985). Lesions by 6-hydroxydopamine of dopamine-containing neurons in the ventral tegmental area resulted in elevated levels of both hypothalamic and pituitary beta-endorphin in lean Zucker rats, but food intake and body weight were not affected (Deutch & Martin, 1983). It was suggested that beta-endorphin levels in these regions are probably regulated by mesolimbic dopamine, but that increases in these levels do not contribute to elevations in feeding (Deutch & Martin, 1983). The authors speculated that mesencephalic dopamine may function permissively in beta-endorphin release in response to environmental stimuli. Under this hypothesis, the elevated concentrations of this ligand following dopamine lesions would reflect an accumulation of peptide due to attenuated release rather than enhanced release and synthesis as appears to have been assumed by other investigators.

These results coupled with findings of an extended latency to initiate feeding following hypothalamic administration of putative mu agonists (Grandison & Guidotti, 1977; Leibowitz & Hor, 1982; McLean & Hoebel, 1983; Tepperman, Hirst, & Gowdey, 1981a) suggest that hypothalamic beta-endorphin may regulate feeding by an inhibitory and not a permissive function. This may explain the failure of morphine in the present investigation to elicit feeding from the paraventricular nucleus. If mu or delta agonists inhibit feeding then it is possible

that the genetically obese Zucker rat is relatively insensitive to the regional inhibitory effect of beta-endorphin. Rats with 6-hydroxydopamine lesions may develop a supersensitive response to remaining beta-endorphin release, so that total food intake remains unchanged. At the same time, genetically obese animals and the rats made obese by dietary manipulations were reported to be more sensitive than their lean conspecifics to the feeding-attenuating effects of opioid antagonists (Cooper et al. 1985b; Margules et al. 1978). In view of these observations and the findings of the present investigation, it is suggested that a comparatively less efficient ingestive regulatory system combined with perhaps normal activity of endogenous opioids such as dynorphin in other brain regions probably contributes importantly to the hyperphagia characteristic of obese rats.

Hypothalamic Mu-Agonists and Stress-Induced Feeding

Both hypothalamic norepinephrine (Anisman & Sklar, 1979) and beta-endorphin (Millan, et al., 1981) are released during stress. Enhanced norepinephrine release in the paraventricular nucleus leads to a combined hyperglycemia and hyperinsulinemia, reportedly indicative of adrenal epinephrine release and consistent with the autonomic response to stress (Luiten et al., 1987). In unstressed, freely moving animals, the mu opioid agonist Tyr-D-Ala-MePhe-Gly-Ol (DAGO: Kosterlitz & Paterson, 1980) in the paraventricular nucleus elicits a number of autonomic responses, including tachycardia, that are typical of a sympathetic response to stress. Strangely however, at the highest dose the effect on cardiac rate was reversed. Among stressed animals, DAGO microinjected into the paraventricular nucleus was found to attenuate the increase in heart rate, and locally administered naloxone exacerbated stress-induced increases in plasma epinephrine content. The

time course of the recovery of peripheral measures to baseline levels after DAGO injection into the paraventricular nucleus (Kiritsy-Roy et al., 1986) is consistent with the latency to feed following administration of morphine or beta-endorphin into this region (Leibowitz & Hor, 1982; Woods & Leibowitz, 1985). Apparently the increase in paraventricular nucleus norepinephrine in response to stress may be modulated locally by beta-endorphin. Under non-stressful conditions when norepinephrine release is not markedly enhanced however, beta-endorphin administration seems to be capable of eliciting some of the autonomic effects typical of a stress response.

A possible beta-endorphin--norepinephrine interaction in the paraventricular nucleus has been proposed in relation to the influence of this structure on feeding. A long delay typically follows opioid injection before feeding is initiated (90 minutes for morphine, Woods & Leibowitz, 1985; 45 minutes for DALA, McLean & Hoebel, 1983; > 60 minutes for B-endorphin, Leibowitz & Hor, 1982). In response to local norepinephrine administration however, feeding began 1 to 2 minutes after injection (Leibowitz & Hor, 1982), suggesting that norepinephrine in the paraventricular nucleus may be directly involved in feeding. Naloxone blocked beta-endorphin- but not norepinephrine-produced eating. In contrast, the alpha-adrenergic antagonist, phentolamine, blocked both beta-endorphin- and norepinephrine-elicited feeding (Leibowitz & Hor, 1982; Tepperman et al., 1981). In the former study, beta-endorphin-elicited feeding apparently took place during the second hour of testing and did not persist beyond this period. However, if norepinephrine was injected within 5 minutes after beta-endorphin the effect on feeding was additive. Presumably the norepinephrine treatment bypassed the delay of feeding seen with beta-endorphin alone, so that eating also occurred

during the first hour. If norepinephrine was injected 2 hours after beta-endorphin the effect on feeding during the second 2-hour period was not additive (Leibowitz & Hor, 1982). The authors suggested that beta-endorphin and norepinephrine in the paraventricular nucleus may be related, but that the effects on feeding of these neurochemical systems appear to be independent. An alternative interpretation is that norepinephrine alone in the paraventricular nucleus produces feeding and beta-endorphin suppresses norepinephrine release. When this influence subsides following high dose administration of beta-endorphin, norepinephrine may be released in a rebound fashion, producing feeding. A similar effect was observed in the ventromedial nucleus of the hypothalamus (Tepperman et al., 1981a), and norepinephrine modulation of feeding at the level of the ventromedial nucleus of the hypothalamus was proposed by the investigators.

Efferents from the paraventricular nucleus project to most hypothalamic areas, and to sympathetic and parasympathetic nuclei, as well as to the median eminence and midbrain areas including the periaqueductal gray, ventral tegmental area, and several raphe and parabrachial nuclei. Luiten et al. (1987) suggested that the longer efferents rather than the intrahypothalamic connections are responsible for the effect of paraventricular nucleus mechanisms on feeding. Both the autonomic stress response and the effect of paraventricular nucleus efferents on pituitary endocrine systems were proposed to participate in paraventricular nucleus-associated feeding. The influence of opioids in the paraventricular nucleus on feeding may be functionally separate from the effects on this behavior of opioids in the ventral tegmental area or the nucleus accumbens.

Animals eat in response to pharmacological manipulations in

hypothalamic areas such as the paraventricular nucleus. Food deprivation is considered stressful, and produces some of the neurochemical alterations concomitant with stress. Possibly hypothalamic mechanisms regulate feeding by mediating stress-related neurochemical conditions including those associated with food deprivation, whereas the rewarding neurochemical aspects of feeding may occur at other levels including the ventral tegmental area and nucleus accumbens.

Methodological Considerations in CNS Studies of Behavior

Gradual beta-endorphin release during feeding may act not to facilitate ingestion as suggested by Dum et al. (1983), but rather to attenuate feeding over time. A 28% decrease in ^{3}H-etorphine binding in hypothalamus following 20 minutes of eating an apparently highly palatable chocolate-covered candy was believed to represent bound endogenous beta-endorphin that had been released during eating. The corresponding reduction of endogenous hypothalamic beta-endorphin levels was reported to be approximately 10 to 12 femtomoles per mg of tissue (Dum et al., 1983). The dose of beta-endorphin used in the paraventricular nucleus to produce feeding in rats was 1 nmole (Leibowitz & Hor, 1982). If we assume that the injected drug may have directly reached 10 mg tissue in the paraventricular nucleus (total ex-cortical brain weight in the adult rat is approximately 935 mg: Will, Rosenzweig, Bennett, Hebert, & Morimoto, 1977), this represents a factor of 8,300 to 10,000 times the endogenously released peptide. In addition, it should be noted that the release of endogenous beta-endorphin took place over a period of 20 minutes during feeding (Dum et al., 1983) whereas the period of drug delivery, although not reported (Leibowitz & Hor, 1982), can be presumed to have been typical of most intracranial

drug studies at approximately 30 to 60 seconds. It would be expected that if one of the primary functions of beta-endorphin in the paraventricular nucleus is the inhibition of norepinephrine, the effect on spontaneous noradrenergic activity of an acute injection of beta-endorphin would be quite abrupt. Moreover, given the quantitative differences between endogenous release and exogenous administration plus the reported extremely high potency and slow dissociation rate of bound beta-endorphin (Akil, Hewlett, Barchas, & Li, 1980), a concentration of 1 nmole could reasonably be considered excessive. Finally, the long latency to feeding and the antagonism of eventual beta-endorphin-elicited eating by phentolamine suggest that this behavioral response cannot be directly attributed to the action of mu-agonistic opioids in the paraventricular nucleus. Instead, the abrupt suppression of spontaneous norepinephrine neurotransmission by beta-endorphin may be followed by a rebound release of norepinephrine that is responsible for the observed feeding behavior.

Tepperman and Hirst (1982) concluded that the effect of morphine in the ventromedial nucleus of the hypothalamus on feeding was mediated by mu receptors. This was by virtue of a comparison of single, equimolar doses of the putative mu agonists levorphanol and morphine, the putative kappa agonist ketocyclazocine, and phencyclidine which binds to kappa and the proposed sigma receptors. Overlooked was the fact that ketocyclazocine and its analog ethylketocyclazocine bind to kappa receptors with nearly as high affinity as dynorphin and with about 2,500 times higher affinity than morphine (see Goldstein, 1984). Although binding affinity alone cannot be used as an index of pharmacological potency of a ligand (Goldstein & James, 1984), it can in the absence of other information provide an indication of the range of doses that may

be likely to produce a behavioral effect. The ketocyclazocine-treated animals in Tepperman and Hirst's (1982) study exhibited behavioral depression and sleeping at the single 5.3 nmoles dose. Rats receiving morphine also showed behavioral depression but recovered after 30 to 60 minutes. Lower drug concentrations apparently were not considered. Even in studies where a broad range of drug doses has been employed for intracerebral injection, however (Leibowitz & Hor, 1982), in the effective dose range feeding occurred only after long delays during which locomotor behavior was depressed. This suggests that feeding could not be directly attributed to drug activation of opioid receptors at the injection target site. The previous section on stress-induced feeding attempted to explain why this behavior may have followed the administration of high doses of mu and delta agonists. On the other hand, Tepperman and Hirst (1982) may have missed a possible kappa agonist-mediated enhancement of feeding by using equimolar doses of all drugs and therefore overdosing the animals.

A particularly dramatic demonstration of the potentially misleading effects of overdosing was the induction of motor dysfunction, including hindlimb paralysis, following intrathecal administration of 3 to 20 nmoles dynorphin in rats (Stevens, Weinger, & Yaksh, 1987; Stevens & Yaksh, 1986). The apparent analgesia formerly attributed to kappa mechanisms in the spinal cord was observed only at the doses that impaired motor function. This effect was not opioid-mediated, as demonstrated by similar responses to 30 nmoles des-Tyr-dynorphin and the failure of up to 100 nmoles of the kappa agonist U50,488H to induce a comparable inhibition of motor function. Morphometric analysis revealed damage to ventral horn interneurons (Caudle & Isaac, 1986; Stevens et al., 1987). The dynorphin doses used by these authors were at least

10,000 to 200,000 times higher than the peak doses producing feeding in the present investigation. Recall that feeding in the present study was attenuated when the highest dose of dynorphin$_{1-13}$ was increased by a factor of 10. Although Stevens and colleagues may be correct in assuming that dynorphin in the spinal cord does not mediate analgesia, it is inappropriate to assume that the function of endogenous dynorphin in the spinal cord is to produce motor dysfunction, or that it has no purpose. First of all, as demonstrated by Stevens and colleagues, the doses used were excessive and produced non-opioid mediated debilitation and cell damage. Clearly, the antinociception paradigm was not sensitive to the effects of intrathecally delivered dynorphin. In addition, this mode of administration may produce competitive effects, particularly if a ligand or its metabolites are biologically active at more than one site. A further illustration of these assertions can be derived from the observation by Gosnell, Levine, and Morley (1986) that intracerebroventricular administration of nanomolar concentrations of opioid ligands including dynorphin eventually elicited feeding, but sedation, catalepsy, and postural abnormalities were also produced. The highest concentration of dynorphin (10 nmoles) in that study resulted in death of one rat within an hour of injection. The femtomolar doses used in the current evaluation effectively produced feeding that was not masked by unidentified, perhaps nonspecific effects that may have occurred at only slightly higher doses.

The present study found that dynorphin$_{1-13}$ but not morphine elicited feeding from the paraventricular or the substantia nigra within a short time following microinjection. This demonstrated first, that the behavioral effects of kappa vs. mu and perhaps delta agonists in these brain regions are probably incompatible and second, that mu and

delta agonists in this region may not be directly involved in the enhancement of feeding. These results further support the proposition that long duration paradigms, coupled with high concentrations of ligands injected directly into brain tissue, may lead to misconceptions about the processes contributing to the ultimate behavioral findings.

It is proposed that as techniques in behavioral pharmacology become more sophisticated and specific (for example, the analysis of the effects on behavior of pharmacological manipulations limited to a narrowly circumscribed target area in brain tissue), the parameters of the behavioral tests must also change. New considerations are required, including an understanding of the ligand binding and pharmacological profiles that contribute to determining the appropriate dose range and characteristic duration of action in nervous tissue for each drug used. Preferably wide dose ranges, expressed in molar concentrations for interligand comparison purposes, should be included. Single-dose studies of ligands and equimolar dose approaches to ligand comparisons are inappropriate for the generation of meaningful behavioral data following intracranial microinjection of ligands.

In all probability the nature of behavioral paradigms will also require reevaluation. Specifically, it is recommended that examination of the behavioral effects of drug microinjection into brain tissue focus on the short term effects of the treatment. A behavior that emerges only an hour or longer following injection into a specific brain region is less likely to represent a direct response to the drug. It is further suggested that contemporary intracranial injection studies, properly conducted, will challenge some and verify other findings of earlier investigations carried out using behavioral measures that are appropriate mainly when drug administration is systemic. From this

perspective, it appears that the observations in Experiments 1 and 4 (i.e., that morphine in the ventral tegmental area elicited dose-dependent feeding within 15 minutes of injection) validated the findings of Experiment 2 (i.e., that morphine in the ventral tegmental area produced a dose-dependent increase in the quantity of food consumed), and not the reverse.

Opioid Reward and Feeding Behavior

The Dopamine Connection

The apparent enhancement of locomotor activity produced by morphine in the ventral tegmental area or substantia nigra was consistent with the findings of other laboratories (Broekkamp et al., 1979; Joyce & Iversen, 1979; Kalivas, Taylor, & Miller, 1985; Kelley, Stinus, & Iversen, 1980; Vezina, Kalivas, & Stewart, 1987; see review by Iwamoto & Way, 1979). This behavior was typical of increased dopaminergic neurotransmission in either the nucleus accumbens (Brudzynski & Mogenson, 1985; Costall, Domeney, & Naylor, 1984; Pijnenburg & Van Rossum, 1973; Pijnenburg et al., 1976) or striatum (Helmeste, 1983; Kelley, Seviour, & Iversen, 1975; Pert & Sivit, 1976), respectively.

The well-documented modulation of central dopamine systems by opioids reflects and supports an opioid/dopamine interaction in the expression of locomotor behavior. A similar implication that mesolimbic opioid/dopamine interactions may contribute substantially to the function of central reward processes (Bozarth, 1987b; DiChiara, Imperato, & Mulas, 1987; Wise & Bozarth, 1984, 1987), and potentially to naturally rewarding behavior such as feeding, is an important consideration from the perspective of the present investigation. Putative kappa and mu agonists have different effects on dopamine transmission and metabolism, however. Observations in the present study

are in agreement with the proposal that opioid-elicited feeding appears to be mediated primarily through kappa receptors. Potentially different yet compatible roles may be implicated for mesolimbic dopamine and dynorphin in naturally rewarding behavior.

Physiological Effects of Opioids on Dopamine Systems

The neurochemical effects of opioids on nigrostriatal dopamine function have been the most widely studied. In most but not all respects the findings of these investigations may serve as a model for opioid modulation of ventral tegmental area--nucleus accumbens dopamine neurons. Systemic (Wood, Sanschagrin, Richard, & Thakur, 1983; Wood, Stotland, Richard, & Rackham, 1980), intrastriatal, and intranigral (Wood & Richard, 1982) morphine administration produced increases in striatal dopamine metabolism as measured by levels of the major dopamine metabolites 3,4-dihydroxyphenylacetic acid and homovanillic acid, but not in neostriatal dopamine release as determined by levels of 3-methoxytyramine (see Westerink, 1978; Wood, Kim, & Marien, 1987; Wood, Nair, & Bozarth, 1982). In addition, intranigral morphine reduced levels of 3-methoxytyramine, suggesting an inhibition of spontaneous nigrostriatal dopamine release (Wood & Richard, 1982). Co-ordinates for these injections (Konig & Klippel, 1963) indicated that morphine may have acted directly on dopamine cell bodies in the substantia nigra - pars compacta (Lindvall & Bjorklund, 1978). In contrast, the locomotor activity observed following morphine microinjection into the substantia nigra - pars reticulata in the present investigation, attributed to striatal dopamine release (Pert & Sivit, 1976) may have resulted from net disinhibition of dopamine neurons in the compacta by opioid action at inhibitory interneurons in the reticulata. Different peptide distributions (Watson et al., 1982) and opioid receptor binding profiles

(Mansour et al., 1987) in these two regions support this possibility. The effects of systemic opioids on nigrostriatal dopamine cell firing probably take place at several levels. These could include a facilitatory influence in the substantia nigra pars reticulata, an inhibitory influence in the substantia nigra pars compacta, and possibly a modulatory influence at presynaptic dopamine terminals (Murrin, Coyle, & Kuhar, 1980; Pollard et al., 1977b; Pollard, Llorens, Schwartz, Gros, & Dray, 1978) and even at postsynaptic sites (Antkiewicz-Michaluk, Havemann, Vetulani, Wellstein, & Kuschinsky, 1984; Murrin et al., 1980). Recent evidence indicates that the effect of systemic morphine on mesocortical dopamine projections is similar to that on nigrostriatal dopamine function (Kim, Iyengar, & Wood, 1986).

In partial contrast to its effects on striatal and cortical dopamine, systemic morphine administration increased not only the metabolism but also the release of dopamine in the nucleus accumbens (Westerink, 1978; Wood, 1982, 1983). Similarly, morphine or DAGO injections into the ventral tegmental area were reported to increase dopamine release in the nucleus accumbens (Kalivas, 1985; Kalivas & Richardson-Carlson, 1986; Latimer, Duffy, & Kalivas, 1987), consistent with the effects of this treatment on locomotor activity (Kalivas, Widerlov, Stanley, Breese, & Prange, 1983). Mu receptors in the ventral tegmental area were found to be located primarily on interneurons (Dilts & Kalivas, 1987). Given the enhancement by morphine and enkephalin analogues of dopamine function and dopamine-associated behavior, it can be presumed that these interneurons are inhibitory and that mu and delta opioid agonists in the ventral tegmental area produce a net disinhibition of DA neurons in this region.

The significance of the foregoing to this discussion is that this

body of evidence is consistent with the concept of dopaminergic modulation of locomotor behavior and of opioid effects on dopamine function. Studies of the ventral tegmental area--nucleus accumbens dopamine projection in particular have also yielded convincing evidence in favour of its critical participation in central reward processes. The ability of opioids to activate this system strongly implicates an opioid-dopamine interaction at the level of the ventral tegmental area and the ensuing enhancement of dopamine release in the nucleus accumbens in both opioid and dopamine-mediated reward and in feeding.

Opioids, Dopamine, and Reward.

A relationship between opioid-related feeding and reward was further supported by findings that food deprivation increased responding for lateral hypothalamic rewarding stimulation in a manner dependent on the period of deprivation (Carey, Goodall, & Lorens, 1975). Naloxone not only reversed the reduction in frequency thresholds for lateral hypothalamic stimulation by food deprivation, but it further increased thresholds compared to both normal and food deprived rats (Carr & Simon, 1983a). In addition, intra-accumbens amphetamine was reported to elicit feeding in food-satiated rats (Evans & Vaccarino, 1986).

Electrical stimulation of the lateral hypothalamus produces feeding in some rats. All rats responsive to stimulation-induced feeding will also perform operant responses for brain stimulation reward (BSR); however not all BSR rats feed in response to lateral hypothalamic stimulation (Roberts, 1980). Ventral tegmental area--nucleus accumbens dopamine has long been considered a common substrate for both phenomena. BSR of lateral hypothalamic fibers produced a reduction in dopamine content in the nucleus accumbens (Bozarth, 1987b), suggesting enhanced dopamine utilization. Administered alone, systemic morphine or

amphetamine each reduced stimulation thresholds for BSR. Administered concurrently these drugs produced an additive effect in decreasing thresholds (Hubner, Bain, & Kornetsky, 1987) suggesting an opioid-dopamine interaction in BSR. Systemic naloxone reversed the reduction in stimulation threshold by amphetamine (Esposito, Perry, & Kornetsky, 1980). Morphine in the ventral tegmental area reduced the stimulation threshold for both rewarding lateral hypothalamic stimulation alone (Broekkamp et al., 1976; 1979; Jenck, Gratton, & Wise, 1987a), and stimulation-induced feeding (Jenck, Quirion, & Wise, 1987b), whereas systemic opioid antagonists increased thresholds for stimulation-induced feeding (Carr & Simon, 1983b, 1984) or BSR (West & Wise, 1986).

A reduction in stimulation threshold implies that directly or indirectly, the treatment has enhanced the excitability of either the fibers being stimulated or of neurons receiving input from those fibers. An important role for nucleus accumbens dopamine in both lateral hypothalamic stimulation-induced feeding and BSR can be perceived from the potentiation of these behaviors by those treatments that directly enhance either ventral tegmental area opioid systems or dopamine, and by the increase in dopamine utilization in the nucleus accumbens during BSR. Localization of the dopamine contribution in the nucleus accumbens was further confirmed by ipsilateral but not contralateral spiroperidol inhibition in the nucleus accumbens of lateral hypothalamic stimulation-induced feeding. Moreover, stimulation of the same electrodes produced alterations in the spontaneous discharge rates of dopamine neurons in the ventral tegmental area (Mogenson & Wu, 1982). From these data, it is tempting to speculate that lateral hypothalamic stimulation contributes to an increase in the excitability of ventral tegmental area--nucleus accumbens dopamine neurons. It should be recognized,

however, that treatments such as amphetamine that increase the synaptic availability of dopamine result in a reduction, not an increase, in dopamine neuronal excitability (Groves, Fehster, Tepper, Nakamura, & Young, 1981; Skirboll, Grace, & Bunney, 1979; Wang, 1981). Clearly, the contribution of the mesolimbic dopamine system to lateral hypothalamic stimulation-induced feeding and BSR is critical. Its precise role remains to be determined, however. Similarly, the distinct roles of opioids in the ventral tegmental area in these processes require further elucidation.

Mu and Kappa Agonists in Dopamine-Mediated Behavior and Feeding.

In studies using isolated receptor populations, dynorphin was shown to bind to mu receptors with an affinity approximately equivalent to that of morphine (James & Goldstein, 1984). It was pointed out, however, that although binding affinity may contribute to the pharmacological potency of a ligand, this alone does not predict the potency of the ligand's biological activity at the receptor (Goldstein & James, 1984). Similarly, in vivo selectivity may vary among tissues. Given these considerations, it was important to attend to the possibility that the effects of dynorphin$_{1-13}$ on feeding may have resulted from a highly potent action at mu receptors resulting in enhanced nucleus accumbens dopamine release, particularly in the ventral tegmental area where dynorphin$_{1-13}$-elicited feeding was greatest. Neurochemical and behavioral evidence, however, suggests that kappa and not mu receptors were responsible for the elicitation of feeding behavior by both dynorphin$_{1-13}$ and morphine.

As discussed previously, morphine and enkephalin analogues acting at mu and perhaps delta receptors produce increases in ventral tegmental area--nucleus accumbens dopamine metabolism and release. Studies using

the benzomorphan class of drugs, including ketocyclazocine, ethylketocyclazocine, and bremazocine, all putative kappa agonists, found that these drugs failed to affect dopamine function in the nucleus accumbens or striatum when given alone. When administered concurrently with morphine the benzomorphans inhibited the facilitatory influence of morphine on striatal and nucleus accumbens dopamine function (Wood, 1982, 1983; Wood & Richard, 1982; Wood et al., 1983). Similarly, dynorphin$_{1-13}$ or the kappa selective agonist U50,488H administered alone had no effect on dopamine function. In contrast to the benzomorphans however, these ligands failed to antagonize the morphine enhancement of dopamine (Wood, Kim, Cosi, & Iyengar, 1987; Wood & Richard, 1982; Wood et al., 1983). Similar antagonism of mu and delta-mediated dopamine electrophysiological activity by the benzomorphans but not by U50,488H was observed by Dunwiddie, Johnson, and Proctor (1987). These findings suggest that the inhibition of morphine-enhanced dopamine function by the benzomorphans occurs through their direct antagonism of mu receptors and not by kappa modulation of mu activity on the same cells. Indeed, endogenous striatonigral dynorphin levels were found to be mediated directly by dopamine activity in the striatum, not the reverse (Nylander & Terenius, 1987). The absence of any effect on dopamine by the benzomorphans alone suggests that the spontaneous activity of nigrostriatal and mesolimbic dopamine neurons may be altered by endogenous endorphinergic or enkephalinergic mechanisms only under specific conditions, such as the presence of an environmental stimulus. Otherwise ventral tegmental area enkephalinergic activity appears to be determined to a great extent by circadian variations (Glimcher, Giovino, Margolin, & Hoebel, 1984). Furthermore, kappa-mediated neuronal activity appears to be independent of the effects of mu receptors or of

ventral tegmental dopaminergic mechanisms.

A Bridge Over Troubled Neurochemicals.

The foregoing raises the problem of reconciling the burgeoning evidence in favour of mesolimbic dopamine involvement in feeding behavior with the less extensive yet consistently encountered evidence from several different sources, including the present study, of kappa agonist-elicited feeding. Clearly mu and possibly delta activation in the ventral tegmental area increases dopamine neurotransmission in the nucleus accumbens. This effect, to the extent that dopamine is released, is consistent with feeding. Indeed, low doses of amphetamine microinjected into the nucleus accumbens elicit feeding (Evans & Vaccarino, 1986). On the other hand, enhanced synaptic availability of dopamine beyond a certain critical limit produces locomotion to an extent that can interfere with feeding (Evans & Vaccarino, 1987; Salisbury & Wolgin, 1985). One potential resolution to the question of ventral tegmental area kappa involvement in feeding is that kappa activation in this region may act only on a small, discrete subset of dopamine neurons projecting to the nucleus accumbens. Considering this as a possibility, if a neurochemical assay included the entire structure of the nucleus accumbens, any dynorphin-produced alterations would be masked and the treatment would appear to have no significant effect.

Dopamine in the nucleus accumbens frequently has been associated with behavioral response initiation, particularly with respect to goal-directed behavior (Brudzynski & Mogenson, 1985; Jones & Mogenson, 1980; Kelley & Domesick, 1982; Koob, Riley, Smith, & Robbins, 1978; Neill & Justice, 1981). This system may be important in mediating approaches to -- and initiation of interactions with -- appetitive stimuli such as food as well as the initiation of escape responses when appropriate

(Brudzynski & Mogenson, 1985). Mogenson's model implies that environment-appropriate responses in general may be a primary function of the nucleus accumbens. Examination of behavioral alterations provoked by dopamine antagonists or by pharmacological lesions of dopamine systems have helped to elucidate this role.

Deutch and Martin (1983) observed that 6-hydroxydopamine lesions of ventral tegmental area neurons produced no alterations in total food intake or body weight. No time course data were reported on these measures however. Moreover, the rats were confined to their home cages and no behavioral task was involved. Contrary to these findings, a timed open field task revealed both locomotor deficits and hyperphagia among rats following 6-hydroxydopamine lesions of dopamine neurons in the nucleus accumbens. By 6 weeks post-lesion the locomotor debilitation had recovered, but the hyperphagia during the test persisted. The hyperphagia was attributed to a reduction in responsiveness to the environment, measured as decreases in behavioral changes during the test, and reflected by a persistence in feeding behavior (Evenden & Carli, 1983). Phillips and colleagues found that pimozide produced a dose-related impairment of rats' typical anticipatory behaviors to a conditioned stimulus that preceded the onset of food delivery. Deficits included extended latencies and reduced frequencies of approaches to the food. The duration of contact with the food delivery area was increased by pimozide however, and total food intake was identical to that of controls (Blackburn, Phillips, & Fibiger, 1987). An examination of the effects on food intake of central microinjections of opioid agonists in these paradigms would be interesting.

Morphine probably activates both mu and kappa receptors, and may

act simultaneously on both dopaminergic and non-dopaminergic mechanisms in the ventral tegmental area. Few studies to date have examined the effects of the relatively selective mu agonist, DAGO, on behaviors other than locomotion. A separation of mu- and kappa-mediated feeding-related behaviors has been achieved, however, by comparing the effects of morphine and U50,488H. In food-satiated deer mice, systemic morphine administration increased both hoarding and food intake, whereas U50,488H increased feeding and reduced hoarding. In contrast, morphine-elicited hoarding was reduced in food-deprived animals relative to both morphine-treated freely feeding mice and controls. Among food deprived mice U50,488H further increased the difference between time spent feeding and time spent hoarding that was observed in freely feeding animals treated with the kappa agonist (Kavaliers & Hirst, 1986). Feeding apparently was competitive with hoarding during the time period of the test, and feeding predominated in both food-deprived and U50,488H-treated animals. These findings support the possibility that endogenous dynorphin release plays a role in enhancing ingestive behavior following a period of fasting. The increase in hoarding behavior produced by morphine is probably related to morphine-elicited increases in mesolimbic dopamine release. A relationship of this dopamine system to hoarding arises in part from the observation that lesions of the ventral tegmental area produced a disappearance of hoarding as well as a disorganization of feeding behavior (Stinus, Gaffori, Simon, & LeMoal, 1979).

A further dissociation of mu- and kappa-mediated behavioral effects at the level of the ventral tegmental area arose from the finding that morphine, the selective delta agonist, D-Pen^2, D-Pen^5-enkephalin (DPDPE: Mosberg, Hurst, Hruby, et al., 1983), and U50,488H all reduced frequency thresholds for lateral hypothalamic stimulation-induced feeding (Jenck

et al., 1987b), but that only morphine and DPDPE reduced thresholds for lateral hypothalamic BSR (Jenck et al., 1987a). This suggests that all three opioid receptor subtypes in the ventral tegmental area may be involved in feeding evoked by stimulation of descending lateral hypothalamic fibers, but that only mu and delta receptors in this region may contribute to the rewarding effects of brain stimulation. Compatible with this difference are the findings of Roberts (1980) that animals that fed in response to lateral hypothalamic stimulation showed differences in neuronal activity by much higher density of [^{14}C]2-deoxyglucose fluorescence in descending fibers to the ventral tegmental area, lateral tegmentum, and parabrachial nucleus, than animals that were self-stimulators only. Several neurochemical substances, including dynorphin (Fallon, Leslie, & Cone, 1985), may be a part of this projection. One prediction from these data could be that mesolimbic dopamine may be affected equally in both groups of animals but that ventral tegmental area dynorphin activity may be enhanced only among feeders. Further support for an involvement of endogenous dynorphin in stimulation-induced feeding is derived from findings that intracerebroventricular injection of antibodies specific for dynorphin completely blocked stimulation-induced feeding in rats (Carr, Bak, Gioannini, & Simon, 1987). Given the potentiation by U50,488H in the ventral tegmental area on stimulation-induced feeding, it would be interesting to examine whether dynorphin antibodies microinjected into this region could attenuate or completely inhibit the effect of the stimulation.

It appears that in the stimulation-induced feeding paradigm, both the integrity of the nucleus accumbens dopamine system and the presence of endogenous dynorphin, the natural ligand for the kappa receptor, are

necessary for feeding to occur. Recent evidence suggests that the effects of dynorphin in the ventral tegmental area may extend to interactions with a variety of appetitive stimuli. For instance, two independent laboratories have confirmed that at the same doses that produce feeding, microinjection of dynorphin$_{1-13}$ into the ventral tegmental area enhances measures of sexual behavior in rats (personal communications from J. Mitchell, Concordia University, Montreal, May, 1987, and L. Band, SUNY at Buffalo, September, 1987). Perhaps kappa receptors in the ventral tegmental area participate in modulating a positive subjective response to sensory input from environmental stimuli. These exogenous stimuli probably do not participate in BSR, and it appears that mechanisms independent of mesolimbic dopamine may be recruited by dopamine-mediated interaction with the stimuli.

The findings of the present investigation are consistent with neurochemical observations that dynorphin$_{1-13}$ neither enhances nor impairs dopamine function. Feeding was elicited by dynorphin$_{1-13}$ from all brain sites examined except the periaqueductal gray, whereas locomotor behavior was not affected by dynorphin$_{1-13}$ at any placement during behavioral testing. This is in agreement with other findings of repeated unsuccessful attempts to elicit locomotor activity by dynorphin microinjection into the ventral tegmental area (P. Kalivas, Washington State University, personal communication, February 1, 1988), and a failure of this treatment to produce contralateral rotation (pilot data collected in this laboratory). In contrast, morphine in either the ventral tegmental area or substantia nigra - pars reticulata produced elevations in locomotor activity, consistent with both increased dopamine release and observations that morphine in the ventral tegmental area elicits contralateral rotation (Holmes, Bozarth, & Wise, 1983;

Holmes & Wise, 1985).

In Experiment 4 the highest doses of morphine or dynorphin$_{1-13}$ in the nucleus accumbens produced behavioral sedation in some animals following feeding and near the end of the test session. This is consistent with observations by Havemann and Kuschinsky (1985), and may be related to the occurrence on accumbens dopamine terminals of at least 50% of opioid receptors in this region (Pollard et al., 1977a). In addition, an important role for the nucleus accumbens in opioid modulation of striatal dopamine neurotransmission and forward locomotion has also been identified. In rats with unilateral electrolytic lesions of the striatum, opioids injected into the nucleus accumbens were more effective than neuroleptics in reversing systemic apomorphine-elicited contralateral turning and stereotypy (Polgar, Mate, Till, & Szekely, 1987). This suggests a regulation of striatal dopaminergic neuronal activity by the nucleus accumbens. An opposite influence of opioids and dopamine in the nucleus accumbens is also implicated in striatal dopamine modulation of forward locomotion. Whether the opioid effect occurred at nucleus accumbens dopamine terminals or on non-dopaminergic processes in the nucleus accumbens was not examined. Interestingly, the ED_{50} for nucleus accumbens morphine to produce this effect (Polgar et al., 1987) was within the dose range that elicited feeding in the present study.

A Matter of Taste

More than 20 years ago, Mendelson (1966) suggested that food deprivation in rats "makes food taste better." Food deprivation potentiates brain stimulation reward and stimulation-induced feeding, and this effect is reversible by naloxone. This implicates endogenous opioids associated with feeding in a possible enhancement of the

responsivity of central reward systems to reinforcing events. Observations that food deprivation also enhances responding for both cocaine and opioid intravenous self-administration (Carroll & Boe, 1982, 1984; Carroll, France, & Meisch, 1981) lend further support to this possibility. In addition, several investigators have suggested that opioids increase the reward value of food (Carr & Simon, 1983a; LeMagnen et al., 1980; Lynch & Libbey, 1983; Morley et al., 1986). In obese humans, naloxone reduced food intake without altering perceptions of satiety. Unanticipitated persistence of this effect for up to a week beyond the period of drug treatment led the investigators to suggest that naloxone may have produced a form of conditioned taste aversion (Spiegel, Stunkard, Shrager, O'Brien, Morrison, & Stellar, 1987). Campbell, Capaldi, and Myers (1987) observed that food deprivation in rats produced a conditioned taste preference for novel flavors that were paired with feeding following deprivation. A central mediation of both taste aversion by naloxone and taste preference by endogenous opioids activated under conditions of food deprivation is supported by observations that peripheral actions of opioid agonists may mediate some of their aversive or suppressive effects (Carr & Simon, 1983a; Bechara, Zito, & Van der Kooy, 1987). Furthermore, peripheral kappa receptors may participate in this process (Bechara & Van der Kooy, 1987). In contrast, considerable evidence including the data from the present investigation suggests that kappa receptors in a number of brain areas may be primarily involved in mediating opioid-elicited feeding behavior. This may occur in part through taste mechanisms (Lynch et al., 1985).

The present investigation did not specifically examine the effects of opioids on palatability. Notably, however, opioids injected into the ventral tegmental area at the same coordinates that produce the most

robust opioid-induced conditioned place preference (Bozarth, 1987b) and intracranial morphine self-administration (Bozarth & Wise, 1981) also produced the greatest feeding. In addition, dynorphin -- the endogenous ligand that demonstrates highest affinity for the kappa receptor (Chavkin & Goldstein, 1981a, 1981b) -- produced the most robust feeding response, characterized by dose-related increases in feeding bout durations. The prolonged ingestion periods may have reflected an enhancement of rewarding properties of the food. Opioids in the nucleus accumbens have also been reported to be rewarding, and this region also supported morphine- and dynorphin$_{1-13}$-elicited feeding. Responses were weaker and less consistent than in the ventral tegmental area, however. If the argument that increased feeding durations may be representative of opioid-enhanced reward value of the food is accepted, it appears that opioid action in the nucleus accumbens may be less important in this respect than in the ventral tegmental area.

It has been suggested that opioids in the nucleus accumbens are more important for opioid reward than in the ventral tegmental area. These conclusions were based on the relative ability of a hydrophilic opioid antagonist microinjected into either the ventral tegmental area or the nucleus accumbens to produce increases in intravenous heroin self-administration in rats (Vaccarino, Bloom, & Koob, 1985). Cannula placements in the nucleus accumbens were essentially compatible with those used in the present study. Ventral tegmental area placements were both dorsal and lateral to those shown to produce morphine self-administration, conditioned place preference, enhancement of BSR, and feeding, however. The authors reported that a higher dose of antagonist was required in the ventral tegmental area than in the nucleus accumbens to increase rates of heroin self-administration. This was interpreted

as representative of the relative importance of the two brain regions in opioid reward. In fact, the difference in dose requirement was small, and the effect of the opioid antagonist on response rate for heroin was actually slightly more robust in the ventral tegmental area than in the nucleus accumbens (Vaccarino et al., 1985). Combined with the difference in ventral tegmental area cannula placements, these findings do not justify the conclusions proposed. The results of the present study are consistent with other findings in this laboratory, that opioids in the ventral tegmental area play an important role in reward-related behavior.

The finding that in both the ventral tegmental area and nucleus accumbens dynorphin$_{1-13}$ was 50,000 times more potent than morphine in producing feeding is consistent with the proposals by other investigators that kappa receptors are primarily responsible for central opioid-elicited feeding. Furthermore, the association of kappa-mediated systems with palatability is compatible with the present suggestion that dynorphin$_{1-13}$ and morphine may have produced feeding in the ventral tegmental area by means of a kappa opioid reward substrate that is distinct in function from mu-mediated activity of dopamine neurons in this region.

Conclusions

The effects of opioids in the central nervous system on behavior are complex and frequently opposite in nature, depending on both the site and subtype of the receptors activated. At different brain sites, opioids produce sedation and analgesia, locomotor enhancement or suppression, sympathetic responses to stressors, and reward. Feeding is considered to be a naturally rewarding behavior, and endogenous opioids have been shown to play an important role in the regulation of feeding.

Opioid involvement in feeding appears to occur at a number of different levels in the brain. Data from several investigations including the present study suggest that the enhancement of feeding by opioids occurs primarily through kappa receptor activation. The endogenous kappa ligand dynorphin$_{1-13}$ was 50,000 times more potent than morphine in both the ventral tegmental area and nucleus accumbens in eliciting feeding. In addition, feeding responses to dynorphin$_{1-13}$ microinjections into the ventral tegmental area were greater than to either ligand at any site examined, further supporting the probability of an important role for kappa receptors in feeding. Both neurochemical and behavioral evidence indicates that in intact preparations where different opioid receptor types may coexist, the relative selectivity of dynorphin$_{1-13}$ for its preferred kappa receptor site may be even greater than that suggested by binding studies. In contrast, the relative preference of morphine for mu receptors may be the same or perhaps slightly lower than <u>in vitro</u> binding indications predict.

The differential effects of morphine and dynorphin$_{1-13}$ in the paraventricular nucleus and the substantia nigra demonstrated that the coexistence of different opioid receptor subtypes in the same brain region does not necessarily predict complementary functions for these substrates. Mu receptor activation by morphine in the ventral tegmental area may play a complementary role with kappa-elicited feeding, however. Enhancement of mesolimbic dopamine function by mu and perhaps delta opioid receptors in the ventral tegmental area may also contribute to feeding behavior by intensifying the animal's interactions with the environment. The integrity of the mesolimbic dopamine system appears to be important in mediating environment-appropriate behaviors, whereas the function of endogenous dynorphin in the ventral tegmental area may

serve to maintain behavior by enhancing the positive subjective response to sensory input from interaction with the stimulus.

The majority of previous investigations of central opioid-elicited feeding have involved intracerebroventricular administration of ligands, or the microinjection of opioids into hypothalamic nuclei traditionally associated with the central control of feeding behavior. Most frequently employed were high doses of drug that elicited feeding only after extended time periods following injection. These delays either were ignored or were attributed to sedation and motor suppression. Potential behavioral effects of opioid agonist diffusion to other brain regions or possible rebound release of endogenous neurotransmitters inhibited by the treatment were not considered. The present investigation demonstrated that in studies using intracranial injections of ligands, neither high doses of drug nor long duration paradigms are necessary to detect measurable behavioral responses. In fact conventional procedures may even be inappropriate, producing results that are subject to misinterpretation. To reiterate, if a ligand at the target injection site is involved in the behavior of interest, this effect should be apparent within a relatively short time following injection. If the delay to behavioral expression is 30 to 90 minutes, the behavior may not be attributed directly to ligand-receptor interaction at the target site, and the substrate responsible for the treatment is probably remote from the injection in terms of function or distance, or both.

Hypothalamic mechanisms and dopamine projections to the nucleus accumbens may be involved in the initiation and perhaps the termination of feeding. The maintenance of feeding behavior apparently does not rely solely on these substrates, however, and involves sensory feedback

from the food, including taste. This may include the recruitment of kappa opioid mechanisms in the ventral tegmental area and of both dopaminergic and non-dopaminergic processes in the nucleus accumbens. It is proposed that in the naturally behaving organism both dopamine and different classes of opioids play complementary roles in the expression of goal-directed behavior.

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