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The Effect of Opiate Injections into the Ventral Tegmental Area on Feeding

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Abstract

The Effect of Opiate Injections into the Ventral Tegmental Area on Feeding.

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Concordia University, 1992

Increases in food intake are observed when opiates are injected into the ventral tegmental area (VTA). The purpose of the present study was to investigate the effects on feeding of opiates that are selective for the mu, delta, or kappa opioid receptors that are found in this region of the brain. The present investigation was designed to determine if the effect of VTA opiates on feeding was the result of changes in the motivation to feed. A second goal was to determine if repeated injections of opiates into the VTA would result in a progressive attenuation or enhancement of the initial effect.

Rats were implanted with stainless steel cannula aimed at the VTA. Feeding behavior was quantified daily after 18 hours of food deprivation; food was presented in 18 meal segments consisting of five 45 mg food pellets per segment. VTA injections of the prototypical opiate (morphine), the selective mu (DAGO), or the selective delta (DPDPE) opiates caused a dose dependent acceleration of feeding. These opiates increased feeding by enhancing the motivation to eat. Injections of morphine or DPDPE into sites dorsal to the VTA had no effect on feeding. Injections of

DAGO into sites dorsal to the VTA facilitated feeding but larger doses of DAGO were needed and the magnitude of the facilitation was less in comparison to VTA injections of DAGO. DAGO was at least 100 times more effective in enhancing feeding than DPDPE or morphine. VTA injections of the selective kappa opiate, U-50,488H, failed to have any significant effect on feeding behavior. While repeated injections of a low dose of DAGO resulted in progressively greater accelerations of feeding, repeated injections of a dose of DPDPE 10 times larger did not. These data suggest that mu opioid receptors play a greater role in the regulation of feeding than do delta receptors and suggest that the potentiation of feeding, like the locomotor effects of mu opioids, is sensitized by repeated injections of a mu opiate.

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For centuries humans have used the sap of the opium poppy for medicinal purposes. Morphine—the active ingredient of this sap—and its various congeners are collectively known as opiates. Opiates can produce a variety of effects in animals including changes in feeding, drinking, thermoregulation, sexual activity, respiration, and sleep patterns (see Olson, Olson & Kastin, 1989 for review). Research on opiate-induced and opiate-modulated behaviors increased dramatically in the 1970s when it was found that morphine binds to specific receptors in the brain (Pert & Snyder, 1973; Simon, Hiller & Edelman, 1973; Terenius, 1973). Following the discovery of opiate receptors, endogenous compounds that serve as natural agonists for the opioid receptors were identified (Hughes et al., 1975; Pasternak, Goodman & Snyder, 1975; Terenius & Wahlstom, 1975). It is now known that there are at least three types of opioid receptors in the rat brain (Chang & Cuatrecasas, 1979; Zukin & Zukin, 1981; Goldstein & Naidu, 1989). These are termed the mu, delta, and kappa opioid receptors. The recent development of opiates with highly selective properties for the mu, delta, and kappa receptors permits the examination of the role of endogenous opioid peptides in the regulation of behavior at a finer level than was previously possible. The present thesis explores the role of the mu, delta, and kappa classes of opioid receptors in the brain mechanisms subserving feeding.

Central Opioid Pathways are Implicated in Feeding

Holtzman (1974) observed that peripheral administration of the opiate antagonist naloxone decreases food intake in rats—despite

the absence of exogenously applied opiates—and first suggested that endogenous opioid peptides are involved in the regulation of ingestive behavior. Since Holtzman's finding, naloxone has been shown to reduce food intake reliably in a variety of species including pigeons, mice, guinea pigs, cats, rabbits, sheep, wolves, monkeys, and humans (Deviche & Schepers, 1984a; Brown & Holtzman, 1979; Schulz, Wuster & Herz, 1980; Foster et al., 1981; Sanger & McCarthy, 1981; Baile et al., 1981; Baile et al., 1987; Morley et al., 1983; Kyriakides et al., 1980; Herling, 1981; Locke, Brown & Holtzman, 1982; Atkinson, 1981; Atkinson, 1982; Cohen et al., 1985). Even the slug *limax maximus* decreases its food intake when given naloxone (Kavaliers & Hirst, 1987). Several other opiate antagonists have effects similar to those of naloxone. Naltrexone, MR2266, MR1452, GP1843, WIN 44,441, nalorphine, and diprenorphine have all been found to suppress food intake reliably (Lowy, Starkey & Yim, 1981; Ostrowski et al., 1981; Sanger et al., 1983). The fact that they do so in the absence of exogenous opiates suggests that opiate antagonists decrease food intake by antagonizing physiological actions of endogenous opioid peptides.

Following Holtzman's finding that opiate antagonists decrease food intake, the converse has been well documented; exogenously applied opiates increase food intake. Opiates can increase food intake in both sated and food-deprived rats (Martin et al., 1963; Kumar, Mitchell & Stolerman, 1971; Thornhill, Hirst & Gowdey, 1979; Jalowiec et al., 1981; Sanger & McCarthy, 1981a; Morley et al., 1983; Cooper et al., 1985; Jackson & Cooper, 1986a; 1986b; Ramarao & Bhargava, 1989). Opiates also facilitate feeding that is induced by

electrical stimulation of the lateral hypothalamus (Carr & Simon, 1983). Stimulation-induced feeding shares many of the same characteristics as deprivation-induced feeding and is thought by some investigators to be mediated by the same system or systems that mediates naturally occurring feeding (Wise, 1974).

Several lines of evidence suggest that peripherally administered opiates or opiate antagonists have their effect on food intake by acting at the brain. Quaternary opiate antagonists (which do not cross the blood brain barrier) have no effect on the consummatory behavior of sated animals, food deprived animals (Brown & Holtzman, 1981; Deviche & Wohland, 1984), or rats feeding in response to electrical stimulation of the lateral hypothalamus (Carr & Simon, 1983). Complementary results have been obtained with opiates that do not cross the blood barrier; no increase in food intake is obtained with these opiates (Carr & Simon, 1983). These results suggest that systemically administered opiate antagonists have their effect by acting on central mechanisms.

A central mode of action can also be inferred from demonstrations that the direct administration of opiates or opiate antagonists into the brain alters food intake. Injections of naloxone administered into the brain decrease food intake at doses that are ineffective when administered systemically (Jones & Richter, 1981; Thornhill et al., 1982; Segall & Margules, 1989). Other opiate antagonists (ICI 174,864, ICI 154,129, MR2266, and nor-binaltorphamine) also decrease food intake when injected into the cerebral ventricles (Jackson & Sewell, 1985; Calcagnetti, Calcagnetti & Faneslow, 1990). Intra-ventricular injections of morphine, or of the

endogenous opioid peptides beta-endorphin or dynorphin, reliably increase food intake (Belluzzi & Stein, 1977; Tseng & Cheng, 1980; Katz, 1980; McKay et al., 1981; Morley & Levine, 1981). The observation that the hyperphagia produced by injections of opiates or endogenous opioid peptides into the brain is achieved with doses that are relatively ineffective when administered peripherally supports the suggestion that these drugs are having their effect centrally (Belluzzi & Stein, 1977; Morley & Levine, 1981; 1983).

Multiple Systems are Involved in Opioid Feeding

In order to elucidate which brain sites are involved in opioid modulation of feeding, some investigators have applied opiates to discrete brain regions while others have evaluated opiate-induced feeding following damage to specific brain regions caused by electrolytic lesions, chemical lesions, or knife cuts.

From these approaches it seems clear that multiple brain sites are implicated in opioid regulation of feeding. An enhancement of feeding has been observed following administration of opiates into the dorsal medial nucleus of the hypothalamus (Gosnell, 1988; Stanley, Lanthier & Leibowitz, 1989). Lesions of this site attenuate the decrease in feeding caused by systemic naloxone (Bellinger, Bernardis & Williams, 1983). Injections of opiates into the paraventricular nucleus also facilitate feeding (Leibowitz & Hor, 1982; McLean & Hoebel, 1983; Stanley, Lanthier & Leibowitz, 1989; Woods & Leibowitz, 1985; Stanley, Lanthier & Leibowitz, 1989) and lesions of the paraventricular nucleus attenuate morphine-induced feeding (Shor-Posner et al., 1986). Administration of opiates into the

amygdala (Stanley et al., 1989; Gosnell, 1988), the nucleus accumbens (Majeed et al., 1986; Mucha & Iversen, 1986; Hamilton, 1988), or the ventromedial hypothalamus (Grandison & Guidotti, 1977; Tepperman & Hirst, 1982; 1983; Woods & Leibowitz, 1985) of sated rats also causes an increase in food intake. Injections of opiates into the ventral tegmental area (VTA) facilitate feeding in sated rats (Cador et al., 1986; Mucha & Iverson, 1986; Hamilton & Bozarth, 1988a; 1988b) and in rats eating in response to electrical stimulation of the lateral hypothalamus (Jenck, Gratton & Wise, 1986b; Jenck, Quirion & Wise, 1987). These findings suggest that opioid regulation of feeding is not restricted to any one brain site but that multiple brain sites are involved in opioid mediation of feeding.

There is evidence suggesting that multiple opioid systems as well as multiple brain sites are involved in the regulation of feeding. Opiates that bind preferentially to mu, delta, or kappa receptors have all been found to increase food intake, thus implicating all the opioid receptor sub-types in the regulation of feeding. Opiates vary in their ability to bind to the receptor sub-types. For example, morphine binds preferentially to mu receptors, has little affinity for delta receptors, and less still for kappa receptors (Takemori, Ikeda & Portoghese, 1986; Goldstein & Naidu, 1989). In contrast, U-50,488H is highly selective for kappa receptors but binds minimally to mu and delta receptors (Von Voightlander, Lahti & Ludens, 1983; Clark & Pasternak, 1988).

The affinity of an opiate for receptor sub-types is important because the distribution of opioid receptor sub-types is not homogeneous throughout the brain (Mansour et al., 1986; 1987;

1988). For example, the paraventricular nucleus has undetectable levels of mu and delta receptors but is moderately dense with kappa receptors (Mansour et al., 1986; 1987; 1988). Injections of the endogenous putative kappa agonist dynorphin to the paraventricular nucleus induce feeding in sated (Katz, 1980; Hamilton, 1988) animals, whereas injections of morphine to this site are ineffective in increasing food intake (Hamilton,1988). This suggests that in the paraventricular nucleus kappa receptors are of primary importance in mediating opiate-induced feeding. In the amygdala, opiates that bind preferentially to mu receptors increase food intake (Gosnell, 1988; Stanley, Lanthier & Leibowitz, 1989). Opiates that bind preferentially to delta or kappa receptors do not increase food intake when injected into the amygdala (Gosnell, 1988) despite the presence of delta and kappa receptors in this site (Mansour et al., 1986; 1987; 1988). Thus, opiate-induced feeding is limited by the ability of an opiate to bind to opioid receptor sub-types. In summary, both multiple opioids and multiple brain sites are involved in the regulation of feeding and brain sites differ from one another in the distribution of opioid receptors and with respect to which opioid receptor mediates feeding.

VTA Opioids and Feeding

Of the brain sites where opiates induce feeding, the VTA is particularly sensitive. Typically, opiate injections into the VTA produce increases in feeding with smaller doses of drug and with shorter latencies than are required when the same drugs are injected into other sites. An increase in the total time spent feeding is

obtained with smaller doses of dynorphin or morphine when these drugs are injected into the VTA than when they are injected into the nucleus accumbens or the paraventricular nucleus (Hamilton, 1988). The time an animal takes to initiate feeding is less when opiates are administered to the VTA than when they are injected into the nucleus accumbens, the ventromedial hypothalamus, or the paraventricular nucleus (Grandison & Guidotti, 1977; Tepperman, Hirst & Gowdey, 1981; McLean & Hoebel, 1983; Cador et al., 1986; Mucha & Iversen, 1986; Hamilton, 1988; Hamilton & Bozarth, 1988a; 1988b). These results suggest that the VTA is a sensitive site for opioid-mediation of feeding.

Controls for diffusion confirm that the VTA is sensitive to opiate-induced feeding. Injections of naloxone into the VTA, but not dorsal to the VTA, decrease food intake in food deprived rats (Segall & Margules, 1989). This suggests that the VTA, and not the area surrounding the VTA, is the site of action for opiate-induced feeding. The fact that injections of opiates into the VTA increase feeding with much shorter delays than when opiates are injected into other brain sites (Leibowitz & Hor, 1982; Woods & Leibowitz, 1985; Cador et al., 1986; Mucha & Iversen, 1986; Hamilton & Bozarth, 1988a) further suggests that the drug is having its effect in the VTA; the shorter the delay the less the likelihood that the drug will have diffused away from the site of injection.

When opiates are injected into the VTA behaviors that are incompatible with feeding are rarely observed. Increasing the dose of opiate injected into the VTA generally produces corresponding increases in feeding. This is not the case when opiates are injected

into either the paraventricular nucleus or the ventral medial hypothalamus; small and moderate doses of opiates administered into these sites increase food intake (Grandison & Guidotti, 1977; Tepperman, Hirst & Gowdey, 1981; Tepperman & Hirst, 1982; Leibowitz & Hor, 1982), but large doses decrease food intake (Leibowitz & Hor, 1982; Woods & Leibowitz, 1985; Gosnell, Morley & Levine, 1986; Stanley, Lanthier & Leibowitz, 1989). Large doses of opiates to these sites decrease spontaneous activity; sedation results from injections into the paraventricular nucleus (Leibowitz & Hor, 1982; Gosnell, Morley & Levine, 1986), and masks changes in feeding. No sedation is observed following administration of high doses of opiates to the VTA making it a good site tc investigate the effects of opiates on feeding. Collectively, these results suggest that the VTA is sensitive to the effect of opiates and may be a primary site of action for opioid-mediated feeding.

At present it is not known which of the opioid receptor sub-types in the VTA is primarily involved in mediating opiate-induced feeding. The VTA is moderately dense with kappa and mu opioid receptors and has minimal levels of delta receptors (Mansour et al., 1986; 1987; 1988). Opiates that bind preferentially to mu, delta or kappa opioid receptors have been reported to enhance feeding when injected into the VTA (Cador et al., 1986; Jenck, Gratton & Wise, 1986b; Mucha & Iversen, 1986; Jenck, Quirion & Wise, 1987; Hamilton & Bozarth, 1988a), thus all three of the receptor sub-types have been implicated in opiate-induced feeding in the VTA. However, with the exception of one study by Jenck et al., (1987) all of the opiates that have been administered to the VTA are now

considered to be non-selective opiates. While binding preferentially to one receptor they are capable of binding to multiple receptor sub-types. Therefore it is impossible to determine which of the receptor sub-types are involved in opiate-mediated feeding and which receptor sub-types are not involved. Injections of selective opiates into the VTA are necessary to evalu. the contribution of mu, delta, and kappa opioids in mediating feeding at the level of the VTA.

Dopamine-Opiate Interactions

The behavioral functions associated with injections of morphine into the VTA are largely functions that are attributed to the mesocorticolimbic dopamine system. This system originates in the VTA and projects primarily to the nucleus accumbens as well as to other limbic structures and the frontal cortex (Dahlstrom & Fuxe, 1964). Dopaminergic activation is associated with locomotion (Ungerstedt, 1971; Arbuthnott & Crow, 1971), feeding (Hernandez, Parada & Hoebel, 1983; Evans & Eikelboom, 1987; Colle & Wise, 1988), and sexual behavior (Tagliamonte et al., 1974; Ågmo & Fernandez, 1989); injections of morphine into the VTA facilitate each of these behaviors (Joyce & Iversen, 1979; Mucha & Iversen, 1986; Mitchell & Stewart, 1990). Thus the feeding induced by administration of morphine into the VTA has been considered in terms of dopamine-opiate interaction. Evidence for a dopamine-opiate interaction comes from behavioral, electrophysiological, neurochemical, and anatomical studies.

Behavioral Evidence

Three lines of behavioral evidence suggest a dopamine-opiate interaction. First, microinjections of opiates into the VTA increase spontaneous motor activity in a fashion that is reminiscent of the increase in behavioral activation produced by systemic injections of the dopamine agonists amphetamine and apomorphine (Joyce & Iversen, 1979; Broekkamp & Phillips, 1980; Kelley, Stinus & Iversen, 1980; Stinus et al., 1980; Joyce et al., 1981). The suggestion of dopamine involvement in this opiate-induced behavioral activation is supported by the demonstration that dopamine antagonists such as haloperidol or pimozide block the behavioral stimulant effect of opiates (Joyce & Iversen, 1979; Vezina & Stewart, 1984). Opiate-induced increases in behavioral activation can also be blocked by selective damage to the dopaminergic systems; this is achieved by administration of 6-hydroxydopamine to the terminal fields of the dopaminergic systems (Kelley, Stinus & Iversen, 1980; Stinus et al., 1980; Kalivas & Bronson, 1985; Latimer, Duffy & Kalivas, 1987). The blockade of opiate-induced behavioral activation by dopamine antagonists or 6-hydroxydopamine lesions implies that normal dopaminergic function is necessary for the increase in behavioral activation induced by opiates and confirms the interaction of opiates and dopamine.

A second line of behavioral evidence for a dopamine-opiate interaction comes from the demonstration that a greater increase in behavioral activation is obtained when opiates are administered in conjunction with dopamine agonists than when either drug is administered alone. Injections of low doses of the opiate agonist DALA into the VTA and low doses of dopamine into the nucleus

accumbens synergise to produce a significantly higher level of behavioral activation than can be obtained with either drug alone (Kalivas et al., 1983). A similar result is obtained when VTA injections of DALA are given in conjunction with systemically applied amphetamine (Kelley, Stinus & Iversen, 1980).

Lastly, data from feeding studies also support the idea of a dopamine-opiate interaction. Opiate-induced feeding is attenuated following reductions in dopaminergic transmission. The increase in food intake that is induced by systemic administration of opiates such as butorphanol or bremazocine is blocked when rats are pretreated with large doses of the dopamine antagonist haloperidol (Morley & Levine, 1983; Morley et al., 1985). Opiate-induced feeding is also attenuated by 6-hydroxydopamine lesions (Gosnell et al., 1984). This suggests that normal dopaminergic transmission is necessary for opiate-induced feeding.

Electrophysiological Evidence

The administration of opiates into the VTA leads to increases in cell firing in the mesocorticolimbic dopamine system. Systemically administered morphine increases the firing rate of dopamine neurons in the VTA (Iwatsubo & Clouet, 1977; Nowycky et al., 1978; Gysling & Wang, 1983; Hommer & Pert, 1983; Mathews & German, 1984). These effects are blocked by systemic naloxone.

Iontophoretic application of morphine into the VTA increases the firing rate of dopamine cells in this region. Iontophoretic application of morphine to the VTA also alters the firing rate of non-dopaminergic cells in the VTA. Unlike the firing rate of dopamine

cells, the firing rate of non-dopaminergic cells in the VTA are decreased by morphine (Gysling & Wang, 1983; Matthews & German, 1984). Based on these results some researchers (Gysling and Wang, 1983) have suggested that opiates interact with dopamine cells in the VTA but that this interaction may be indirectly mediated by non-dopaminergic neurons.

Not all opiates increase dopaminergic cell firing. Intravenous injections of the selective kappa opiate, U-50,488H, decrease dopamine cell firing in the substantia nigra. This effect can be attenuated by naloxone administration although larger doses of naloxone are required to block the effect of U-50,488H than are required to block morphine's activation of these dopamine cells (Thompson et al., 1986; Walker et al., 1987; Thompson & Walker, 1990). This is probably due to naloxone's weak antagonism of kappa receptors and preferential antagonism of mu receptors (Goldstein & Naidu, 1989). Iontophoretic application of U-50,488H into the substantia nigra also decreases the firing rate of dopamine cells in this region. U-50,488H has mixed effects on the firing rate of non-dopaminergic cells. Some cells show decreased firing rate in response to U-50,488H while others are unaffected (Thompson et al., 1986). It is not clear from these results whether the inhibition of dopamine cells in the substantia nigra by kappa opiates is a directly mediated effect or occurs indirectly through non-dopaminergic neurons. At present it is not known if kappa opiates also inhibit the firing of dopamine cells in the VTA. What is clear is that kappa opiates are capable of inhibiting dopaminergic activity and thus, in at

least one dopaminergic system, act in the opposite manner from mu and delta agonists.

Neurochemical Evidence

The ability of opiates to increase dopamine activity is relevant when one considers that the act of feeding can increase dopamine activity. Neurochemical studies, which involve the measurement of changes in the release and metabolism of neurotransmitters such as dopamine, have provided evidence for increased dopamine release and metabolism in the nucleus accumbens when an animal anticipates and initiates eating (Blackburn et al., 1986a; Radhakishun, van Ree & Westerink, 1988; Rose & Gratton, 1990). Dopamine activity remains high while an animal is eating (Rose, Mitchell & Gratton, in preparation) and following the consumption of a meal (Heffner, Hartman & Seiden, 1980; Blackburn et al., 1986b). This suggests that the release of dopamine may be important in initiating and in maintaining feeding behavior. Thus, it may well be that opiates induce and enhance feeding because they increase mesocorticolimbic dopamine activity.

Neurochemical studies confirm that opiates alter mesocorticolimbic dopaminergic activity. Systemic or intracerebroventricular injections of opiates that bind to mu or delta receptors increase dopamine release and metabolism in the nucleus accumbens. These increases are blocked by systemic or intracerebroventricular injections of opiate antagonists (Westernik and Korf, 1976; Di Chiara & Imperato, 1988; Iyengar et al., 1989; Spanagel, Herz & Shippenberg, 1990).

Opiates also increase dopamine activity when injected directly into the VTA. VTA injections of DAGO, the selective mu opiate, or DPDPE, the selective delta opiate, increase dopamine release and metabolism in the nucleus accumbens (Kalivas et al., 1983; Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a; 1991b; Spanagel et al., 1991). These effects are blocked by administration of injections of a selective mu or delta opiate antagonist, respectively, into the VTA or by systemic injections of naloxone (Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a; Spanagel et al., 1991). These results are consistent with the electrophysiological data demonstrating that mu and delta opiates activate dopamine neurons at the level of the VTA.

The VTA and not the nucleus accumbens appears to be the site where mu opiates increase dopamine activity. While injections of DAGO into the VTA increase dopamine release and metabolism in the nucleus accumbens injections of DAGO into the nucleus accumbens itself, have no effect on the release or metabolism of dopamine in this brain site (Spanagel et al.,1991). Thus, it appears that mu opiates have their effect by acting at dopamine cell bodies and not at dopamine terminals.

Mu opiates are more effective in activating dopamine than are delta opiates. It has been estimated that DAGO is at least 100 times more effective than DPDPE in elevating levels of dopamine and dopamine metabolites in the nucleus accumbens following VTA injections (Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a). The greater potency of the mu opiate can not be attributed to differences in metabolic or dispositional factors between the mu and delta opiates (Latimer, Duffy & Kalivas, 1987).

Kappa opiates decrease dopamine release. Peripheral or intracerebroventricular administration of kappa agonists such as U-50,488H or E-2078 decrease dopamine release in the nucleus accumbens (Di Chiara & Imperato, 1985; 1988; Spanagel, Herz & Shippenberg, 1990; Devine et al., 1991b). This confirms the electrophysiological data demonstrating that kappa opiates inhibit dopamine cell firing and thus have an effect opposite to that of mu and delta opiates.

Unlike mu and delta opiates, when kappa opiates are injected directly into the VTA they have no effect on the release or metabolism of dopamine in the nucleus accumbens (Spanagel et al., 1991; Devine et al., 1991a). However, injections of a kappa opiate directly into the nucleus accumbens decreases dopamine release from this site. Injections of a kappa opiate antagonist have the opposite effect; they increase dopamine release and metabolism in the nucleus accumbens (Spanagel et al., 1991). This suggests that kappa opiates inhibit dopaminergic transmission and that they do so by acting at dopamine terminals rather than dopamine cell bodies.

To summarize, neurochemical studies confirm that opiates alter the activity of the mesocorticolimbic dopaminergic system. Mu or delta opiates increase dopamine activity and kappa opiates inhibit dopamine activity. Mu and delta opiates stimulate dopamine neurons at the level of the VTA while kappa opiates inhibit dopamine activity at the level of the nucleus accumbens.

Anatomical Evidence

Endogenous opioids and dopamine neurons have a similar distribution within the central nervous system. All the dopamine cell body regions and their terminal regions have been found to be moderately dense with opioids (Elde et al., 1978; Johnson, Sar & Stumpf, 1980; Khatchaturian et al., 1985; Quirion, Weiss & Pert, 1983; Mansour et al., 1986; 1987; 1988; Dilts & Kalivas, 1989; 1990).

Opioid terminals and fibers are found in close proximity to dopamine neurons within the mesocorticolimbic system and the adjacent nigrostriatal system (dopamine projections from the substantia nigra to the striatum) (Johnson, Sar & Stumpf, 1980; Moskowitz & Goodman, 1984). The proximity of opioid neurons to dopamine neurons prompted researchers to investigate the possibility that opioid receptors are localized to the dopamine cell membrane. If opioid receptor sites are localized to dopamine neurons, then lesions of dopamine containing neurons should result in a decrease in opiate binding. The results of early studies suggested that naloxone binding in the substantia nigra, VTA, striatum, and nucleus accumbens was decreased following 6-hydroxydopamine lesions. These results suggested that opioid receptors were on mesocorticolimbic and nigrostriatal dopamine neurons (Pollard et al., 1977a; 1977b; Pollard et al., 1978; Llorens-Cortes, Pollard & Schwartz, 1979). Recent results, however, contradict this conclusion.

Recently conducted studies have had the benefit of highly selective opiates and iodinated compounds that are resistant to the quenching that occurs in areas of high fiber density. Thus, recent findings probably provide a more accurate view of the anatomical

relationship between opioid and dopamine neurons than did previous studies. With the aid of a selective mu agonist (^{125}I-DAGO) Dilts and Kalivas (1989) have shown that 6-hydroxydopamine lesions of the mesocorticolimbic or nigrostriatal systems have no effect on mu binding in the dopamine cell body or terminal regions. In fact, lesions of non-dopaminergic neurons decrease mu opiate binding suggesting that mu receptors are on non-dopamine neurons in the VTA (Dilts & Kalivas, 1989). These results support the suggestion that mesocorticolimbic dopamine neurons are indirectly modulated by mu opioid agonists (Gysling & Wang, 1983).

Unilateral 6-hydroxydopamine lesions of dopamine cells increases binding of a selective delta agonist (^{125}I-DPDPE) in the terminal areas on the side opposite the lesion. Increases in delta binding in terminal regions are also seen following damage to non-dopaminergic neurons. These results suggest that delta opioid agonists do not directly modulate dopamine cell firing (Dilts & Kalivas, 1990). In conclusion, neither mu nor delta opioid receptors appear to be located directly on dopamine neurons. However, from electrophysiological and neurochemical studies it is clear that both mu and delta opiates increase dopamine cell firing and release. Thus, dopamine-opiate interactions may occur indirectly via non-dopaminergic neurons.

Present Investigation

We know that mu and delta opiates increase dopamine cell firing and dopamine release when injected into the VTA. We also know that injections of opiates into the VTA can facilitate feeding. If

the administration of opiates into the VTA alters food intake by changing the level of dopamine activation then mu and delta opiates should increase food intake when injected into the VTA. By the same reasoning, since kappa opiates have no effect on dopamine cell firing or dopamine release when injected into the VTA, kappa opiates should have no effect on feeding. However, all three opioid receptor sub-types have been implicated in feeding at the level of the VTA. In general, however, feeding has been investigated following the administration of non-selective opiates to the VTA.

The primary purpose of the present investigation was to identify the opioid receptor sub-types involved in the regulation of feeding at the level of the VTA by administering opiates selective for the mu (DAGO), delta (DPDPE), or kappa (U-50,488H) opioid receptors.

The procedure that was used to investigate the effects of opiates on feeding allowed for repeated measurements of feeding to be taken within a single test session. This was done by breaking a meal into discrete trials or meal segments. The discrete trial approach allows one to assess the consistency with which animals focus their attention on food. Two measures of feeding were taken for each meal segment; the time required to initiate feeding (latency) and the duration of time required to complete a meal segment (duration). Thus, the consistency for the latency measures as well as for the duration measures was assessed. Because this procedure has not been used to evaluate the effects of opiates on feeding, the first experiment was aimed at assessing the effect of VTA injections of the prototypical opiate, morphine. This first experiment was then able to

serve as a baseline against which the effect of the selective mu, delta, and kappa opiates on feeding could be compared to (experiments 2-4).

The final experiment was conducted to investigate the effect of repeated administration of selective mu or delta opiates into the VTA. Previous studies have demonstrated that repeated administration of opiates into the VTA produce a progressive enhancement of behavior. This literature is reviewed at the beginning of experiment 5. On the basis of previous research it was expected that repeated injections of opiates into the VTA would result in a progressive enhancement of feeding.

Experiment 1

Low to moderate doses of systemically administered morphine increase food intake in both sated and food-deprived rats (Sanger & McCarthy, 1980; Jalowiec et al., 1981; Sanger & McCarthy, 1981; Morley et al., 1985; Ramaro & Bhargava, 1989). Increases in food intake have also been reported following central injections of morphine (Mucha & Iversen, 1986; Hamilton & Bozarth, 1988). However, while it is clear that morphine can increase food intake, it is not clear what aspect of feeding is altered by morphine to produce this effect. Two aspects of feeding that can affect the total amount of food consumed are the latency to initiate feeding and the speed of feeding. Decreases in the latency to initiate feeding and increases in the speed of feeding can result in an increase in total food intake.

The latency to initiate feeding and the speed of feeding are usually measured only once during a test session. This approach makes it difficult to assess the consistency of a drug's effects on feeding. A procedure that allows for the repeated measurement of latency and speed within a single test session is advantageous in this regard. Repeated measures can be taken by breaking a single meal down into a number of small meals or meal segments. This approach, called the discrete trials approach, allows one to assess the consistency of a drug's effects as both measures of mean score and the score variability (consistency) are obtained. If both mean score and score variability change following drug treatment, this suggests that the degree to which an animal's interest in food is sustained has changed (Colle & Wise, 1984). If the mean score

changes but score variability remains the same following drug treatment, this suggests that the drug has altered performance capability (Colle & Wise, 1984). For example, if the mean speed of eating is increased and score variability is decreased following the administration of a drug we can say that the drug increases the amount of attention an animal pays to food. If mean speed of eating increases but score variability remains the same we can say that the drug has enhanced the motoric capacity of an animal without substantially alterating the amount of attention an animal pays to food.

The discrete trials approach has been used by Wise and his colleagues to characterize the effects of dopamine antagonists and opiate antagonists on feeding. Dopamine antagonists and opiate antagonists have both been found to inhibit feeding by decreasing the speed of eating and increasing score variability (Colle & Wise, 1984; Jenck et al., 1986a; Wise & Raptis, 1986; Koechling, Colle & Wise, 1988). The decrease in speed of eating accompanied by an increase in score variability is taken as an indication of a decrease in the amount of attention that animals pay to food. Changes in the attentional focus on food are believed to reflect changes in the motivation to eat (Colle & Wise, 1984). A decrease in the focus on food following drug administration is thought to represent a decrease in the motivation to eat (Colle & Wise, 1984). Thus, using the disecrete trials approach it is possible to assess the effect that a drug has on the motivational aspects of feeding.

The primary objective of the present experiment was to determine the effect of VTA injections of morphine on feeding using

the discrete trials approach. In this way the effect of morphine on the latency to initiate feeding and the speed of feeding could be assessed, and the consistency of morphine's effect could be evaluated. A second aim of this study was to provide a baseline against which the effects of VTA injections of selective opiates on feeding could be compared.

METHOD

Subjects and Surgery

The subjects were thirteen naive adult male Long-Evans Old Colony rats individually housed and maintained on a 12 hour light cycle. They weighed between 350 and 480 grams at time of surgery. Seven rats were implanted under sodium pentobarbitol anesthesia (65 mg/kg) with unilateral stainless steel 22 gauge guide cannula aimed at the VTA (AP: -3.4; L: + 2.7; DV: 7.4 mm below dura with the incisor bar 5 mm above the interaural line). The remaining rats were implanted with a unilateral cannula aimed dorsal to the VTA (DV: 5.8 to 6.2 mm below dura; all other co-ordinates were the same as previously stated). All cannula were angled 10 degrees so as to avoid the periaqueductal gray. Four stainless steel screws were threaded into the cranium and dental acylic was used to anchor the cannulae to the screws and the skull. Obturators made from 30 gauge stainless steel wire were inserted to a depth of 1 mm beyond the guide cannula immediately following surgery. Drug was delivered through an injector cannula which was the same length as the obturators and which was made from a hollow 30 gauge

stainless steel wire attached to polyethylene tubing which in turn was connected to a 1 microliter Hamilton syringe.

Apparatus

Meal segments, consisting of five 45 mg Noyes pellets each, were introduced into a 25 x 25 cm test box by an automatic dispensing apparatus. Thirty-six food cups (1.3 cm diameter x 0.8 cm deep) were drilled into a 25 cm (diameter) food-delivery platter which extended 5 cm into the test box. In order to measure feeding only one of the food cups was exposed through a mask at any given time. The platters were indexed one position every 36 seconds; rubber drive wheels on continuously running motors were pulled against the circumference of platters by a solenoid when triggered by a timer. The solenoid circuit was broken by a microswitch when a new food cup reached the correct position. The solenoid noise was clearly audible but not loud; it could just be heard over normal conversation. The aluminum food platters were cleaned with a vacuum when food crumbs accumulated in the food cups. Each test box was dimly lit, the test room was otherwise dark.

Procedure

One week following recovery from surgery all rats were food-deprived and placed daily into test chambers. The animals were weighed daily prior to testing. Testing always occurred at the same time of day and took place during the animals light cycle. All animals were trained to obtain a major portion of their daily food intake in the test chambers where 18 meal segments were offered

for 36 seconds at intervals of 72 seconds. Immediately following testing rats were returned to their home cage and given approximately 15 grams of standard rat food. The animals usually consumed this amount of food within 4-6 hours, and were thus, in effect, maintained on an 18 hour food-deprivation schedule.

When animals ate all of the pellets in the test chamber for at least three consecutive days, testing was initiated the following day. The animals were individually tested and observed in each test. The latency to feed (the time until the first oral contact with food) and the duration of feeding (the time required to eat all five food pellets once contact was made) were recorded using hand operated electronic timers. If all five food pellets were not eaten by the end of the 36 second interval, an arbitrary "ceiling" score of 36 seconds was assigned. The number of uneaten pellets and incidents of grooming, freezing and locomotion were noted for each trial.

Drug and Injection Procedure

Morphine sulphate (Health & Welfare, Canada) was dissolved in isotonic saline and injected centrally in a volume of 0.5 micoliters immediately prior to testing. Each rat received each dose of morphine (0.1,1.0 and 10.0 nmole). The order of drug presentation was counterbalanced. Drug was administered every second day and saline was administered on non-drug days. Each injection was administered over a minimum of 60 seconds. Following administration of the drug, the injector was left in place for a minimum of 45 seconds after which the injector was removed and the obturator replaced.

Histology

Following completion of each experiment the animals were anesthetized with chloral hydrate (400 mg/kg). The obturators were removed and replaced by the injector cannulae. The animals were perfused intracardially with 0.9% saline followed by 10% formalin. The brains were immediatedly removed and stored in a solution of 10% formalin until they were firm. This usually took four days. The brains were then frozen in dry ice, sliced into 40 micron sections with the aid of a microtome and mounted on gelatin coated microscope slides. The sections were stained with formal thionin. The tips of the injectors were located and recorded. Reconstructions of the injector placements were made based on the stereotaxic atlas of Pellegrino, Pellegrino and Cushman (1979).

Statistical Analyses

To determine if there was an effect of drug on the latency and duration scores one-way analyses of variance (repeated measures design) were performed. Post hoc comparisons between individual drug doses were made using Tukey's Honestly Significant Difference tests.

In order to determine whether morphine caused consistent changes in feeding an analysis of the raw scores was performed. Scores were grouped into bins and the number of scores that fell into each bin was recorded. The number of duration scores ranging from 2.5 to 5.4 were recorded and grouped together in the 4 second

bin. Scores ranging from 5.5 to 10.4 were grouped under the 8 second bin. Scores from 10.5 to 14.4 were grouped under the 12 second bin and so on. The exception was the bin of 36 seconds which included scores that were equal or greater to 34.5. Latency scores were grouped in a similar fashion. The 0.3 second bin included scores that ranged from 0.25 to 0.44. Scores from 0.45 to 0.64 were grouped under the 0.5 second bin. Scores from 0.65 to 0.74 were gouped under the 0.7 second bin and so on until the last bin where scores equal to or greater than 1.85 were grouped under >1.70.

'Best' scores are defined as scores in the shortest duration or latency bins (4 and 0.3 seconds, respectively). 'Worst' scores are defined as scores in the longest duration or latency bins (36 and >1.7 seconds, respectively).

F-tests were done on all the frequency distributions to determine if there were any differences between score variability in drug and saline conditions. Changes in motivation were said to occur if a change in mean score value that was accompanied by a change in score variability was obtained.

Results

Injections of morphine into the VTA resulted in a significant acceleration of feeding as reflected by a decrease in the duration of time animals took to complete their meal segments ($F(3,18)=5.27$, $p<0.0088$, Fig. 1). Morphine had a dose-dependent effect on the speed of eating (Fig. 1); the shortest mean duration scores were obtained with the highest dose of morphine. Only the highest dose of

morphine (10.0 nmole) was significantly different from saline at the probability level of 0.01. This dose produced a large leftward shift in the peak of the distribution of frequency scores (Fig. 2). Short duration scores were more frequent in the 10 nmole morphine condition than in the saline condition. This is particularly evident in the duration categories of 4, 8, and 12 seconds (Fig. 2). Animals consistently had fewer long duration scores in the morphine (10 nmole) condition than in the saline condition. However, while score variability was smaller under morphine than under saline, this failed to reach significance.

There was no significant effect of VTA morphine injections on the mean latency to initiate feeding ($F(3,18)=1.89$, $p<0.1679$, Fig 3). In general the distribution of latency scores under morphine was similar to that under saline. However, under all doses of morphine there was a tendancy for animals to initiate feeding faster than under saline. This is reflected by the increase in the frequency of 'best' latency scores. As well, the 10 nmole dose of morphine was consistent in producing a decrease in the number of long latency scores (scores ranging from 1.1 to >1.7 secs; Fig 4).

Injections of morphine dorsal to the VTA had no significant effect on duration scores ($F(3,15)=0.61$, $p<0.619$, Fig 5). There was very little effect of dorsal morphine on the distribution of scores. The largest dose of morphine did increase the frequency of 'best' scores. This dose, however, also increased the frequency of 'worst' scores (Fig 6).

Injections of morphine dorsal to the VTA did not have any significant effect on the latency scores ($F(3,15)=0.95$, $p<0.4406$).

However, in the latter half of the test session there was a tendancy for the largest dose of morphine to increase the latency to initiate feeding (Fig. 7). This is reflected in the frequency distribution; 10 nmole dose of morphine increased the number of 'worst' latency scores (Fig. 8). All doses of morphine injected dorsal to the VTA produced more 'best' scores and more scores in the 0.5 second category than were obtained in the saline condition (Fig 8). However, score variability was no different under dorsal morphine than under saline.

Observations of freezing or startle responses were rare under morphine and were observed equally in the saline and drug conditions. Instances where animals did not finish their food pellets were also rare and were usually a result of the interruption of feeding by grooming or exploratory behavior. This happened equally under saline and drug conditions. Circling behavior was evident when animals received the largest dose (10 nmole) of morphine into the VTA, but circling behavior did not appear to interfere with feeding.

Locations of injector tips aimed at and dorsal to the VTA are shown in figure 9.

Discussion

The main finding of the present experiment is that VTA morphine facilitates feeding in food deprived rats. This finding raises the possibility that systemically administered morphine is acting at the VTA to produce an enhancement of feeding. Several

lines of evidence support this suggestion. First, in the present experiment it was found that injections of morphine dorsal to the VTA had no effect on feeding. Thus, the possibility that VTA morphine diffused up the cannulae shaft and had its effect at a site distal to the VTA is slim. Second, the facilitation of feeding following VTA morphine occurred with very short latencies. Similar observations have also been made by other investigators (Hamilton, 1988; Hamilton & Bozarth, 1988a). The shorter the latencies the more likely it is that morphine has its effect by acting immediately at the site of injection.

The suggestion that systemic morphine facilitates feeding through its action at the VTA is also supported by the fact that feeding is generally more easily enhanced when opiates are injected into the VTA as opposed to other brain sites. In general, smaller doses of morphine are required to obtain a facilitation of feeding when morphine is administered to the VTA than when morphine is injected into other sites (Hamilton, 1988; Hamilton & Bozarth, 1988a). As well, opiate-induced feeding occurs with shorter latencies following VTA injections than when opiates are injected into other brain sites (Hamilton, 1988; Hamilton & Bozarth, 1988a).

Morphine appears to have an effect on the motivational aspect of feeding. The mean duration of time animals took to complete meal segments was shorter in the VTA morphine (10 nmole) condition than in the saline condition. The decrease in mean duration was the result of an increase in the frequency of short scores and a decrease in the frequency of long scores. The effect of VTA morphine was particularly noticeable in the second half of the

test session when duration scores usually increase as a result of the induction of satiety. Morphine produces a noticeable attenuation of this response slowing effect. There appears to be little or no effect of VTA morphine on the motoric capacity of animals. The shortest duration or latency scores following VTA morphine were no different than those following saline. Thus, the limits of performance capability appear to be much the same under morphine as they are under saline.

These findings agree with previous findings suggesting that opiate antagonists affect the motivation to eat but have no effect on performance. It has been found that the opiate antagonist, naloxone, attenuates feeding not by affecting the latency to initiate feeding--'best' scores are the same in naloxone and saline conditions--but by decreasing the speed of feeding (Kirkham & Blundell, 1984; Wise & Raptis, 1986; Kirkham, 1990). These results suggest that opiates regulate feeding by altering the motivation to feed. Thus, VTA morphine may facilitate feeding by enhancing the ability of food to sustain an animals interest.

Morphine's facilitation of feeding may involve an interaction of morphine with the mesolimbic dopamine system. Two lines of evidence support this suggestion. First, drugs that act on the dopaminergic system affect feeding in the same manner as opiates do. The dopamine antagonists SCH 23390 and pimozide have the same effect on feeding as the opiate antagonist, naloxone; each increases the frequency of long scores without changing the value of 'best' scores (Wise & Colle, 1984; Jenck et al., 1986a; Wise & Raptis, 1986). Complementing these results is the finding that nucleus

accumbens injections of the dopamine agonist amphetamine enhance feeding in a manner reminiscent of VTA morphine. Each increases the frequency of short duration and latency scores without having any affect on the value of 'best' scores (Wise, Fotuhi & Colle, 1989). Thus, both opiates and dopaminergic drugs alter the motivation to eat without altering performance. This similarity between the effects of opiates and dopaminergic drugs suggests that the dopaminergic and opioid systems interact to influence feeding through a common mechanism.

Second, systemic or intra-VTA injections of morphine or other mu agonists increase dopamine release and metabolism in the nucleus accumbens (Westerink, 1978; Kalivas et al., 1983; Wood, 1983; Di Chiara & Imperato, 1985; 1988; Devine et al., 1991a; 1991b; Leone, Pocock & Wise, 1991; Spanagel, 1991). The dose of VTA morphine that is required to obtain an increase in dopamine release in the nucleus accumbens is comparable to the dose of VTA morphine required to obtain a facilitation of feeding (Leone, Pocock & Wise, 1991). Thus, morphine is capable of modulating mesolimbic dopamine function and it does so at a dose that is behaviorally relevant.

Based on the findings of the present study it appears that VTA morphine facilitates feeding by enhancing the motivation to eat. This enhancement in motivation may occur as a result of an increase in mesolimbic dopamine. However, because morphine has the ability to bind to all three opioid receptor sub-types (Goldstein & Naidu, 1989) it is not clear which opioid receptors are involved in

morphine's facilitation of feeding. The following experiments will address this issue.

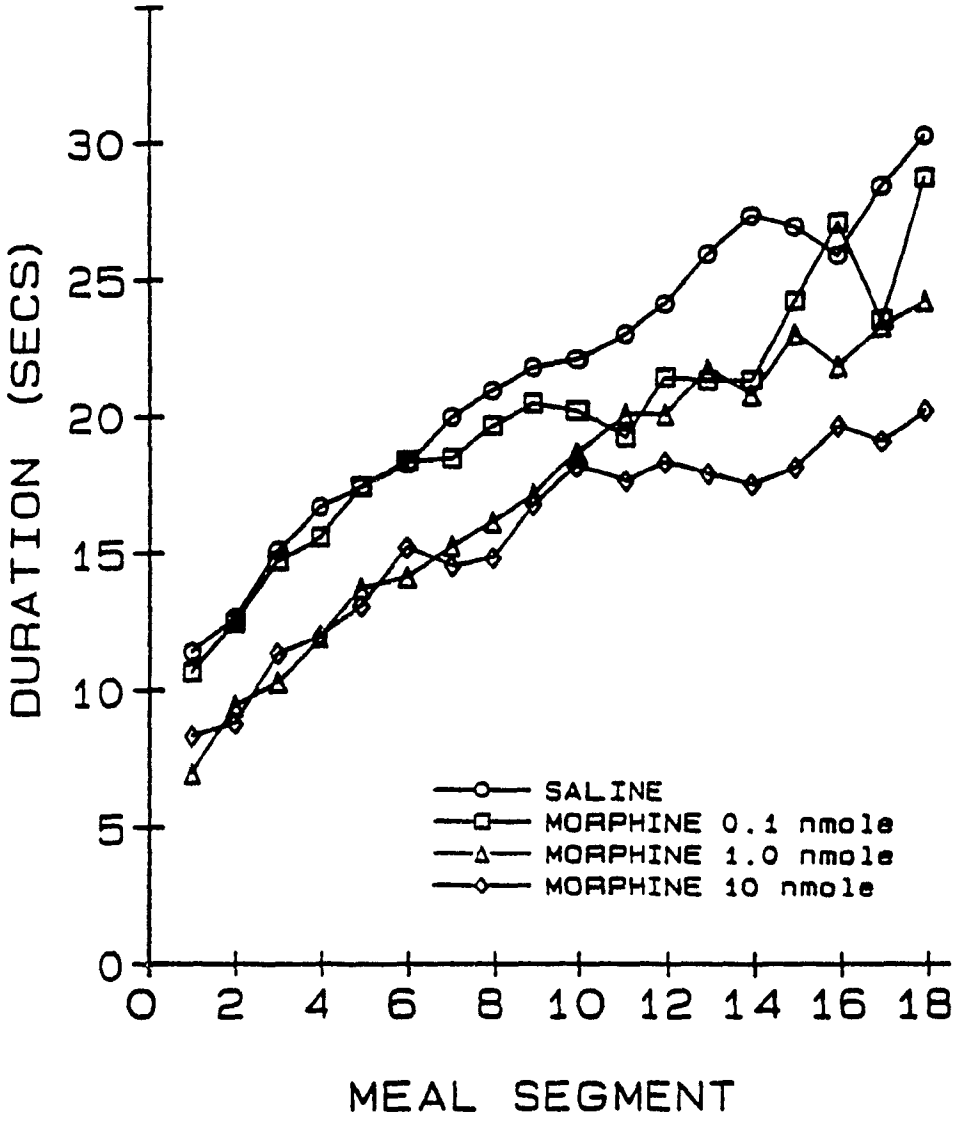

Figure 1. The mean time required to complete meal segments following injections of saline, 0.1, 1.0, or 10 nmole of morphine into the ventral tegmental area (n=7).

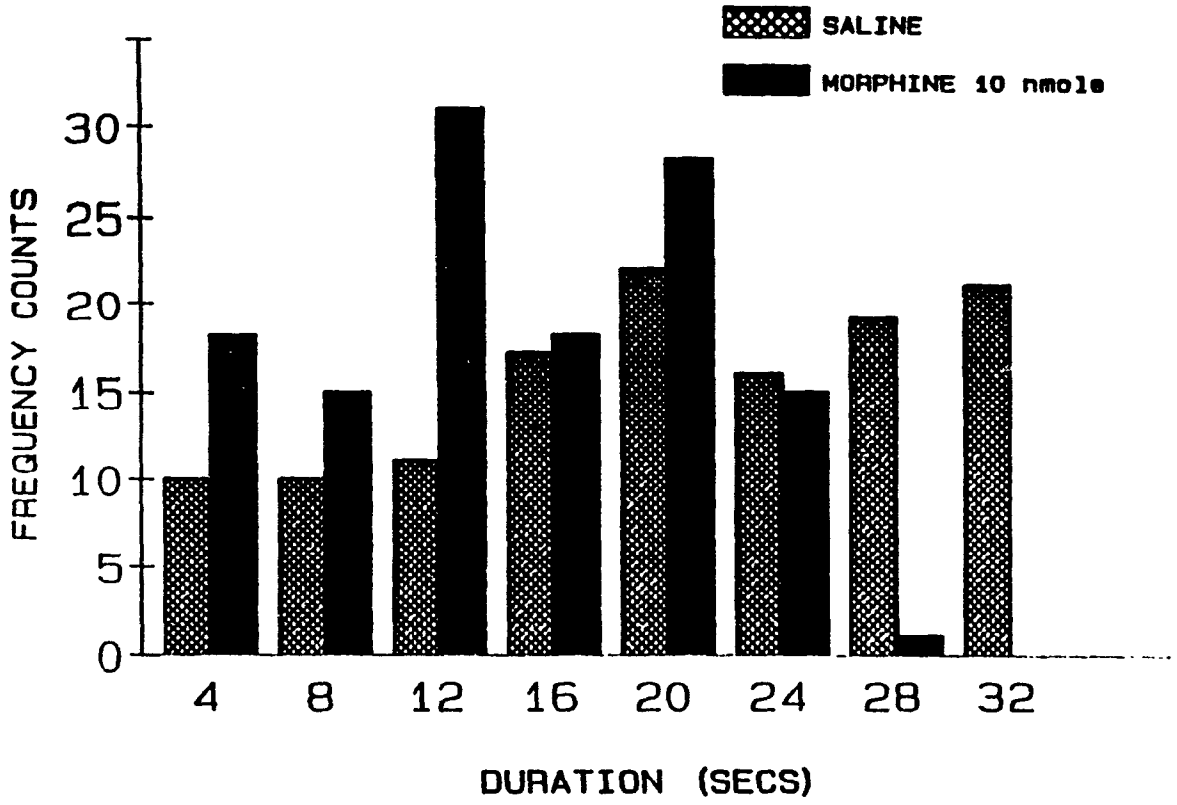

Figure 2. Distribution of duration scores following injections of 10 nmole of morphine or saline into the ventral tegmental area (n=7). Response bins comprise 4 second intervals.

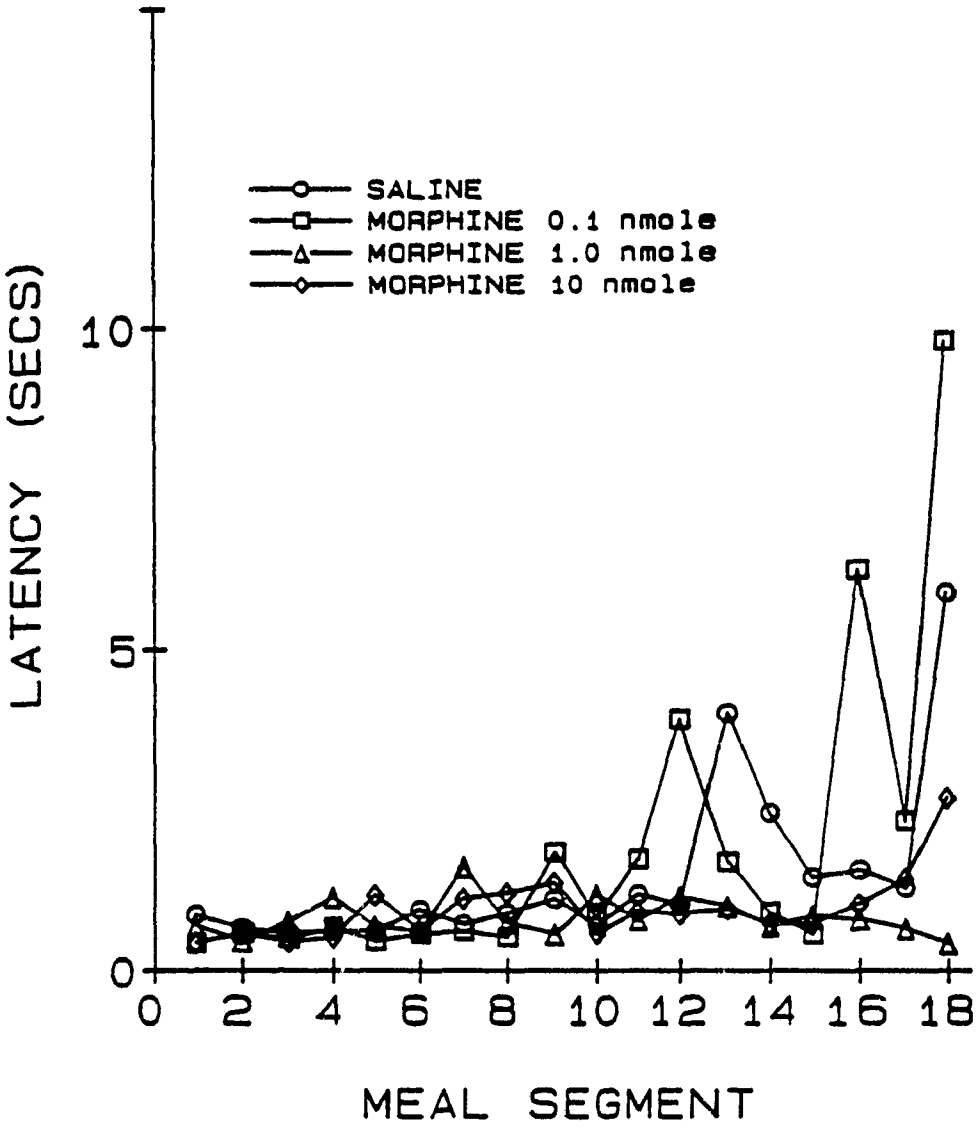

Figure 3. The mean latency to initiate feeding for each meal segment following injections of saline, 0.1, 1.0, or 10 nmole of morphine into the ventral tegmental area (n=7).

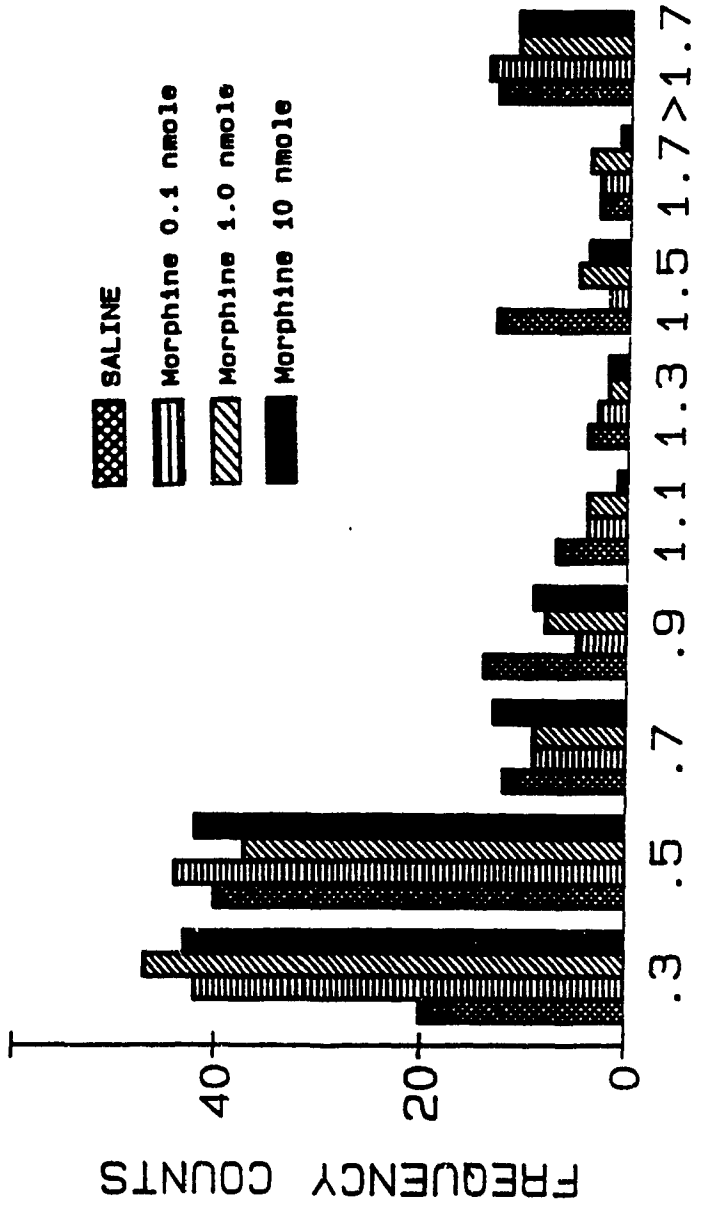

Figure 4. Distribution of latency scores following injections of saline, 0.1, 1.0 or 10 nmole of morphine into the ventral tegmental area (n=7). Response bins comprise 0.2 second intervals.

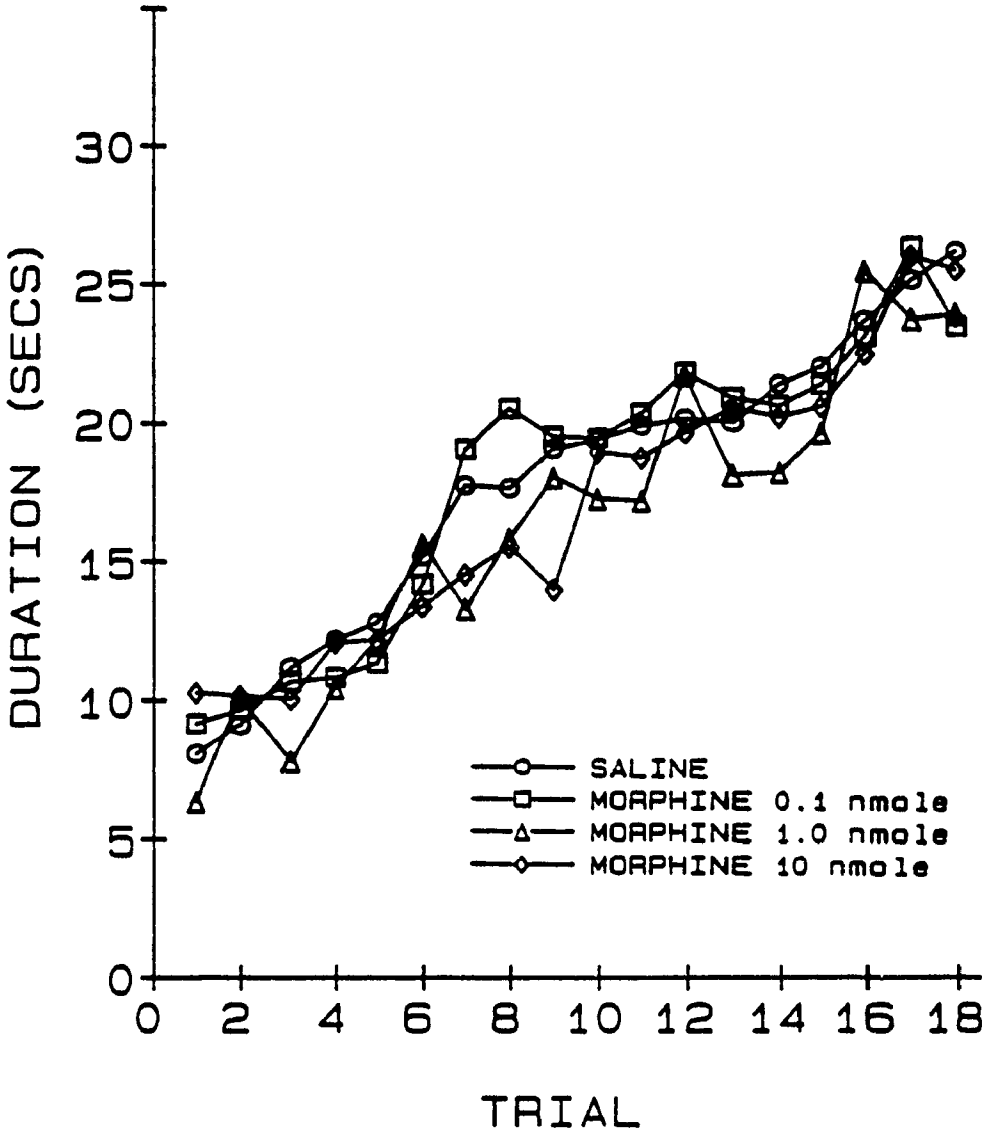

Figure 5. The mean time required to complete meal segments following injections of saline, 0.1, 1.0, or 10 nmole of morphine into sites dorsal to the ventral tegmental area (n=6).

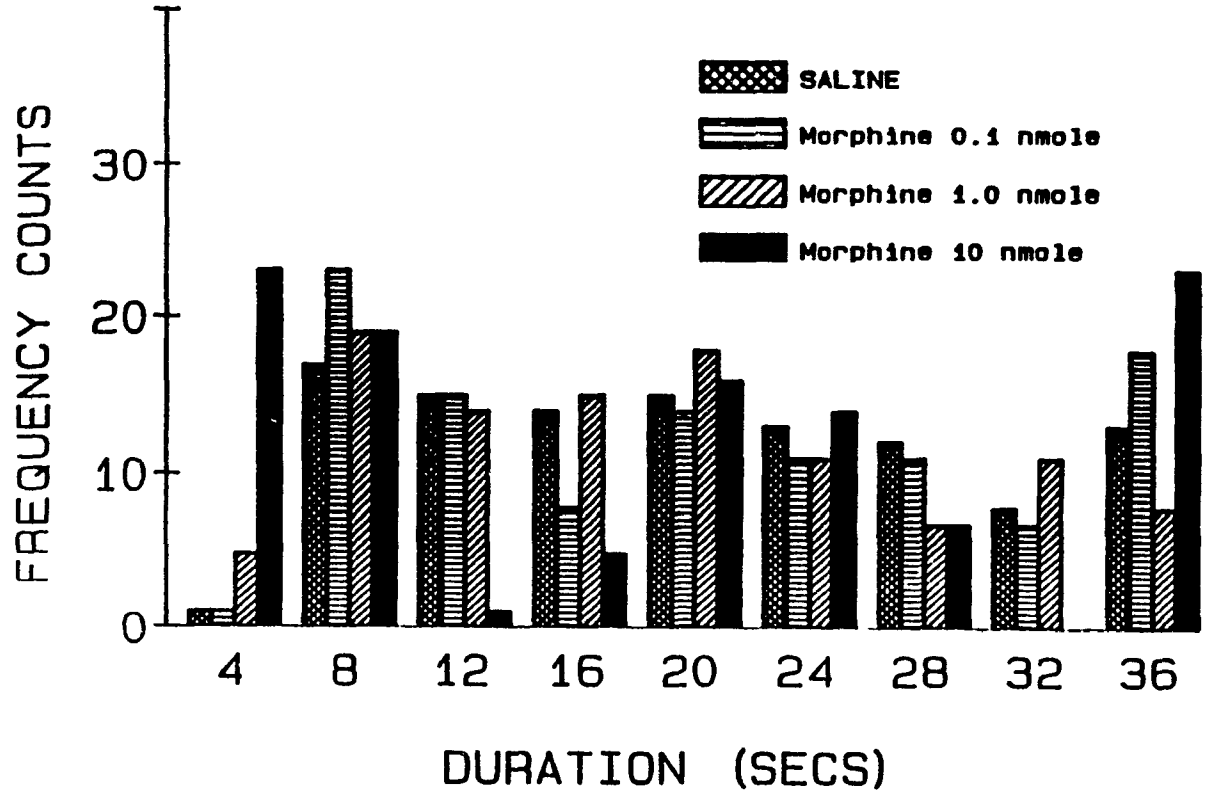

Figure 6. Distribution of duration scores following injections of saline, 0.1, 1.0 or 10 nmole of morphine into sites dorsal to the ventral tegmental area (n=6). Response bins comprise 4 second intervals.

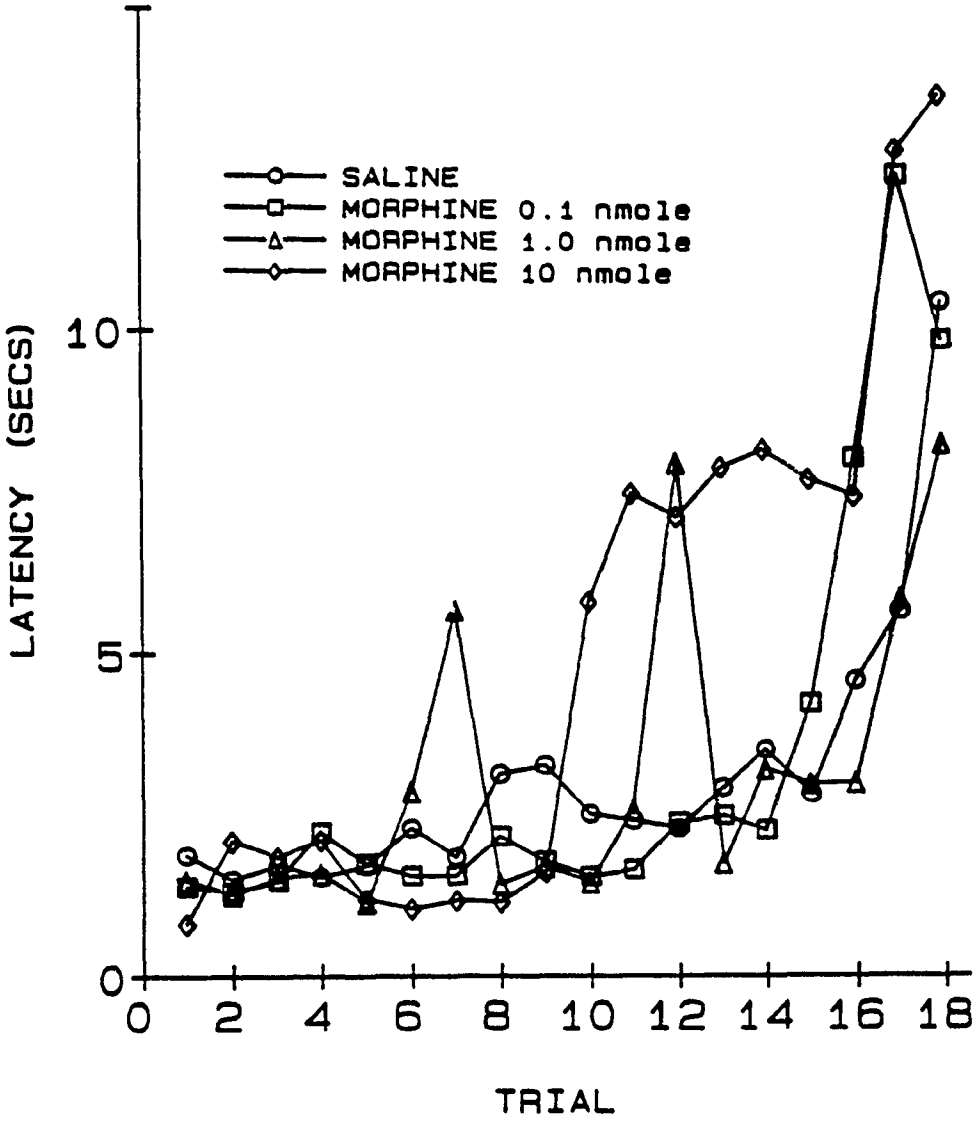

Figure 7. The mean latency to initiate feeding for each meal segment following injections of saline, 0.1, 1.0, or 10 nmole of morphine into sites dorsal to the ventral tegmental area (n=6).

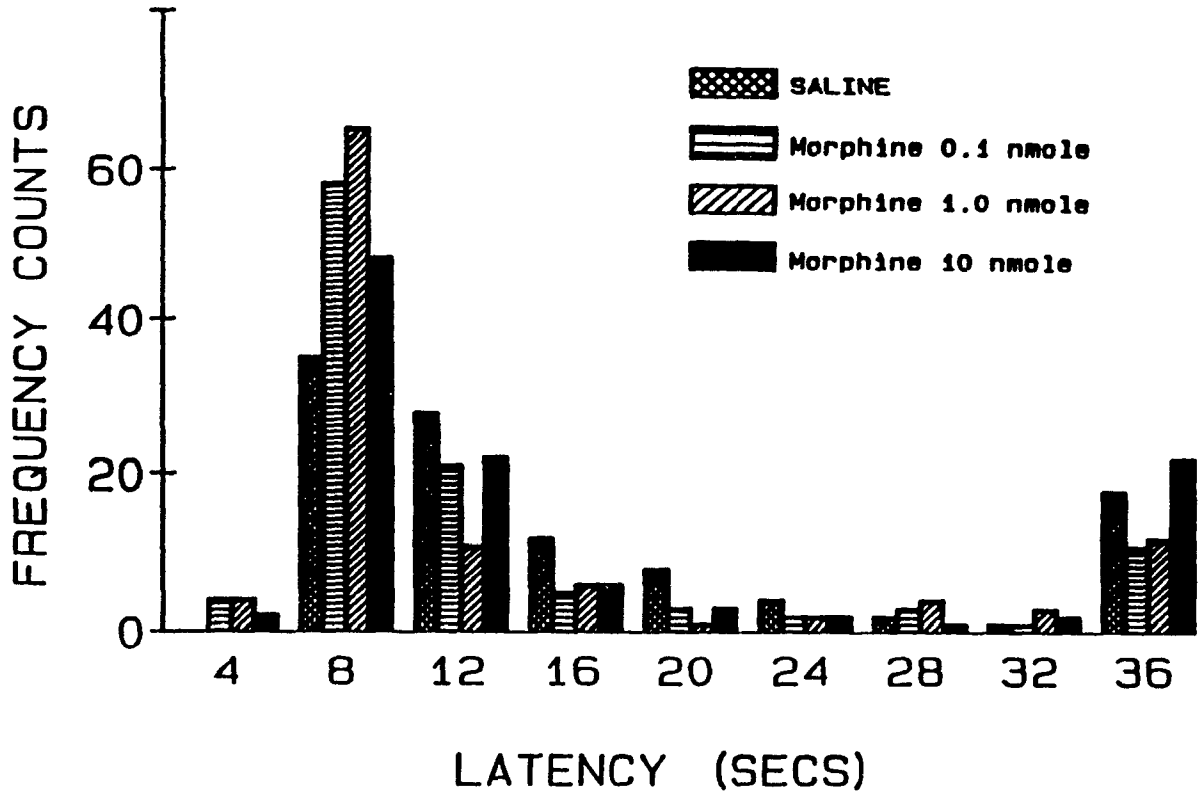

Figure 8. Distribution of latency scores following injections of saline, 0.1, 1.0 or 10 nmole of morphine into sites ɔrsal to the ventral tegmental area (n=6). Response bins comprise 0.2 second intervals.

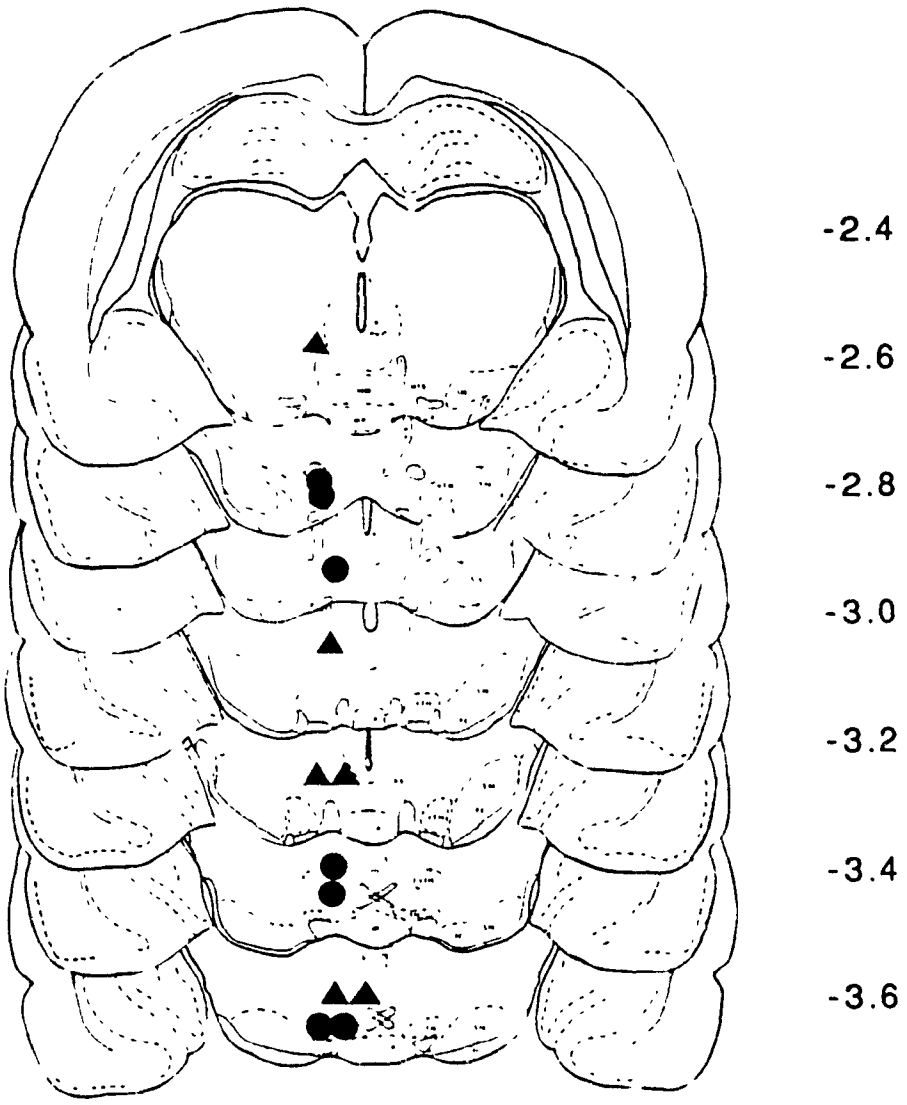

Figure 9. Histological placements for animals with injector tips located dorsal to the ventral tegmental area (triangles, n=6) and animals with injector tips located in the ventral tegmental area (circles, n=7). Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice refers to the distance (in millimeters) posterior to bregma.

Experiment 2

Peripheral administration of kappa agonists have been demonstrated to modulate feeding behavior. The kappa agonists bremazocine, tifluadom, and ketocyclazocine have all been reported to increase food intake when administered systemically (Morley et al., 1982; Hartig & Opitiz, 1983; Morley et al., 1983a; 1983c; Cooper, Jackson & Kirkham, 1985; Ramarao & Bharghava, 1989). However, while these drugs are kappa agonists, they are non-selective (Gillan & Kosterlitz, 1982; Carroll et al., 1984; Gold & Naidu, 1989), acting at mu and delta as well as at kappa opioid receptors. This raises the possibility that the effects of these drugs on food intake may be mediated through non-kappa mechanisms. Against this is the observation that systemic administration of the highly selective kappa opiate, U-50,488H (Lahti, Von Voightlander & Barsuhn, 1982; Von Voightlander, Lahti & Ludens, 1983; Lahti et al., 1985; Clark & Pasternak, 1988), facilitates feeding in sated and food deprived rats (Cooper, Jackson & Kirkham, 1985; Jackson & Cooper, 1986a; 1986b; Bhargava et al., 1989; Ramarao & Bhargava, 1989). This suggests that kappa opioids play a role in the regulation of feeding. Because systemically administered kappa opiates act in the brain as well as in the gut it is not clear where these drugs are acting to produce their facilitation of feeding.

Several lines of evidence suggest that kappa opiates modulate feeding through a central mode of action. First, the facilitation of systemic U-50,488H can be blocked by injections of the selective kappa antagonist, nor-binaltophimine, into the ventricles. Second,

alone nor-binaltorphimine inhibits feeding when administered into the ventricles (Carr et al., 1989; Arjune & Bodnar, 1990; Calcagnetti, Calcagnetti & Faneslow, 1990). These data support a central mode of action but do not shed light on the question of where in the brain kappa opiates have their effects. However, feeding is facilitated when kappa opiates are administered centrally and a number of different brain sites have been implicated in this effect. Dynorphin A $_{(1-17)}$ has been found to facilitate feeding when injected into the ventricles (Katz, 1980; Morley & Levine, 1981), the paraventricular nucleus, the ventromedial hypothalamus (Gosnell, Morley & Levine, 1986) or the VTA (Hamilton & Bozarth,1988a; 1988b). When injected into the VTA, dynorphin A $_{(1-17)}$ is more effective at facilitating feeding than is morphine (Hamilton, 1988; Hamilton & Bozarth, 1988a). Because dynorphin A$_{(1-17)}$ is also a more potent kappa agonist than morphine it has been suggested that VTA morphine's facilitation of feeding is mediated by actions of morphine at kappa receptors (Hamilton, 1988). Recent evidence, however, suggests that dynorphin A $_{(1-17)}$ may bind to mu and delta as well as to kappa receptors, and may even have non-opioid actions. This raises the possibility that dynorphin A's $_{(1-17)}$ facilitation of feeding is not a kappa mediated effect.

Because dynorphin A $_{(1-17)}$ is an endogenous opioid peptide that binds with high affinity to kappa receptors (Goldstein et al., 1979) it was, until recently, considered the kappa opiate of choice. Dynorphin A $_{(1-17)}$ is derived from the endogenous peptide prodynorphin and can be broken down into a number of fragments. These fragments include dynorphin A $_{(1-13)}$, dynorphin A $_{(2-9)}$,

dynorphin A $_{(1-9)}$, dynorphin A $_{(1-8)}$, dynorphin A $_{(1-7)}$ and dynorphin A $_{(1-5)}$, also known as leu-enkephalin (Fallon & Leslie, 1986; Fallon & Ciofi, 1990). While dynorphin A $_{(1-17)}$ and each of its fragments are considered to be endogenous ligands for the kappa receptor (Quirion & Pert, 1981; Corbett et al., 1982; Young et al., 1983). there is evidence suggesting that the dynorphins do not necessarily bind selectively to kappa receptors. The smaller the dynorphin fragment the less preferential it appears to be for the kappa receptor. In other words, binding affinity to the mu and delta receptors increases with decreasing fragment length (Quirion & Pert,1981; Quirion, Weiss & Pert, 1983; Young et al., 1983). There is evidence that leu-enkephalin (dynorphin A $_{(1-5)}$) has a greater affinity for delta receptors than it does for kappa receptors (Zamir et al., 1984; Christenssen-Nylander et al., 1986). Thus, dynorphins are not always highly selective for the kappa receptors, but may also bind to mu and delta receptors.

Further studies on the degradation of Dynorphin A $_{(1-17)}$ have demonstrated that it can be rapidly broken down *in vivo* to dynorphin A $_{(2-17)}$, a behaviorally active fragment which has been demonstrated to have non-opioid actions (Young et al., 1986). Dynorphin A $_{(1-17)}$ and dynorphin A $_{(1-13)}$ have also been demonstrated to have non-opioid actions (Walker et al., 1982; Moises & Walker, 1985). Dynorphin A $_{(1-13)}$ can have a neurotoxic effect through actions on the N-methyl-D-aspartate type of glutamate receptor in the spinal cord (Caudle & Isaac, 1988). Dynorphin A $_{(1-13)}$ can also act directly at the N-methyl-D-aspartate receptors in rat cortex to produce non-opioid mediated effects (Massardier &

Hunt, 1989). These data demonstrate that the dynorphins can have non-opioid actions. Thus, dynorphin A's (1-17) facilitation of feeding may be a non-kappa opioid mediated effect or a non-opioid mediated effect.

If dynorphin A's (1-17) effect on feeding is a kappa mediated effect, then central administration of U-50,488H, an opiate with greater selectivity for kappa receptors than dynorphin A (1-17) (Lahti, Von Voightlander & Barsuhn, 1982; Von Voightlander, Lahti & Ludens, 1983; Lahti et al., 1985; Clark & Pasternak, 1988), should also facilitate feeding. Central administration of the selective kappa opiate U-50,488H has, however, resulted in mixed effects. In sated rats injections of U-50,488H into the VTA have no significant effect on feeding (Badiani & Noel, 1991). VTA injections of U-50,488H have, however, been reported to facilitate feeding induced by electrical stimulation of the lateral hypothalamus (Jenck et al., 1987). These inconsistent findings may be the result of differences between the internal state of the animal or between differences in stimulation-induced feeding and natural feeding. Some researchers have suggested that stimulation-induced feeding shares many of the characteristics of deprivation-induced feeding (Wise, 1974). If this is the case, and if kappa opioids at the level of the VTA are involved in the regulation of feeding, then one would expect U-50,488H to facilitate feeding in food deprived rats.

The present experiment was designed to re-investigate the role of VTA kappa opioids in the regulation of feeding in food deprived rats using a range of doses of the selective kappa opiate, U-50,488H. If the facilitation of feeding that occurs following VTA

administration of dynorphin A(-17) or VTA morphine is mediated by kappa receptors, then injections of U-50,488H into the VTA should facilitate feeding.

Method

Subjects and Surgery

Subjects were eight naive adult male Long-Evans Old Colony rats individually housed and weighing between 330 and 430 grams at time of surgery. Each animal was implanted with a unilateral stainless steel cannula aimed at the ventral tegmental area. Surgery and co-ordinates were identical to those in experiment 1.

Apparatus and Procedure

Same as those described in experiment 1.

Drug and injection procedure

Each rat received each dose (0.1,1.0 and 10.0 nm) of the selective kappa opiate agonist U-50,488H (trans-3,4-dichloro-N-methyl-N[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide; Upjohn Company, Kalamazoo, MI, USA). Drug was dissolved in isotonic saline. The order of drug presentation was counterbalanced across all rats. Drug was administered on alternate days and saline was administered on non-drug days. The injection procedure was identical to that of experiment 1.

Histology and Statistical Analyses

Identical procedures to those used in experiment 1.

Results

Injections of the selective kappa opiate U-50,488H into the VTA did not significantly alter the duration scores for feeding regardless of the dose administered ($F(3,21)=0.03$, $p<0.9940$, Fig 10). Although not significant, U-50,488H tended to increase the frequency of long scores (those over 28 seconds) as well as increasing the frequency of short scores (scores under 12 seconds). This resulted in a flattening in the entire distribution of duration scores (Fig 11).

There was no significant effect ($F(3,21)=0.41$. $p<0.7478$, Fig 12) of U-50,488H on the latency to initiate feeding, although it is of interest to note that all doses of U-50,488H increased the frequency of "best" scores and decreased the frequency of "worst" scores (Fig 13).

Animals receiving injections of U-50,488H into the VTA appeared to spend more time resting and less time exploring and rearing between presentations of meals. This was particularly evident when the largest dose of U-50,488H was administered. The increase in resting, however, was not reflected in any measure of feeding. The distribution of latency scores clearly indicates that the initiation of eating was not affected by the increase in time spent resting.

Histological reconstruction of injector tips in the VTA are illustrated in figure 14.

Discussion

Injections of U-50,488H into the VTA of food deprived rats failed to significantly alter either the latency to initiate feeding or the duration of time required to complete meal segments. These results agree with those of Badiani & Noel (1991). These investigators found that U-50,488H has no effect on feeding when injected into the VTA of sated rats. These results suggest that VTA dynorphin A's$_{(1-17)}$ facilitation of feeding is mediated by non-kappa mechanisms. They also suggest that the facilitation of feeding observed following VTA morphine is not mediated by kappa receptors. Lastly, on the basis of the present results, the facilitation of feeding by systemic U-50,488H appears not to be mediated by U-50,488H's actions at the VTA.

Although the present results and those of Badiani & Noel (1991) suggest that the VTA is not the site of action for systemically administered U-50,488H this does not eliminate the possibility that systemic U-50,488H is acting centrally to produce its facilitation of feeding. Indeed, intraventricular administration of nor-binaltorphimine, a highly selective kappa antagonist (Portoghese, Lipokowski & Takemori,1987), inhibits feeding (Carr et al., 1989; Arjune & Bodnar, 1990; Calcagnetti, Calcagnetti & Faneslow, 1990) and blocks the facilitation of feeding by systemic U-50,488H (Levine et al., 1990). These results suggest that central kappa opioids have a role in the regulation of feeding and that systemic U-50,488H is having its effect on feeding through a central mode of action.

It has been suggested that opiates have their effect on behavior by interacting with dopaminergic systems. This hypothesis was first put forward following the observation that intra-VTA injections of opiates, most notably morphine, induce behavioral activation in a manner reminiscent of the behavioral activation produced by the dopamine agonist, amphetamine (Joyce & Iversen, 1979; Broekkamp & Phillips 1980). Similarly, naloxone has been found to attenuate feeding in the same manner as the dopamine antagonist, pimozide. Both drugs decrease the motivation to eat without altering the ability to perform the responses necessary for feeding (Jenck et al., 1986a; Wise & Raptis, 1986). This suggests the possibility that opioids and dopamine act on a common mechanism to modulate feeding. Finally, opiates are known to activate dopaminergic neurons. Systemic, intraventricular, and VTA injections of opiates that bind to mu or delta receptors increase dopamine release and metabolism in the nucleus accumbens (Westerink, 1978; Kalivas et al., 1983; Di Chiara & Imperato, 1988; Devine et al., 1991a; 1991b; Leone, Pocock & Wise, 1991; Spanagel, Herz & Shippenberg, 1990; Spanagel et al., 1991). These results suggest that opiates and opiate antagonists have their effect on behavior by modulating mesolimbic dopamine activity.

Kappa opiates are known to modulate mesolimbic dopamine activity. But unlike mu and delta opiates, kappa opiates inhibit dopamine activity. Inhibition of the release and metabolism of dopamine in the nucleus accumbens occurs following systemic, intraventricular and nucleus accumbens injections of selective kappa opiates (Di Chiara & Imperato, 1988; Spanagel, Herz &

Shippenberg, 1990; Devine et al., 1991a; Spanagel et al., 1991). Complementing this is the finding that injections of the kappa antagonist, nor-binaltorphimine, into the ventricles or the nucleus accumbens increase dopamine activity within this brain site (Spanagel, Herz & Shippenberg, 1990; Spanagel, et al.,1991). VTA injections of selective kappa opiates or opiate antagonists have no effect on dopamine release in the nucleus accumbens (Devine et al., 1991a; Spanagel et al.,1991). Thus, while kappa opiates modulate mesolimbic dopamine activity, their effect is opposite to that of mu and delta opiates and the inhibition of mesolimbic dopamine by kappa opiates occurs through actions at the dopamine terminals.

It is possible that the inability of VTA kappa opiates to modulate the activity of the mesolimbic dopamine system results in the inability of VTA U-50,488H to modulate feeding. However, if a modulation of mesolimbic dopamine activity is necessary for changes in feeding to be observed, then injections of U-50,488H to the nucleus accumbens should inhibit feeding. Majeed et al., (1986) have demonstrated that nucleus accumbens injections of U-50,488H fail to alter feeding in food deprived rats. In addition, if mesolimbic dopamine activity was of primary importance, then one might expect systemic administration of U-50,488H to inhibit feeding. In fact systemic U-50,488H facilitates feeding. Thus, despite an inhibitory effect of kappa opiates on mesolimbic dopamine, systemic administration of kappa opiates facilitates feeding. These results suggest that kappa regulation of feeding is mediated by non-dopaminergic mechanisms.

The failure of VTA U-50,488H to modulate feeding is in contrast to results obtained by Jenck et al., (1986). These investigators have reported that VTA injections of U-50,488H facilitate stimulation-induced feeding of the lateral hypothalamus. If, as it has been suggested by some researchers (Wise, 1974), stimulation-induced feeding is comparable to deprivation-induced feeding, then one would expect that manipulations that affect stimulation-induced feeding would have similar effects on deprivation-induced feeding. A common mechanism for both feeding behaviors is supported by the observation that intraventricular administration of nor-binaltorphimine inhibits both stimulation-induced feeding (Carr et al.,1989) and feeding of preferred restricted substances (Arjune & Bodnar, 1990; Calcagnetti, Calcagnetti & Fanselow, 1990). However, it is possible that, at the level of the VTA, stimulation-induced feeding involves the recruitment of mechanisms not involved in deprivation-induced feeding. The mechanisms involved in stimulation-induced feeding are most probably non-dopaminergic. A more thorough investigation comparing stimulation-induced and normal feeding is required in order to better understand the mechanisms that underlie these types of feeding.

To summarize, VTA injections of U-50,488H have no effect on feeding in food deprived or sated rats. This may be due to the inability of VTA injections of U-50 to activate the mesolimbic dopamine system. However, it is possible that kappa regulation of feeding is mediated by non-dopaminergic mechanisms. Lastly, differences between stimulation-induced and deprivation-induced

feeding may reflect a difference in the underlying mechanisms that mediate these behaviors.

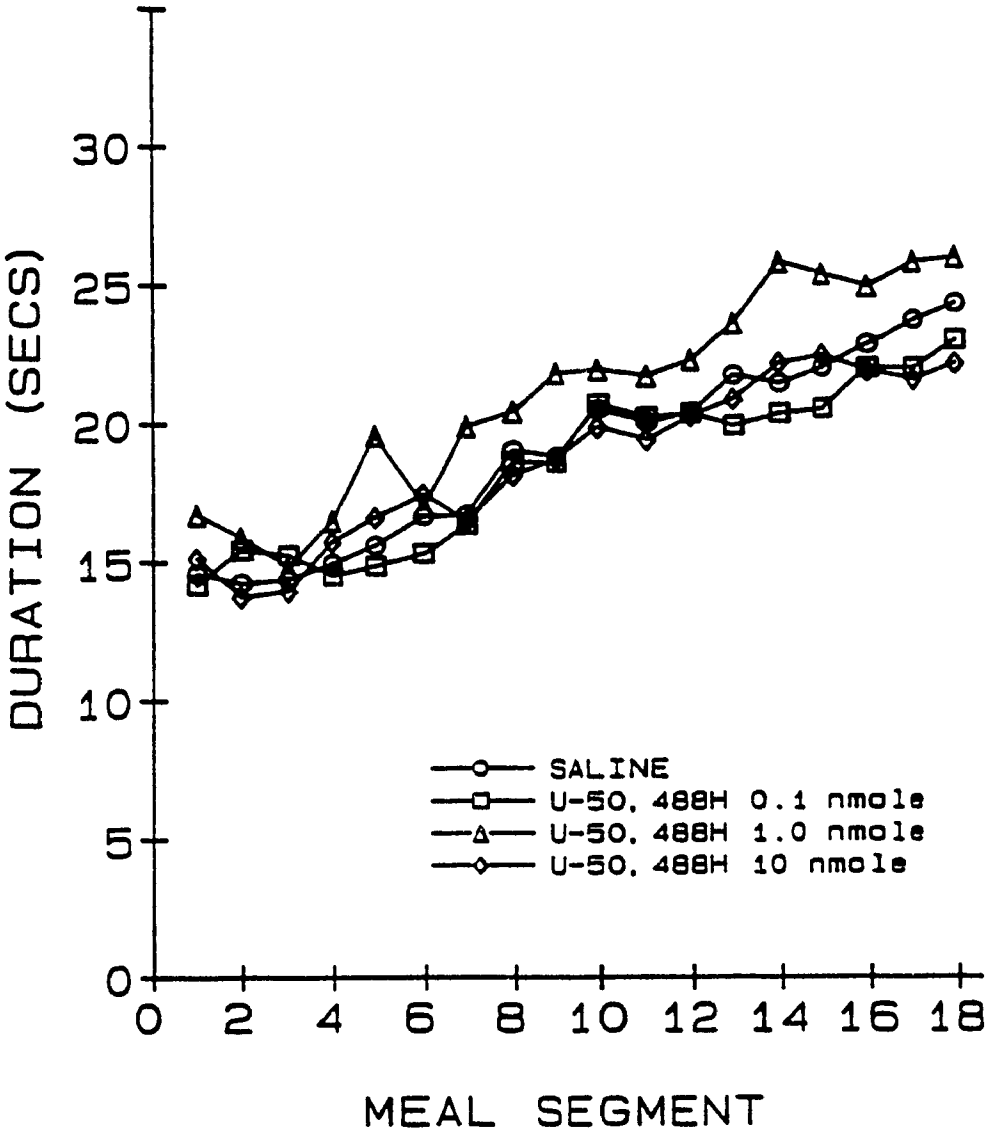

Figure 10. The mean time required to complete meal segments following injections of saline, 0.1, 1.0, or 10 nmole of U-50,488H into the ventral tegmental area (n=8).

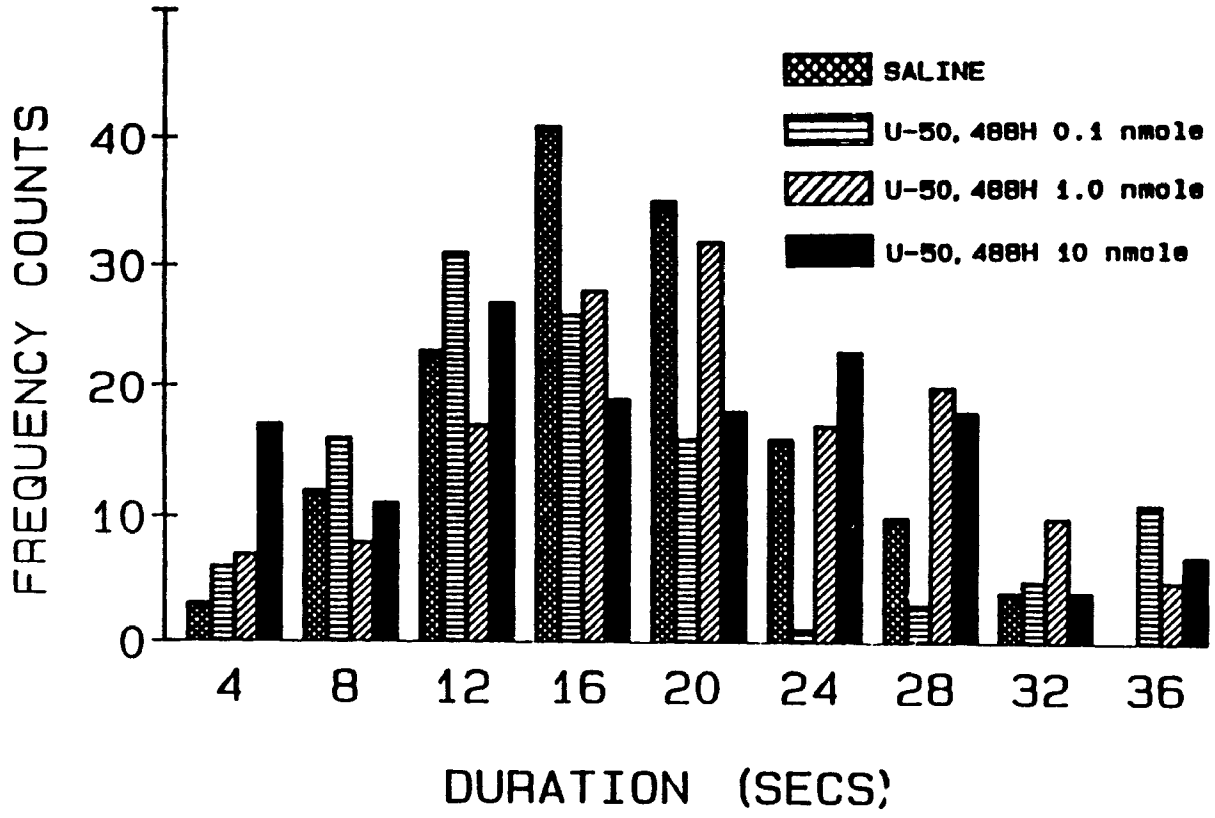

Figure 11. Distribution of duration scores following injections of saline, 0.1, 1.0 or 10 nmole of U-50,488H into the ventral tegmental area (n=7). Response bins comprise 4 second intervals.

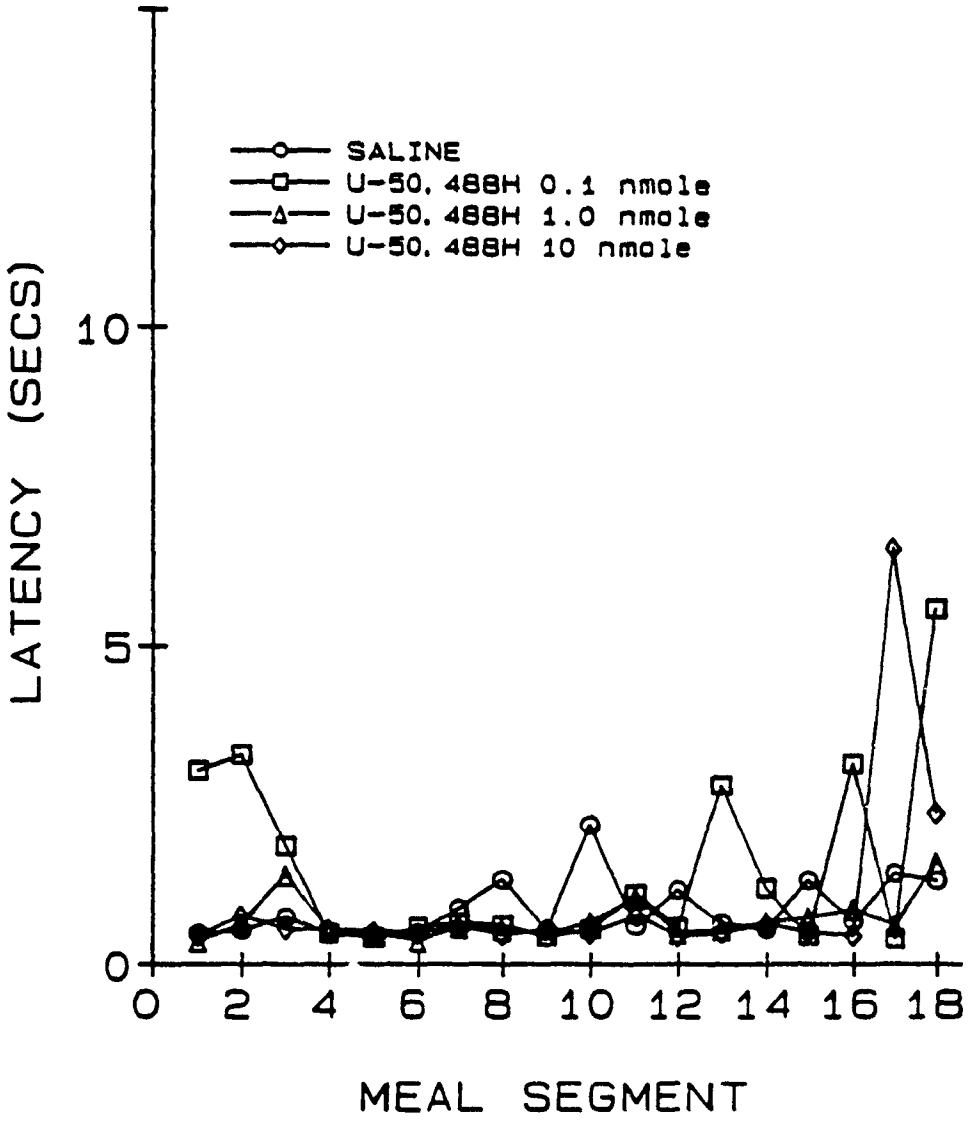

Figure 12. The mean latency to initiate feeding for each meal segment following injections of saline, 0.1, 1.0, or 10 nmole of U-50,488H into the ventral tegmental area (n=8).

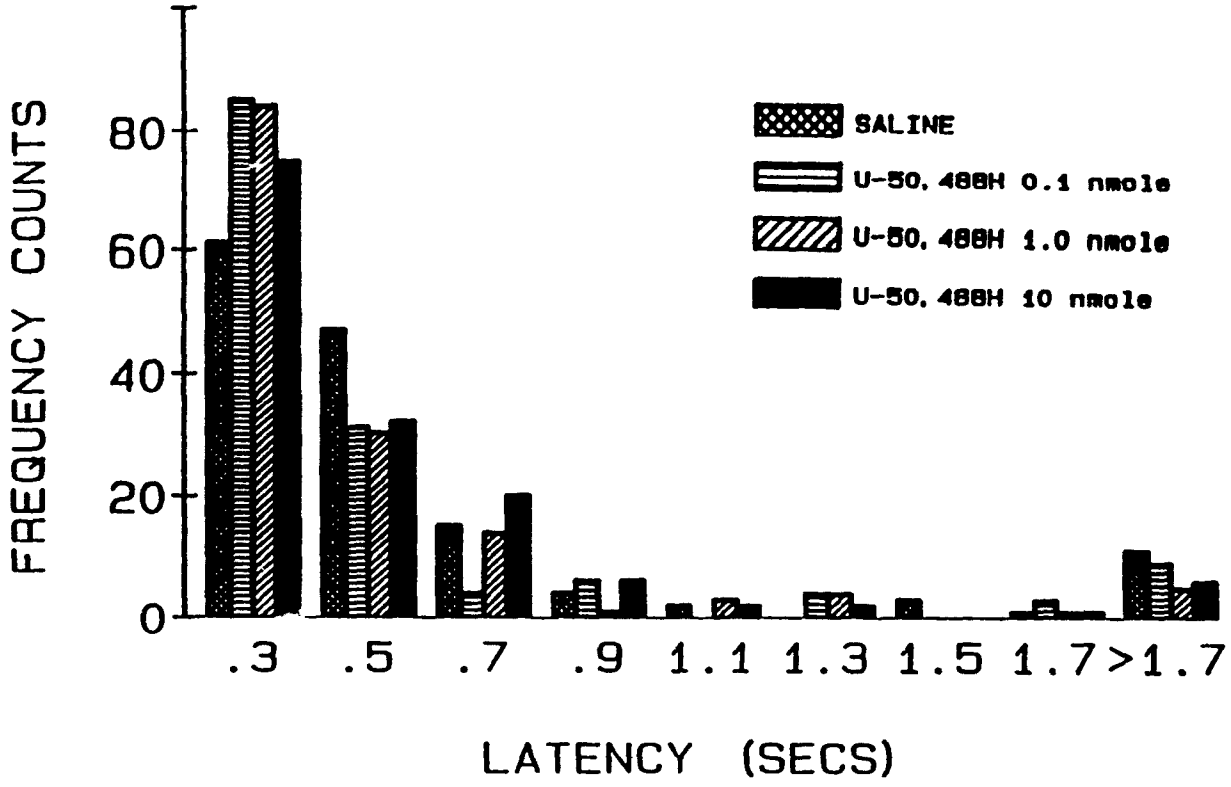

Figure 13. Distribution of latency scores following injections of saline, 0.1, 1.0 or 10 nmole of U-50,488H into the ventral tegmental area (n=7). Response bins comprise 0.2 second intervals.

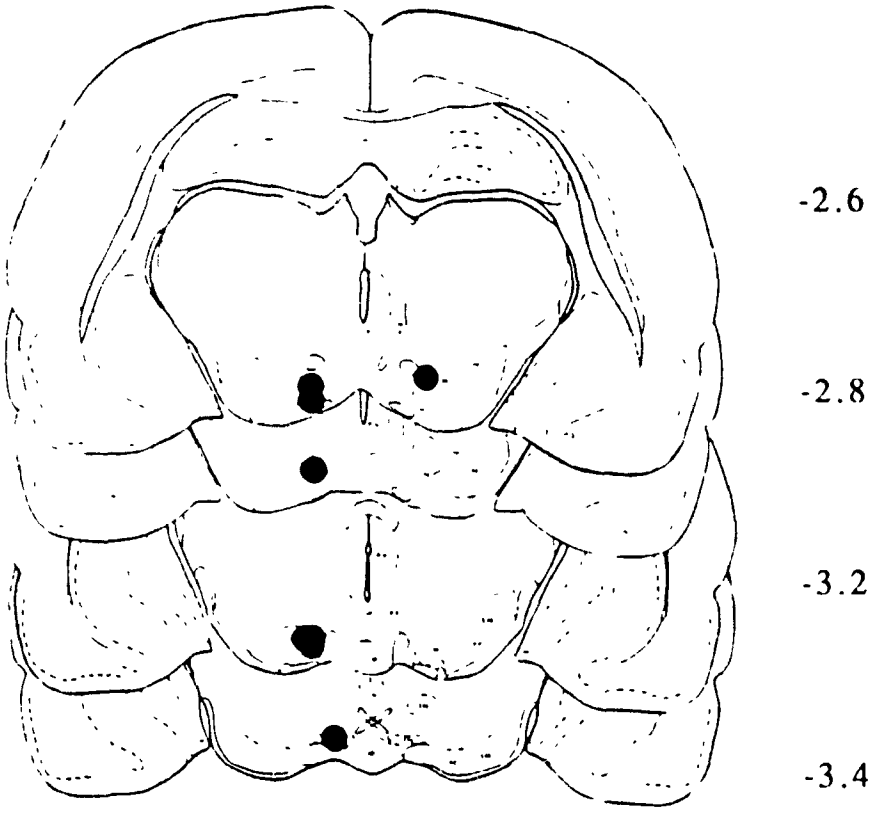

Figure 14. Histological placements for animals with injector tips located in the ventral tegmental area (circles, n=8). Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice refers to the distance (in millimeters) posterior to bregma.

Experiment 3

The synthetic enkephalin, D-Ala^2,N-Me-Phe^4-Gly^5-Ol-Enkephalin (DAGO) is the most highly selective agonist for the mu opioid receptor available. It has been demonstrated to have an affinity that is 10,000 greater for the mu than for the delta receptor with minimal binding at the kappa receptor (Handa et al., 1981; Lutz et al., 1985; Goldstein & Naidu, 1989). This drug is the opiate of choice in determining the role of VTA mu receptors in the modulation of feeding behavior.

Method

Subjects and Surgery

Subjects were sixteen naive adult male Long-Evans Old Colony rats individually housed and weighing between 370 and 430 grams at time of surgery. Ten animals were implanted with a unilateral stainless steel cannula aimed at the ventral tegmental area. The remaining animals were implanted with cannula aimed dorsal to the ventral tegmental area. Surgery and co-ordinates were identical to those in Experiment 1.

Apparatus and Procedure

Same as those used for Experiment 1.

Drug and injection procedure

Each animal received each dose (0.01, 0.1, 1.0 and 10.0 nmole) of the selective mu agonist D-Ala^2,N-Me-Phe^4-Gly^5-Ol-Enkephalin (DAGO). The drug was dissolved in isotonic saline. The order of drug presentation was counterbalanced across all rats. Drug was administered on alternate days and saline was administered on non-drug days. The injection procedure was identical to that of Experiment 1.

After all doses of drug had been tested, some of the animals that received injections of DAGO into the VTA received additional testing. This consisted of intraperitoneal naloxone administered in conjunction with intracranial injections of 1.0 nmole of DAGO or intraperitoneal saline in conjunction with intracranial saline. Naloxone was dissolved in isotonic saline and administered at a dose of 2 mg/kg. Animals received intraperitoneal injections ten minutes prior to intracranial injections. Testing procedure was identical to that previously described.

Histology and Statistical Analyses

The histological procedure and statistical analyses were identical to those described in Experiment 1.

Results

VTA injections of DAGO decreased the duration of time required to complete meal segments [$F(4,36)=17.17$, $p<0.00002$]. The three largest doses of DAGO were significantly different from both

saline and the smallest dose of DAGO ($p< 0.001$). The maximum effect on duration was obtained with a dose of 0.1 nmole of DAGO. Increasing the dose of DAGO to 1.0 or 10.0 nmole did not result in any further reduction in duration scores (Fig. 15). The three largest doses of DAGO produced a significant leftward shift in the mean distribution of duration scores relative to the distribution of saline duration scores (F test $p<0.05$). The number of 'best' scores was dramatically increased in the 0.1, 1.0 and 10 nmole of DAGO conditions (Fig. 16). Similar distributions of duration scores were obtained following injections of 0.01 nmole of DAGO or saline into the VTA.

DAGO into the VTA increased the latency to initiate feeding [$F(4,36)=5.44$, $p<0.0016$]. This increase was due to the largest dose (10 nmole) of DAGO which was the only dose of DAGO that was significantly different from saline ($p<0.05$; Fig. 17). More 'worst' latency scores were obtained following 10 nmole of DAGO than were obtained with saline or any other dose of DAGO. There was a tendancy for all doses of DAGO to produce more 'best' latency scores and thus shift the mean distribution of latency scores to the left, however, this failed to reach significance (Fig. 18).

Injections of DAGO into sites dorsal to the VTA also significantly decreased the duration of time required to complete meal segments [$F(4,20)=5.91$, $p<0.0026$]. However, unlike VTA DAGO, only the 1.0 and 10 nmole doses of dorsal DAGO had this effect ($p<0.05$; Fig. 19). The decrease in duration that occurred following 1.0 or 10 nmole of DAGO did not occur immediately, but rather, only became apparent on the fourth meal segment. The magnitude of the

decrease in duration scores obtained following dorsal injections of DAGO was not as large as the decreases obtained following VTA injections of DAGO. Although not significant, injections of DAGO dorsal to the VTA had the effect of shifting the mean distribution of duration scores to the left (Fig. 20). More scores in the 4, 8 and 12 second categories were obtained with dorsal injections of DAGO than were obtained when saline was administered.

There was no significant effect of dorsal injections of DAGO on the latency to initiate feeding [$F(4,20)=1.55$, $p<0.2252$; Fig. 21]. The distribution of latency scores was similar for saline and all doses of dorsal DAGO, although there was a tendancy for all doses of dorsal DAGO to produce more scores in the 'best' category than occurred following saline injections (Fig. 22) This trend failed to reach significance, however.

Pretreating animals with the opiate antagonist naloxone (2 mg/kg) was effective in blocking the decrease in duration produced by 1.0 nmole of VTA DAGO. Naloxone in conjunction with DAGO was no different than intraperitoneal injections of saline in conjunction with VTA injections of saline (Fig. 23).

Observations of freezing or startle responses were seen more often when animals received VTA injections of 1.0 or 10 nmole of DAGO than when they were administered saline, 0.01 or 0.1 nmole of DAGO. Circling behavior was also evident when animals received 1.0 or 10 nmole of DAGO into the VTA. Once an animal had initiated feeding, circling behavior did not appear to interfere with the feeding behavior. Freezing or startle responses were rarely observed in animals administered DAGO into sites dorsal to the VTA,

and when seen they occurred equally in the drug and saline conditions. Instances where animals did not finish their food pellets were rare in either the dorsal or VTA group of rats and were usually a result of feeding being interrupted by grooming or exploratory behavior. This happened equally under saline and drug conditions.

The location of injector tips aimed at and dorsal to the VTA are illustrated in figure 24.

Discussion

Injections of DAGO into the VTA significantly enhance feeding in food deprived rats. DAGO is most effective when injected directly into the VTA. While injections of DAGO into sites dorsal to the VTA clearly have an effect on feeding, a substantially larger dose of drug is required to facilitate feeding than is needed when DAGO is injected directly into the VTA. In contrast to VTA injections of DAGO, the facilitation of feeding following injections of DAGO dorsal to the VTA does not occur immediately. The facilitation of feeding is apparent only after the fourth or fifth meal segment. In addition, the magnitude of the effect is greater following VTA DAGO than it is following injections of DAGO into sites dorsal to the VTA. The short latency and the small dose of drug that is needed to obtain a facilitation of feeding following injections of DAGO into the VTA suggest that the VTA, and not sites adjacent to the VTA, is the primary site for the modulation of feeding by mu opiates.

DAGO is more potent in accelerating the speed of eating in food deprived rats than is morphine. VTA DAGO is at least 100 fold more potent than VTA morphine. The most effective dose of DAGO is 0.01 nmole whereas a dose of 10 nmole of morphine is needed to facilitate feeding. It was found that increasing the dose of DAGO from 0.1 to 1.0 or 10 nmole did not produce any further facilitation of feeding. This suggests that a dose of at least 0.1 nmole of DAGO is necessary in order to facilitate feeding at the level of the VTA. At this dose of DAGO all of the mu opioid receptors at the level fo the VTA may be maximally occupied. The degree of facilitation of feeding was greater following VTA DAGO than following VTA morphine. This suggests that the facilitation of feeding by VTA morphine may be mediated by mu opioid receptors.

The facilitation of feeding produced by 1.0 nmole of VTA DAGO can be attenuated if animals are pretreated with 2 mg/kg of the opiate antagonist, naloxone. This confirms that the effects of VTA DAGO are a result of DAGO's actions at opioid receptors rather than a result of DAGO's physico-chemical properties.

VTA DAGO facilitates feeding by increasing the motivation to eat. An increase in motivation is inferred from the increase in the consistency of performance; more 'best' duration scores where obtained following VTA DAGO than following saline. The three highest doses of VTA DAGO decreased the mean duration by significantly decreasing the variance of duration scores and increasing the consistency of an animal's performance. Thus, it appears that VTA DAGO facilitates feeding by enhancing the ability of food to sustain an animal's interest.

DAGO has no effect on an animal's ability to perform. The lower limits or 'best' scores for either the latency or the duration were the same in drug and saline conditions. This illustrates that animals were capable of performing equally well in the drug and saline conditions. The upper limit for latency scores was longer following injections of 1.0 or 10 nmole of VTA DAGO than following saline. These doses of DAGO produced more 'worst' latency scores than was observed in any other condition. This probably reflects the increase in behaviors such as exploration and circling that were observed following administration of 1.0 or 10 nm of DAGO into the VTA. It is possible that large doses of VTA DAGO make animals more easily distracted and consequently decrease an animal's focus on food. However, while the largest doses of DAGO tended to produce longer latency scores they did not increase the time animals required to complete meal segments. Once an animal had initiated feeding the animal continued to focus on the food until that meal segment had been completed. Thus, long latency scores reflect conflicting behaviors or attentional distraction rather than motoric inability.

Several lines of evidence suggest that VTA opiates have their effect on behavior by interacting with mesolimbic dopamine neurons. First, injections of mu opiates into the VTA result in an increase in spontaneous locomotion (Kalivas et al., 1983; Latimer, Duffy & Kalivas, 1987; Vezina, Kalivas & Stewart, 1987). This effect can be blocked if the mesolimbic dopaminergic system is damaged or if dopamine antagonists are injected into the nucleus accumbens (Kalivas et al., 1983). This suggests that the behavioral activation

induced by VTA mu opiates is dependent on mesolimbic dopamine activity. Second, injections of mu opiates into the VTA increase dopamine release and metabolism in the nucleus accumbens (Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a; 1991b; Spanagel et al., 1991). Complementing this is the observation that intra-VTA mu opiate antagonists produce a decrease in nucleus accumbens dopamine release and metabolism (Spanagel et al., 1991). These results suggest that VTA mu opiates modulate the activity of mesolimbic dopamine. Third, the dose of VTA DAGO that is needed to obtain a facilitation of feeding is the same as that needed to obtain an increase in dopamine release in the nucleus accumbens (0.1 nmole for the former and 0.132 nmole for the latter; Devine et al., 1991a). This suggests that DAGO's facilitation of feeding and DAGO-induced dopamine release are related events. Lastly, the increase in feeding and nucleus accumbens dopamine release induced by VTA mu opiates are attenuated by systemic injections of opiate antagonists (Latimer, Duffy & Kalivas, 1987). This suggests that VTA opiate-induced feeding is dependent on opiate-induced dopamine release in the nucleus accumbens.

Evidence from behavioral studies suggests that the effects of mu opiates can occur independent of normal dopamine functioning. The site of administration of mu opiates is important in this regard. Injections of mu opiates into either the VTA or the nucleus accumbens result in an increase in spontaneous locomotor activity (Pert & Sivit, 1977; Kalivas et al., 1983; Vezina, Kalivas & Stewart, 1987). However, VTA opiate-induced locomotion but not nucleus accumbens opiate-induced locomotion is attenuated following

damage to the mesolimbic dopamine system or by the administration of dopamine antagonists into the nucleus accumbens. This suggests that the increase in behavioral activation resulting from VTA injections of mu opiates is dependent on dopamine, whereas the effect of nucleus accumbens injections of mu opiates are dopamine independent (Kalivas et al., 1983). Neurochemical studies support the lack of effect of nucleus accumbens injections of mu opiates on mesolimbic dopamine activity. Injections of DAGO or the mu antagonist, CTOP, have no effect on nucleus accumbens dopamine release or metabolism when injected into this site (Spanagel et al., 1991). This suggests that an interaction between dopamine and mu opiates occurs at the cell bodies and not at terminals.

Like opiate-induced locomotion, feeding can be facilitated following injections of mu opiates into either the VTA or the nucleus accumbens (Majeed et al., 1986; Mucha & Iversen, 1986). It is possible that opiate-induced feeding may also occur independently as well as dependently on mesolimbic dopamine. If the facilitation of feeding by VTA DAGO is dopamine dependent then one should be able to block this facilitation either by damaging the mesolimbic dopamine system or by pretreating animals with dopamine antagonists injected into the nucleus accumbens. If the facilitation of feeding by injections of mu opiates into the nucleus accumbens is dopamine independent, then manipulations of the mesolimbic dopamine system should have no effect on the facilitation of feeding by injections of mu opiates into the nucleus accumbens. These hypotheses remain to be tested.

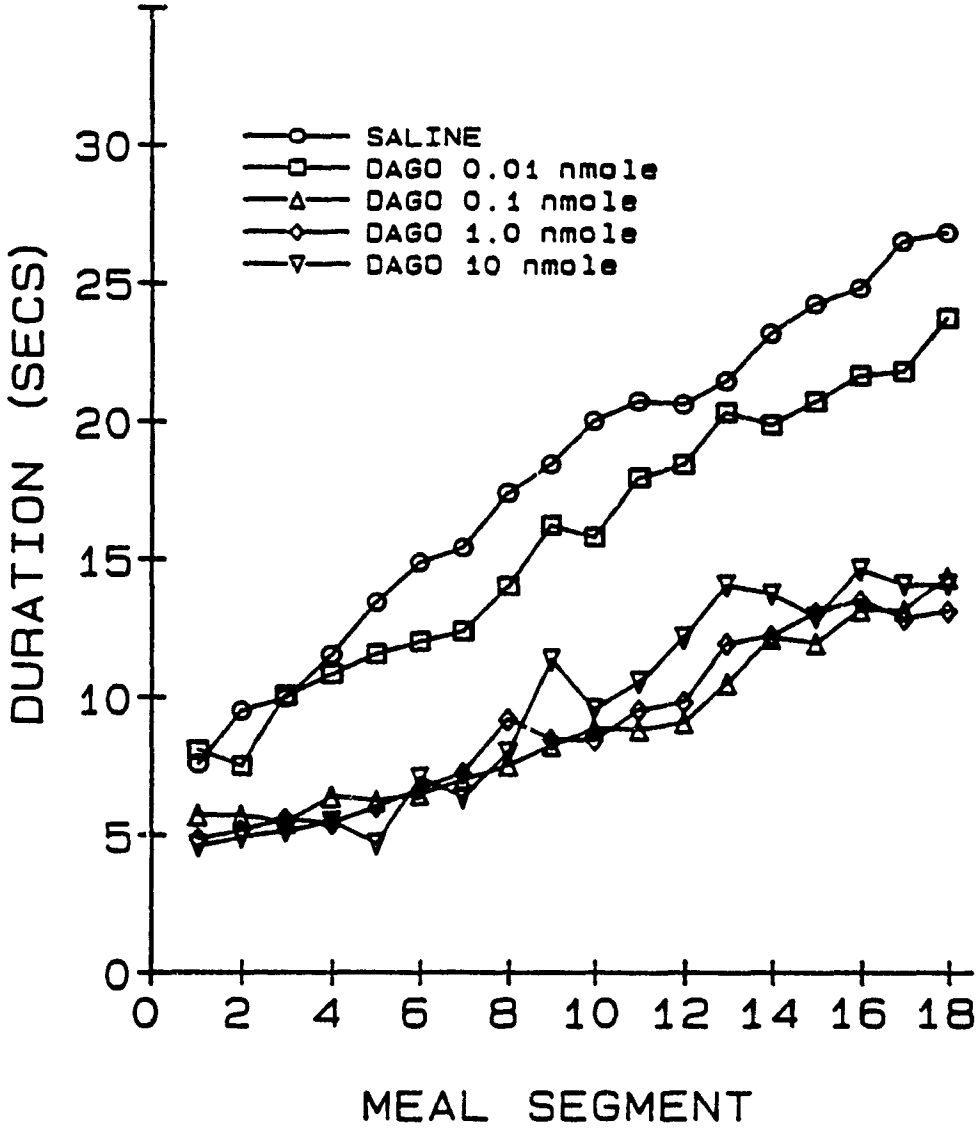

Figure 15. The mean time required to complete meal segments following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into the ventral tegmental area (n=10).

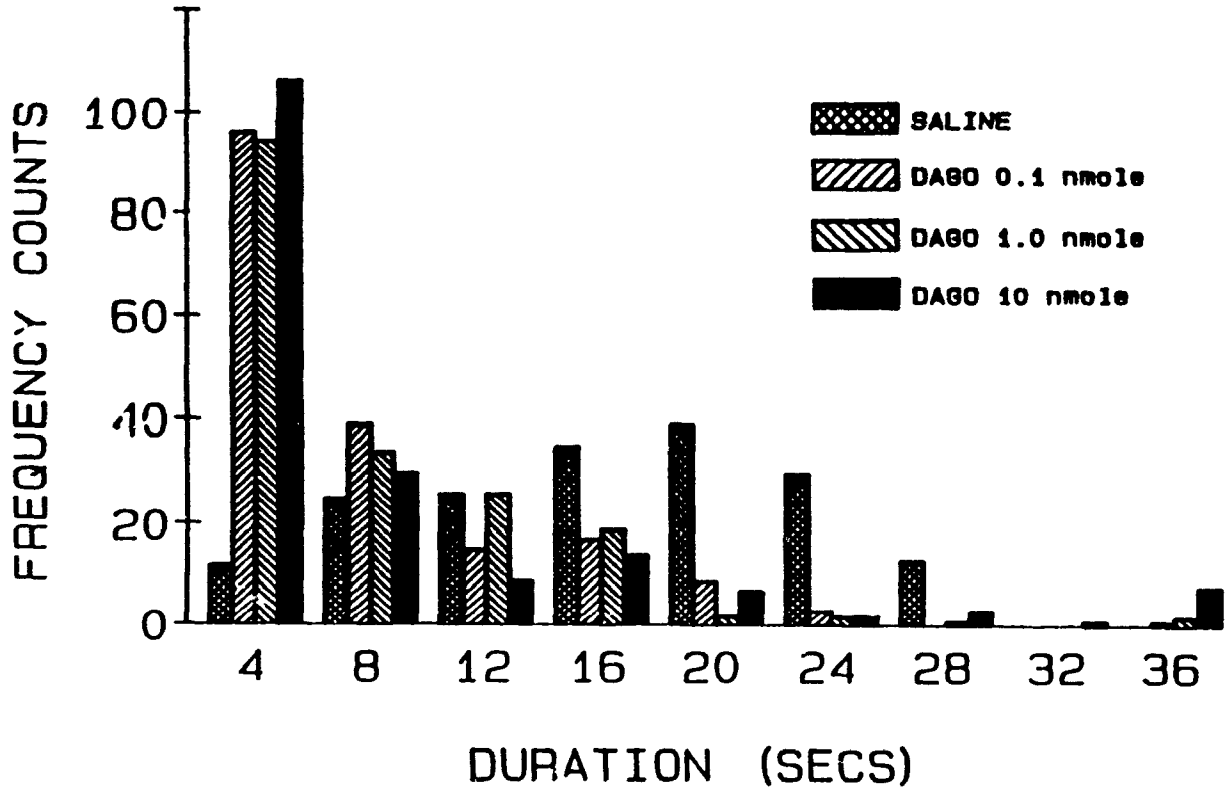

Figure 16. Distribution of duration scores following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into the ventral tegmental area (n=10). Response bins comprise 4 second intervals.

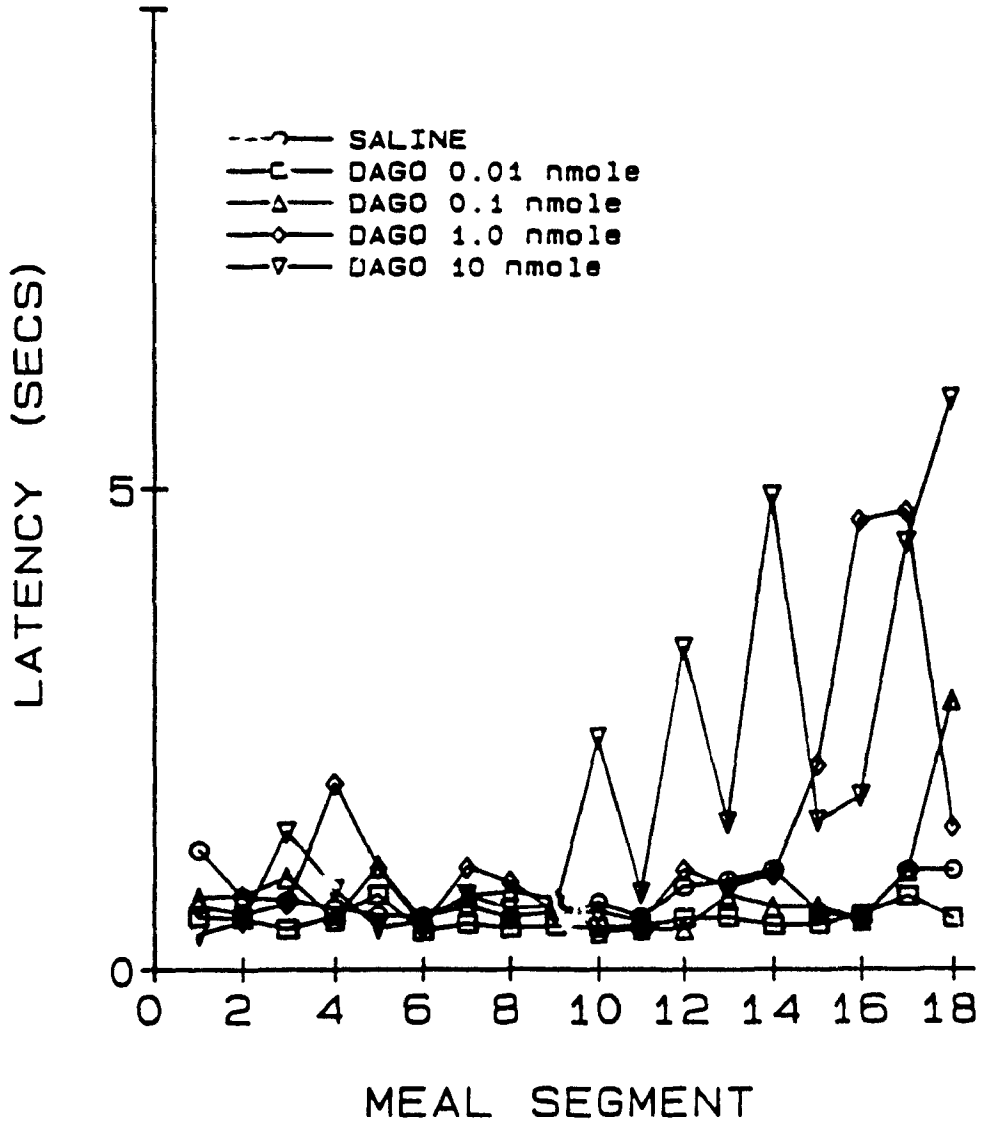

Figure 17. The mean latency to initiate feeding for each meal segment following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into the ventral tegmental area (n=10).

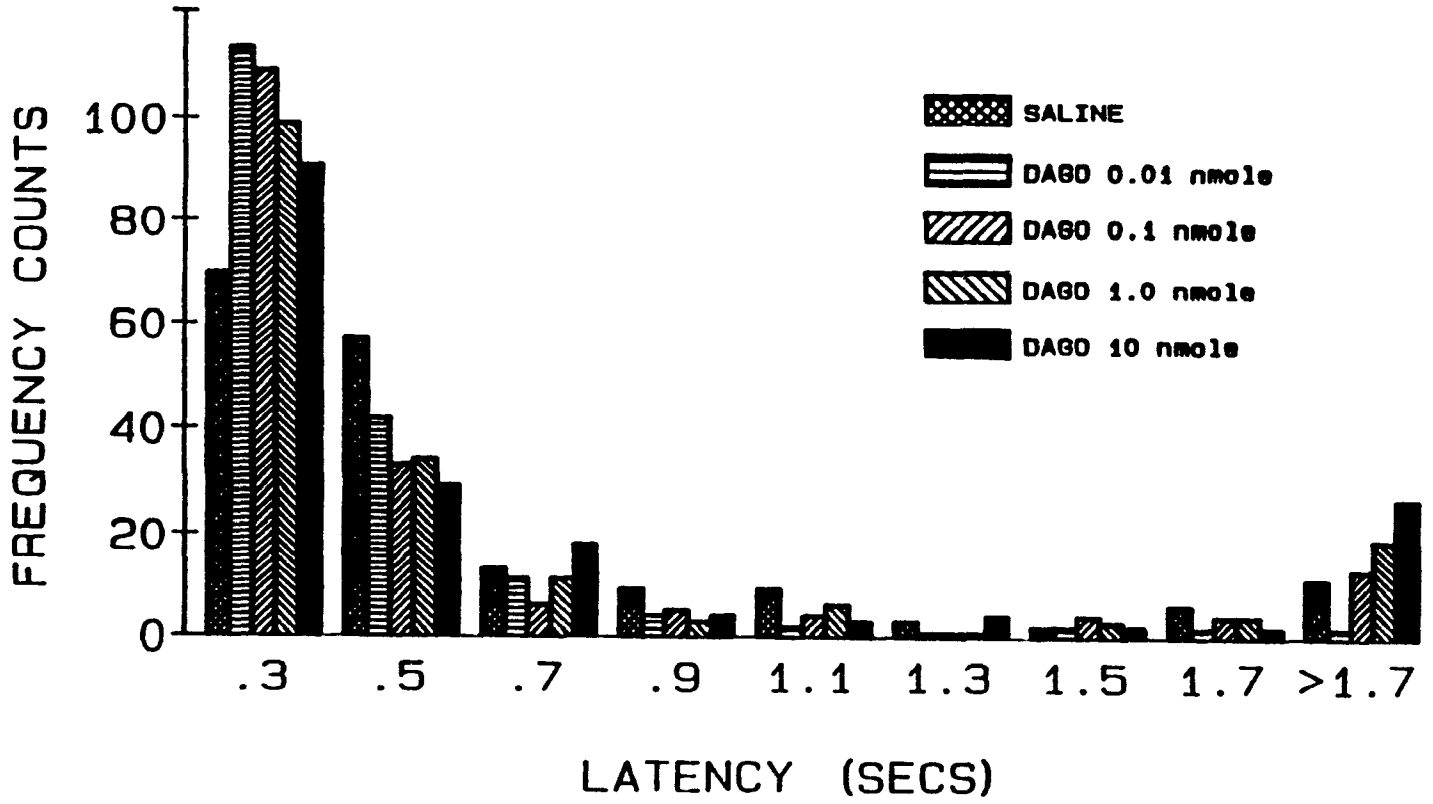

Figure 18. Distribution of latency scores following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into the ventral tegmental area (n=10). Response bins comprise 0.2 second intervals.

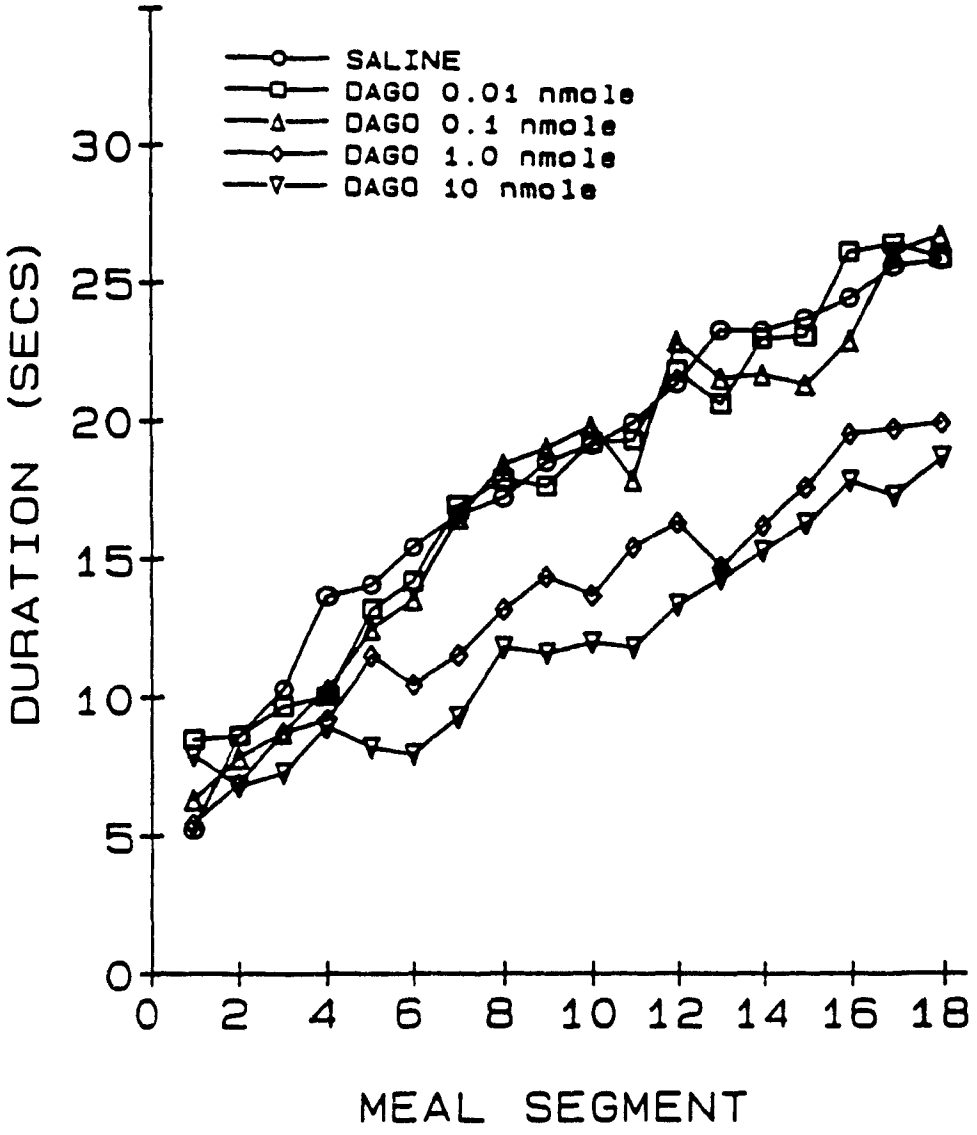

Figure 19. The mean time required to complete meal segments following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into sites dorsal to the ventral tegmental area (n=6).

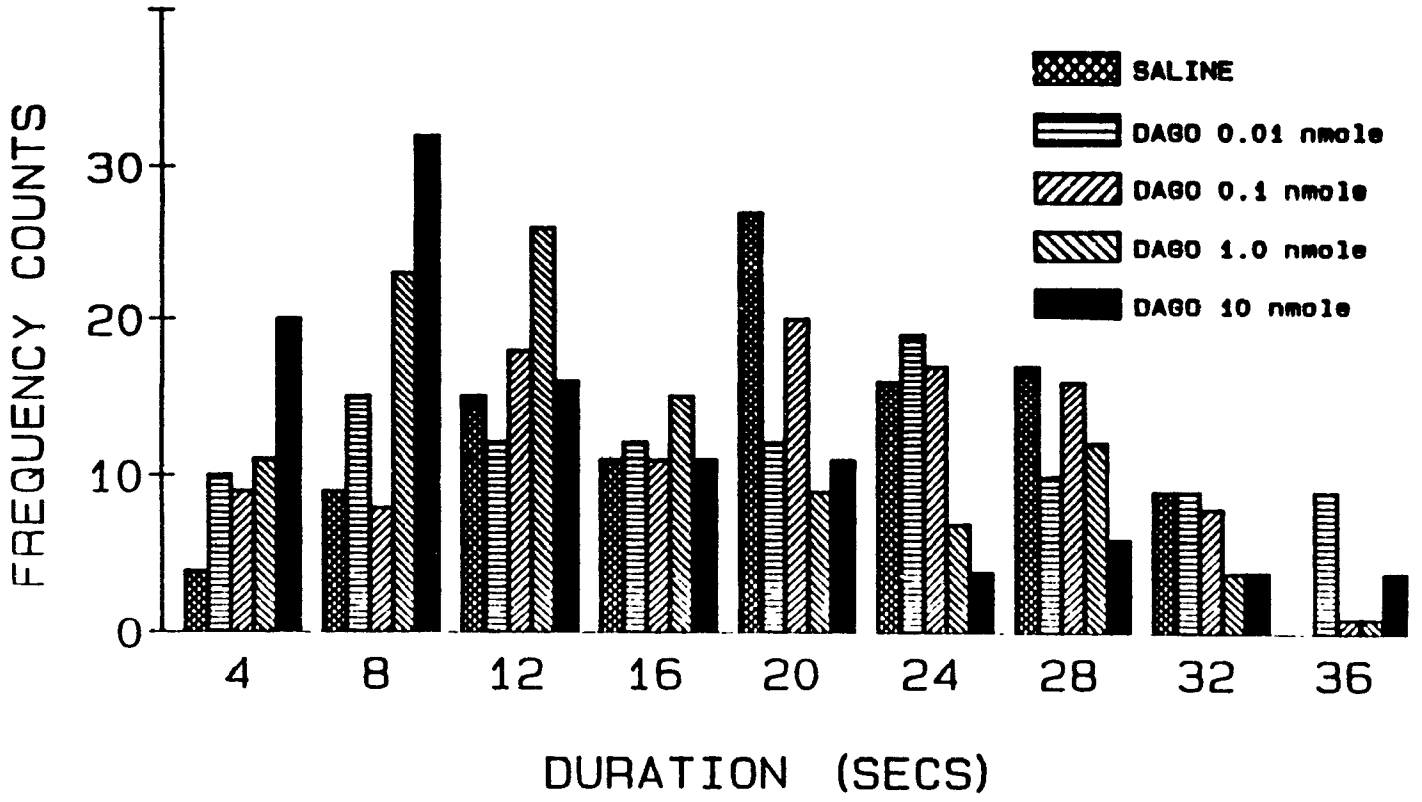

Figure 20. Distribution of duration scores following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into sites dorsal to the ventral tegmental area (n=6). Response bins comprise 4 second intervals.

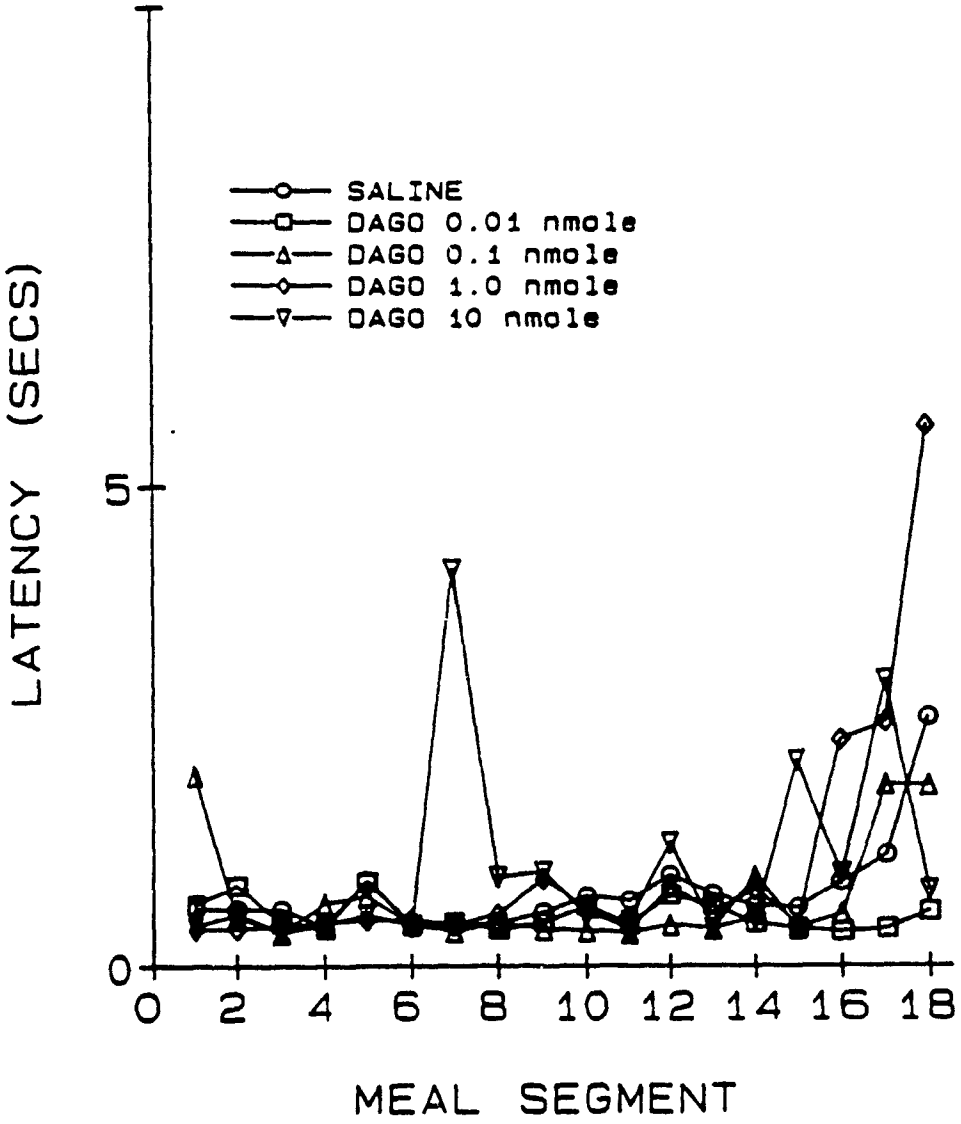

Figure 21. The mean latency to initiate feeding for each meal segment following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into sites dorsal to the ventral tegmental area (n=6).

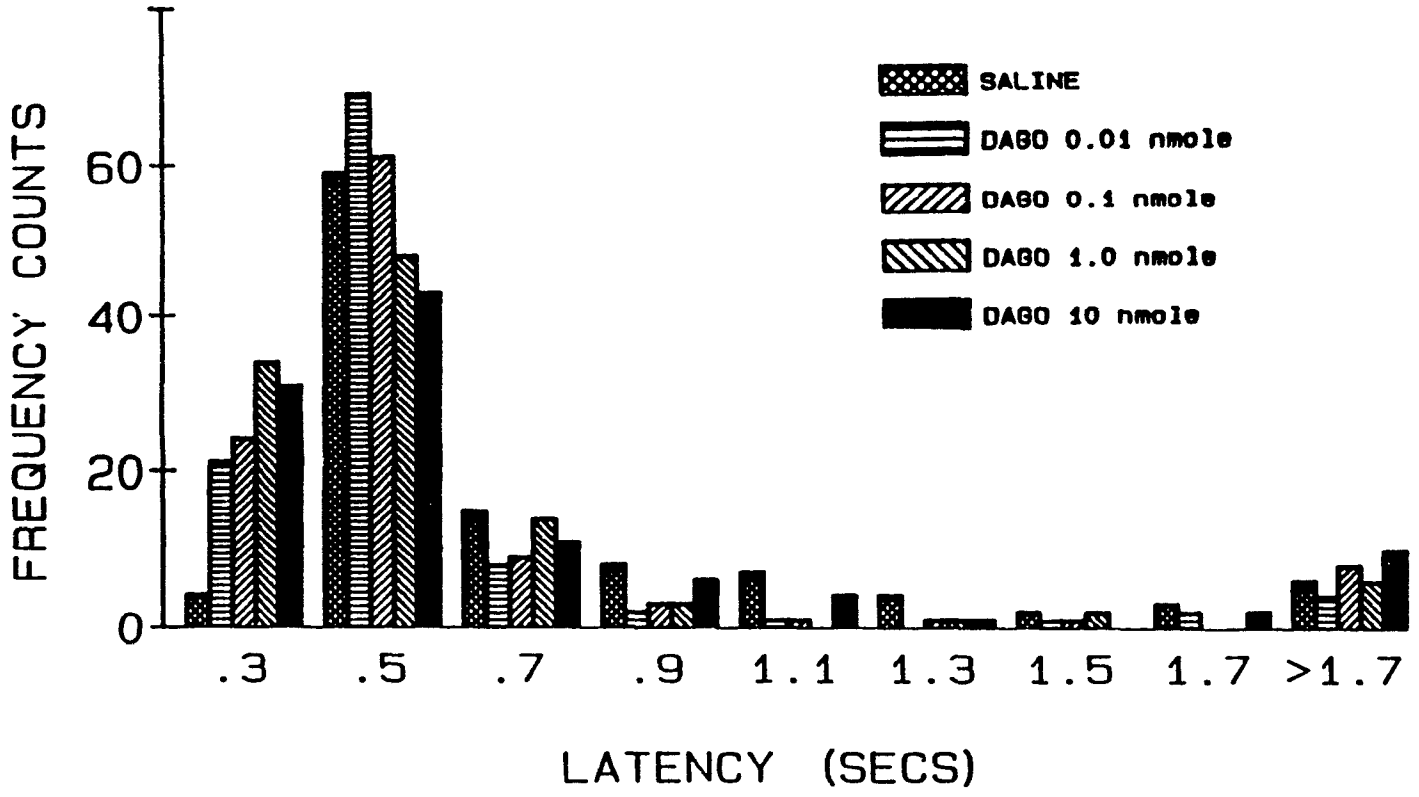

Figure 22. Distribution of latency scores following injections of saline, 0.01, 0.1, 1.0, or 10 nmole of DAGO into sites dorsal to the ventral tegmental area (n=6). Response bins comprise 0.2 second intervals.

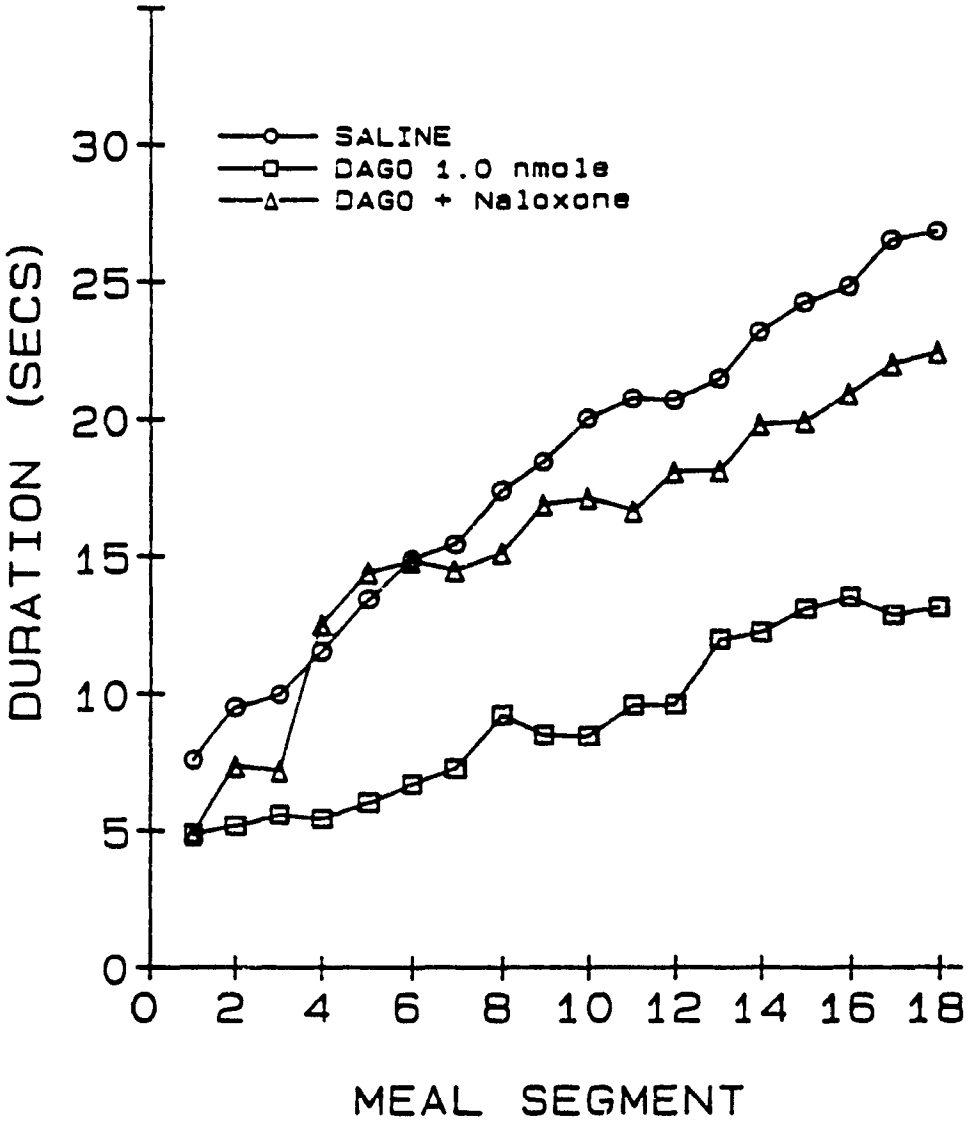

Figure 23. The mean time required to complete meal segments following injections of i.p. saline and VTA saline, 1.0 nmole of VTA DAGO, or 1.0 nmole of VTA DAGO and 2 mg/kg of naloxone (n=6).

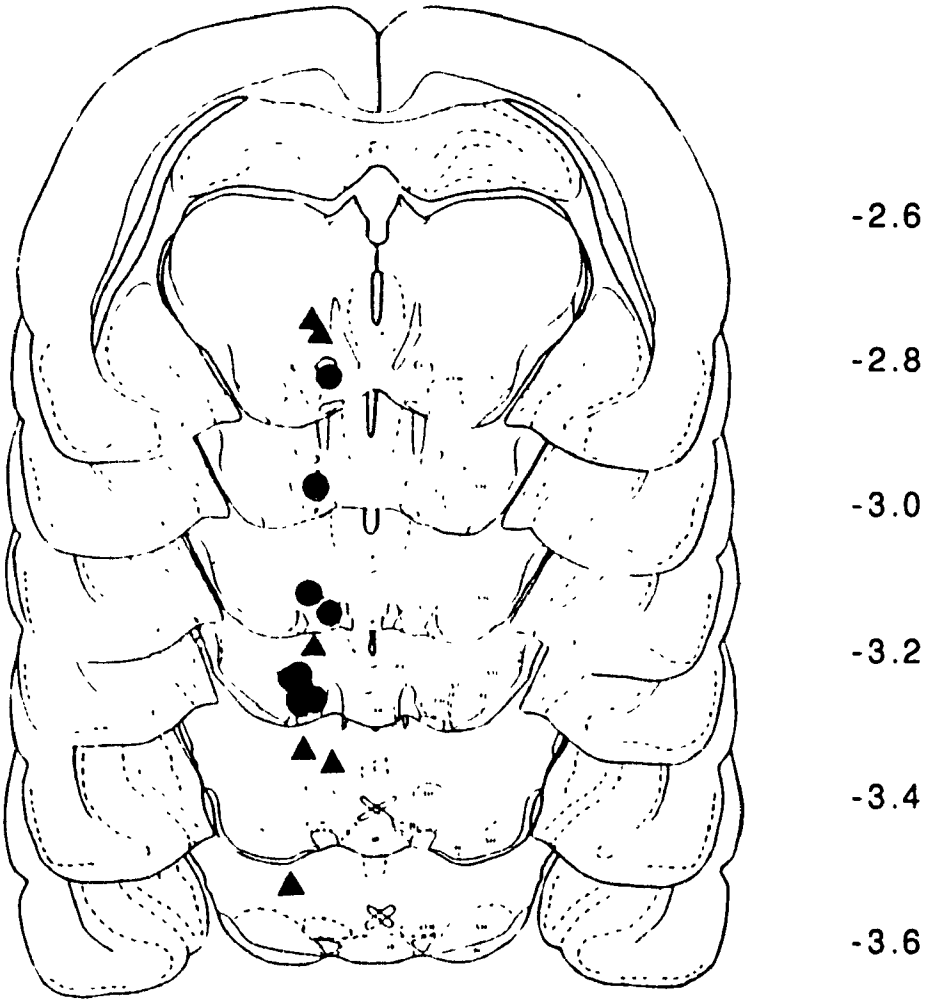

Figure 24. Histological placements for animals with injector tips located dorsal to the ventral tegmental area (triangles, n=6) and for animals with injector tips located in the ventral tegmental area (circles, n=10). Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice refers to the distance (in millimeters) posterior to bregma.

Experiment 4

The synthetic enkephalin D-Pen^2, D-Pen^5 (DPDPE) is the most selective and readily available delta receptor agonist to date. In the mouse vas deferens bioassay DPDPE has been estimated to have approximately 3000 greater times affinity for the delta than for the mu opioid receptor (Mosberg et al., 1983a; 1983b; Corbett et al., 1984; Cotton et al., 1985; Clark et al., 1986; Goldstein & Naidu, 1989). The high selectivity of DPDPE for the delta receptor makes this drug the drug of choice in the determination of the role that the delta receptor plays in the regulation of feeding behavior.

Method

Subjects and Surgery

Subjects were eleven naive adult male Long-Evans Old Colony rats individually housed and weighing between 340 and 380 grams at time of surgery. Six animals were implanted with unilateral stainless steel cannula aimed at the ventral tegmental area. The remaining animals were implanted with cannula aimed dorsal to the ventral tegmental area. Surgery and co-ordinates were identical to those in experiment 1.

Apparatus and Procedure

Please see experiment 1 for details.

Drug and Injection Procedure

All animals received all doses (0.1, 1.0 and 10.0 nmole) of the selective delta agonist D-Pen^2, D-Pen^5-Enkephalin (DPDPE). The drug was dissolved in isotonic saline and drug doses were counterbalanced across all animals. Saline was administered on non-drug days. The injection procedure was identical to that of experiment 1.

After all doses of drug had been tested some of the animals that had received ventral tegmental injections of DPDPE received additional testing of intraperitoneal naloxone in conjunction with intracranial injections of DPDPE (10.0 nmole) or intraperitoneal saline in conjunction with intracranial saline. Naloxone was dissolved in isotonic saline and administered at a dose of 2 mg/kg. Intraperitoneal injections of saline or naloxone were given ten minutes prior to intracranial injections. Testing procedure was identical to that previously described in experiment 1.

Results

VTA injections of DPDPE decreased the duration of time required to complete meal segments [$F(3,15)=4.65$, $p<0.0172$, Fig. 25]. However, only the largest dose of DPDPE (10 nmole) was significantly different from saline ($p<0.05$). There was clearly a tendancy for 0.1 and 1.0 nmole of DPDPE to accelerate feeding, however, this failed to reach significance (Fig. 25). The largest dose of DPDPE produced a slight but nonsignificant leftward shift in the mean distribution or duration scores; more 'best' scores were obtained following injections

of 10 nmole of DPDPE into the VTA than when saline was administered (Fig. 26).

Injections of DPDPE into the VTA had no effect on the latency to initiate feeding [F3,15)=2.43, p<0.1052, Fig 27]. All doses of DPDPE produced a small but non-significant leftward shift in the mean distribution of latency; more 'best' scores and fewer 'worst' latency scores were obtained following injections of DPDPE than when saline was administered (Fig 28).

Injections of DPDPE dorsal to the VTA had no effect on either the duration [F(3,12)=0.58, p<0.6410, Fig 29] or the latency (F(3,12)= 0.85, p<0.5247, Fig 30) scores. The distribution of duration scores remained relatively unchanged by injections of DPDPE dorsal to the VTA (Fig 31). Dorsal injections of DPDPE had a tendency to produce more 'best' latency scores and fewer 'worst' latency scores than saline (Fig 32), however, this was not significant.

Pretreating animals with the opiate antagonist naloxone (2 mg/kg) prior to injecting them with 10.0 nmole of VTA DPDPE was effective in blocking DPDPE's facilitation of feeding (Fig 33). Naloxone in conjunction with DPDPE was no different than intraperitoneal injections of saline in conjunction with VTA injections of saline.

Observations of freezing or startle responses were rare when DPDPE was administered and when seen were observed equally in the saline and drug conditions. Instances where animals did not finish their food pellets were also rare and were usually a result of feeding being interrupted by grooming or exploratory behavior. This happened equally in the saline and drug conditions. Circling

behavior was seen in the case where animals received 10 nmole of DPDPE, however, circling behavior did not appear to interfere with an animal's feeding behavior. The circling behavior seen with DPDPE was not as vigorous as that obtained with VTA injections of DAGO.

Localization of injector tips aimed at and dorsal to the VTA are illustrated in figure 34.

Discussion

Injections of DPDPE into the VTA facilitate feeding in food deprived rats. Injections of DPDPE into sites dorsal to the VTA have no effect on feeding. This suggests that the VTA, and not areas surrounding the VTA, is the primary site of action for delta opiates. The facilitation of feeding by VTA DPDPE is attenuated following administration of systemic naloxone. This suggests that the effects of DPDPE are due to its actions on delta receptors and are not a function of its physico-chemical properties.

VTA DPDPE facilitates feeding to the same degree as VTA injections of morphine. The magnitude of the facilitation of feeding is equivalent following VTA DPDPE and VTA morphine. DPDPE and morphine are also equipotent; both require 10 nmole of drug to produce a significant facilitation of feeding. It is possible that morphine's facilitation of feeding occurs as a result of morphine's actions on delta opioid receptors at the level of the VTA, however, this is not likely for two reasons. The first is that morphine binds preferentially to mu opioid receptors (Goldstein & Naidu, 1989). The

second is that there are a greater number of mu receptors than delta receptors at the level fo the VTA (Mansour et al., 1988; Dilts & Kalivas, 1989; 1990). Together, these facts suggest that morphine is having its facilitation of feeding through its actions at mu receptors.

VTA DPDPE is not as effective as VTA injections of DAGO in facilitating feeding. The most effective dose of DPDPE was 10 nmole whereas a dose of 0.1 nmole of DAGO was found to be maximally effective in Experiment 3. Thus, VTA DAGO is at least 100 more potent than VTA DPDPE. In addition, the magnitude of the facilitatory effect is greater following VTA DAGO than following VTA DPDPE; DAGO attenuates the response slowing effect of feeding to a much greater degree than DPDPE and significantly decreases the variance of duration scores. These results suggest that, at the level of the VTA, mu opioids play a greater role in the regulation of feeding than delta opioids.

VTA injections of DPDPE appear to enhance the motivation to feed. The frequency of short duration scores is increased following VTA DPDPE and the frequency of long scores is reduced. Animals consistently take less time to complete a meal following DPDPE than they do following saline. Thus, VTA DPDPE facilitates feeding by both increasing the mean speed of eating and decreasing score variability. This suggests that DPDPE facilitates feeding by sustaining an animal's interest in food.

DPDPE may facilitate feeding by interacting with mesolimbic dopamine. A role for delta opiates in the modulation of mesolimbic dopamine activity is supported by data from neurochemical studies. Injections of DPDPE into the ventricles or directly into the VTA

increase dopamine release and metabolism in the nucleus accumbens (Spanagel, Herz & Shippenberg, 1990; Devine et al., 1991a; 1991b). Complementing this is the finding that intraventricular or VTA injections of delta antagonists decrease dopamine release and metabolism in the nucleus accumbens (Spanagel, Herz & Shippenberg, 1990; Devine et al., 1991b). Thus, delta opiates and opiate antagonists appear to be able to modulate mesolimbic dopamine.

There are similarities between VTA DPDPE's facilitation of feeding and VTA DPDPE's effect on mesolimbic dopamine activity that support the suggestion that delta opiates have their effect on feeding by modulating dopamine. First, both effects require aproximately the same dose of drug; 10 nmole for the former and 13.2 nmole for the latter. Related to this is the observation that for both the facilitation of feeding and dopamine release the mu opiate, DAGO, is 100 more potent than the delta opiate, DPDPE (Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a). Second, both DPDPE-induced feeding and DPDPE-induced dopamine release are attenuated by administration of an opiate antagonist; feeding by naloxone and modulation of mesolimbic dopamine by naltrindole (Devine; personal communication). These data suggest that the facilitation of feeding by VTA DPDPE may be dependent on DPDPE's ability to activate the mesolimbic dopamine system. If VTA DPDPE-induced feeding is dopamine dependent then damaging the mesolimbic dopamine system or pretreating animals with dopamine antagonists into the nucleus accumbens should attenuate the feeding effect. This remains to be tested. In conclusion, injections of DPDPE into the VTA

facilitate feeding and they appear to do so by increasing mesolimbic dopamine.

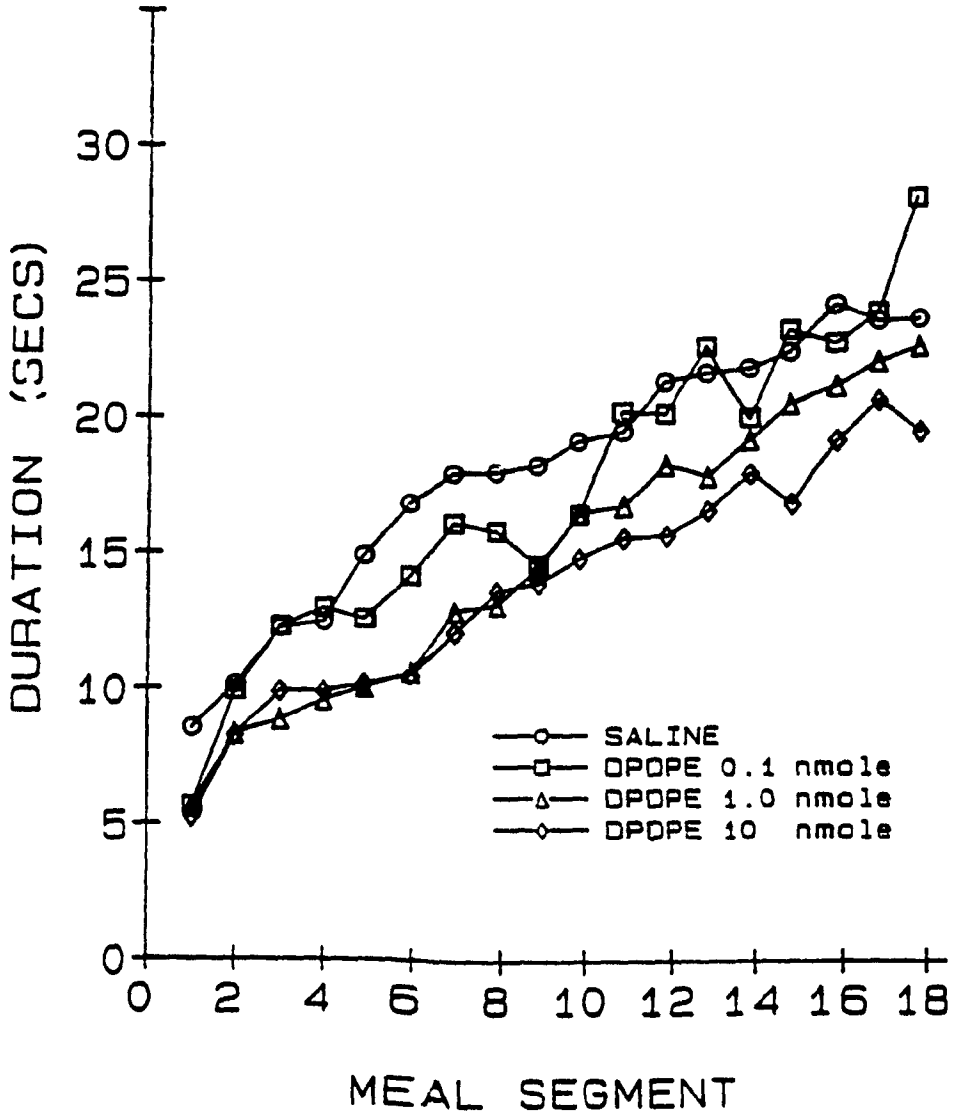

Figure 25. The mean time required to complete meal segments following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into the ventral tegmental area (n=6).

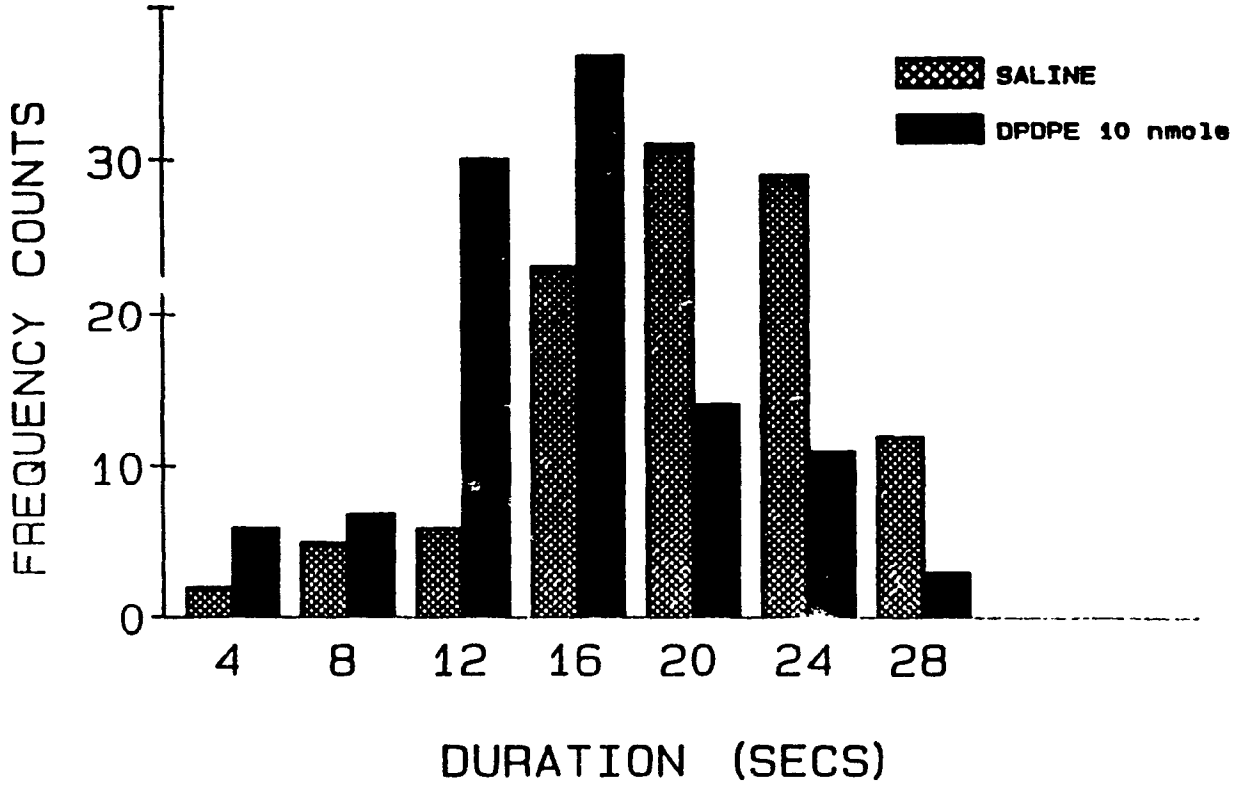

Figure 26. Distribution of duration scores following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into the ventral tegmental area (n=6). Response bins comprise 4 second intervals.

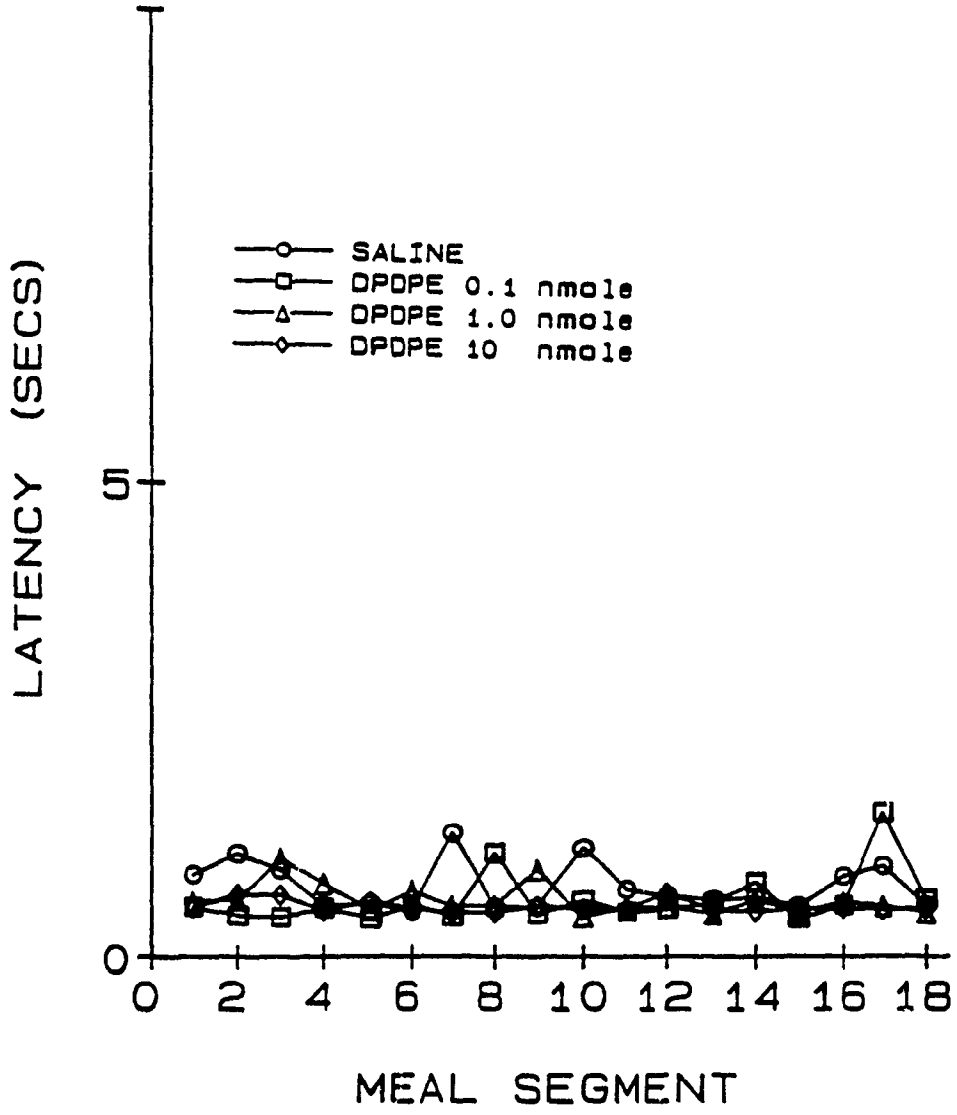

Figure 27. The mean latency to initiate feeding for each meal segment following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into the ventral tegmental area (n=6).

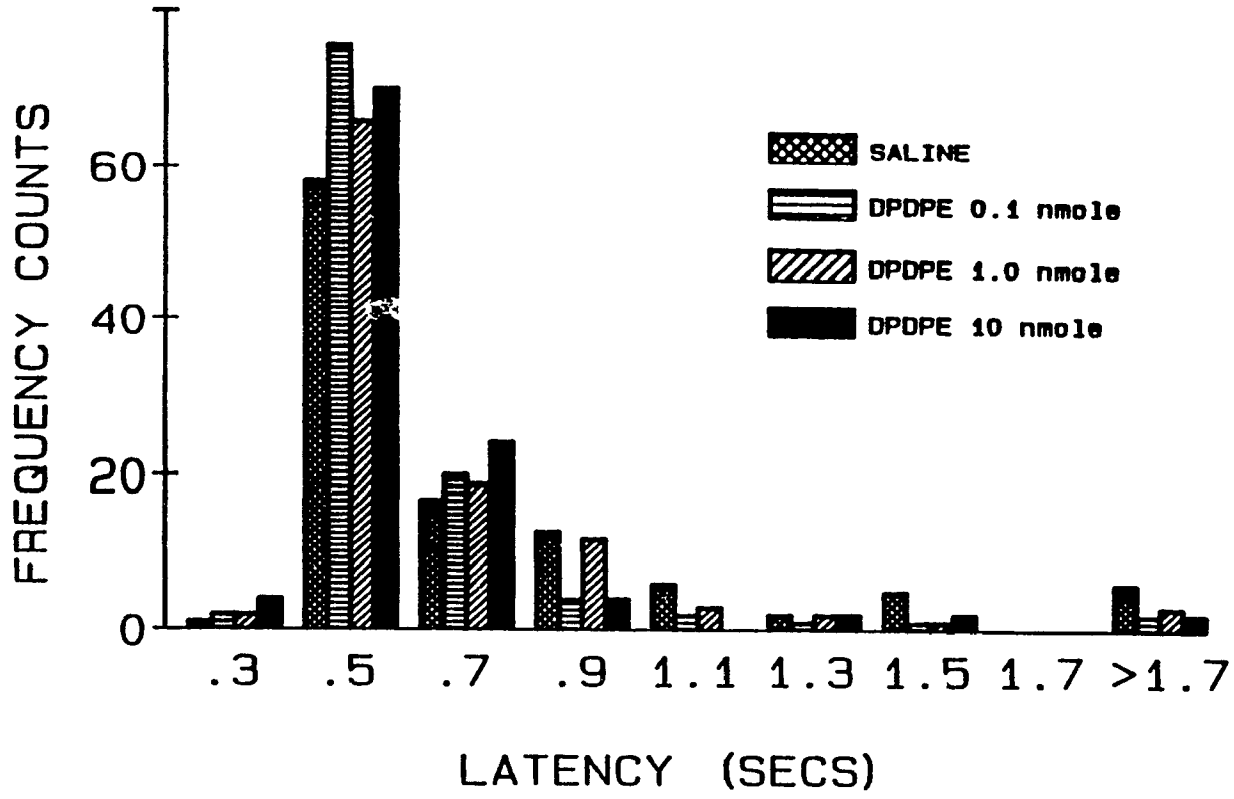

Figure 28. Distribution of latency scores following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into the ventral tegmental area (n=6). Response bins comprise 0.2 second intervals.

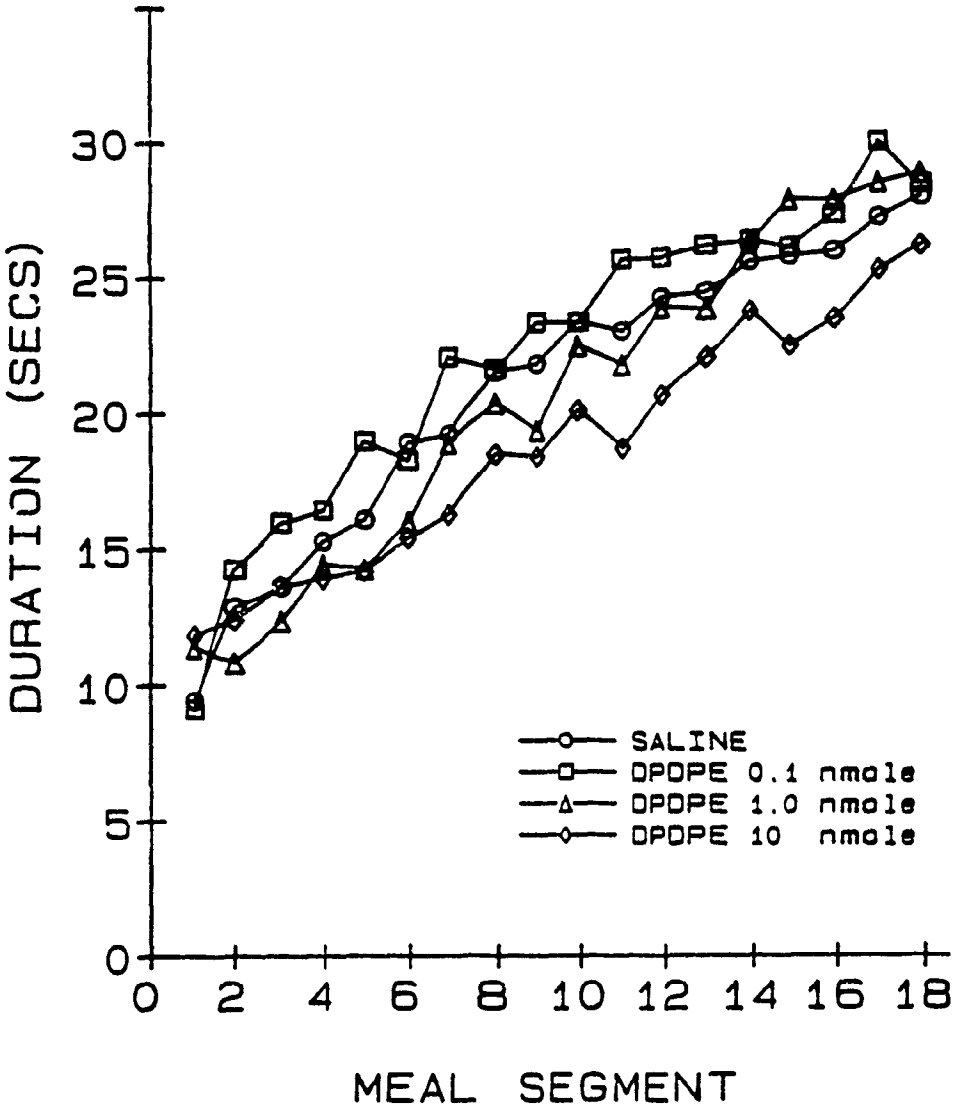

Figure 29. The mean time required to complete meal segments following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into sites dorsal to the ventral tegmental area (n=5).

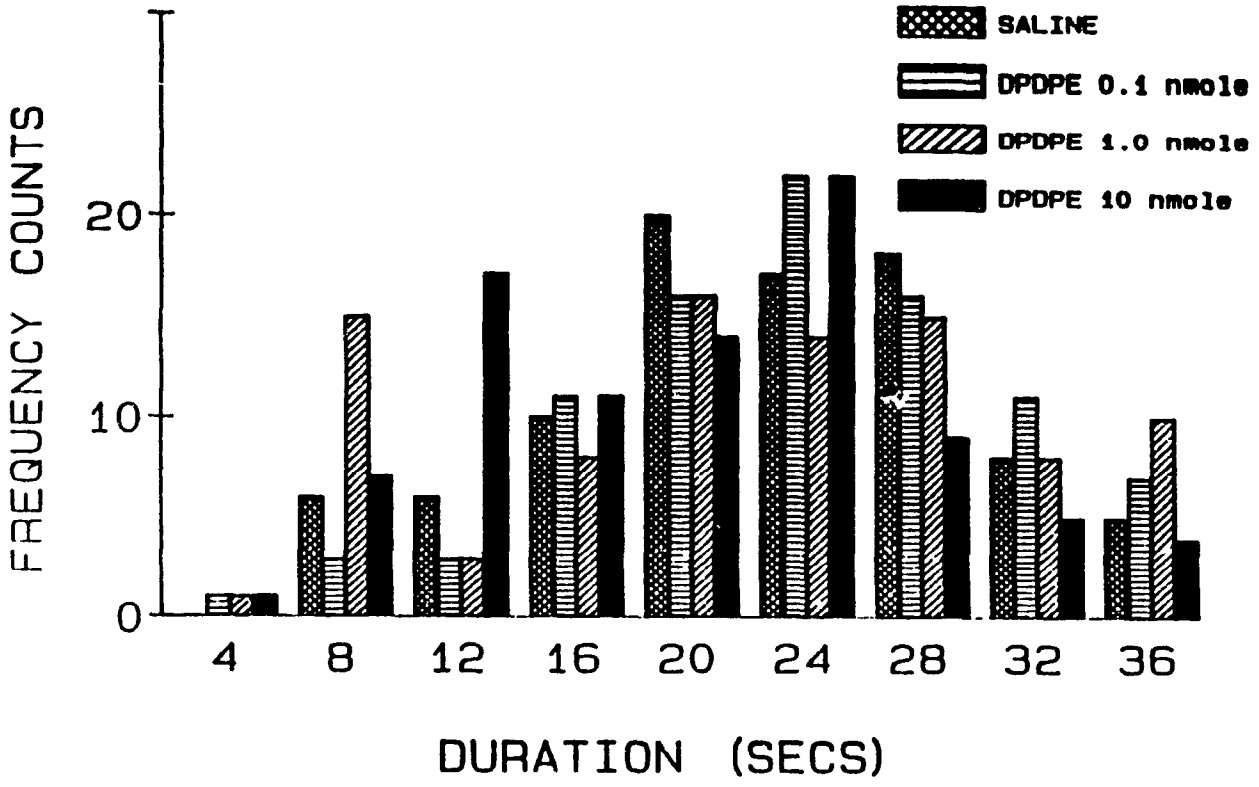

Figure 30. Distribution of duration scores following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into sites dorsal to the ventral tegmental area (n=5). Response bins comprise 4 second intervals.

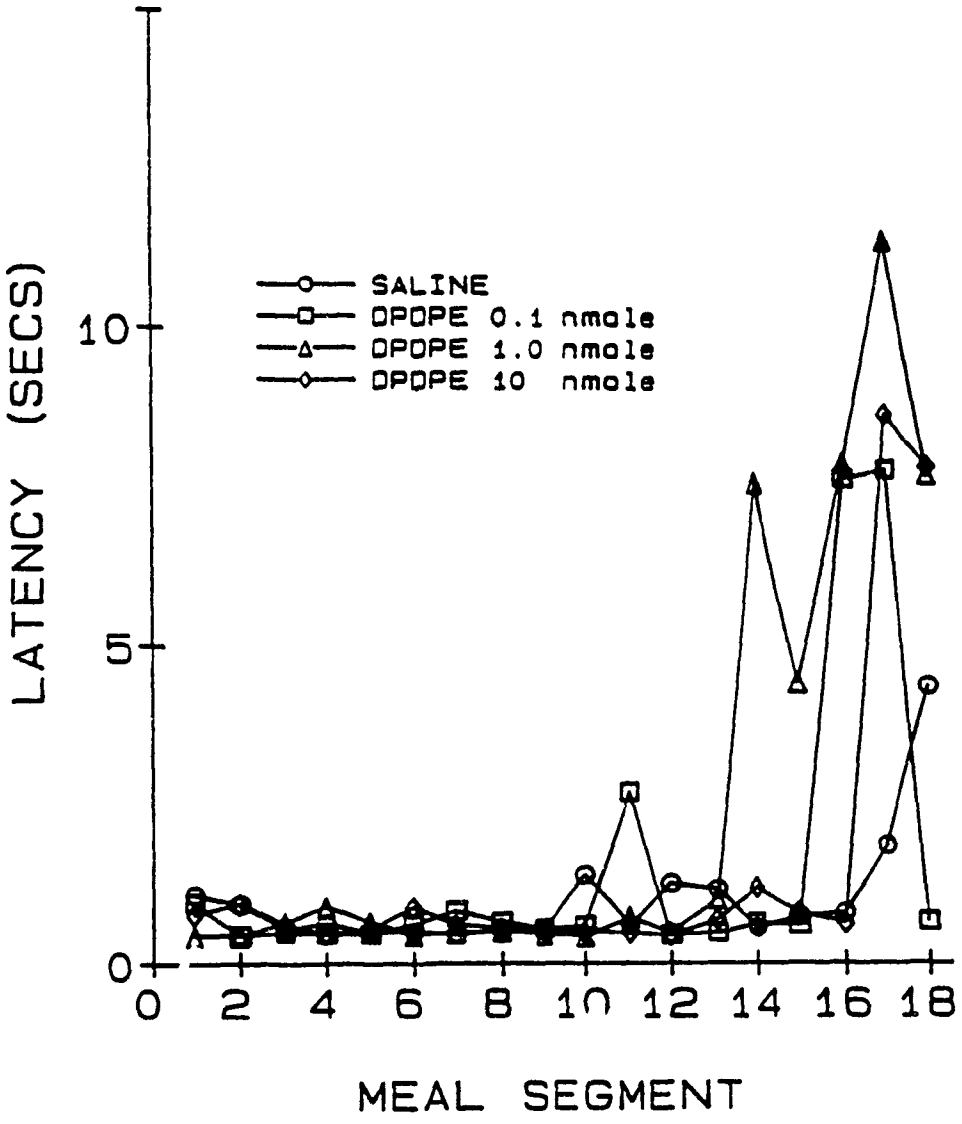

Figure 31. The mean latency to initiate feeding for each meal segment following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into sites dorsal to the ventral tegmental area (n=5).

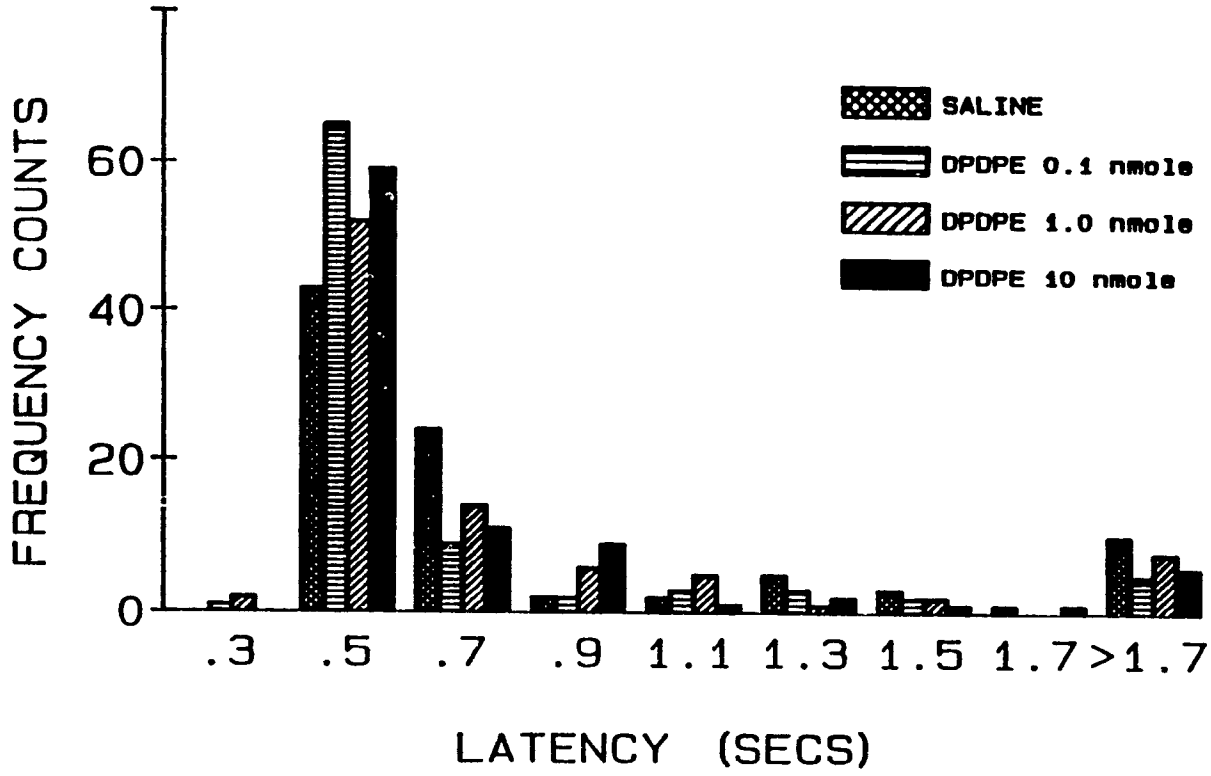

Figure 32. Distribution of latency scores following injections of saline, 0.1, 1.0, or 10 nmole of DPDPE into sites dorsal to the ventral tegmental area (n=5). Response bins comprise 0.2 second intervals.

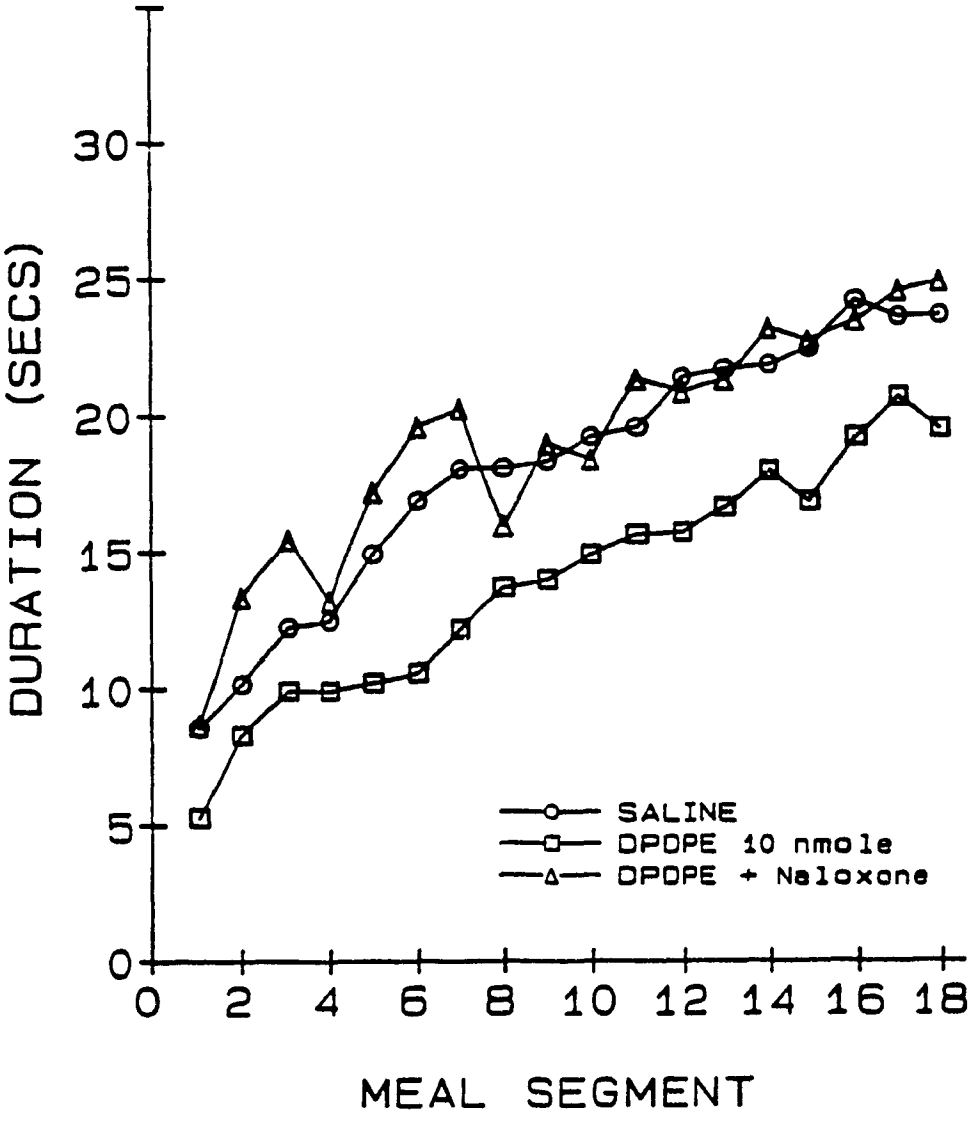

Figure 33. The mean time required to complete meal segments following injections of i.p. saline and VTA saline, 10 nmole of VTA DPDPE, or 10 nmole of VTA DPDPE and 2 mg/kg of naloxone (n=5).

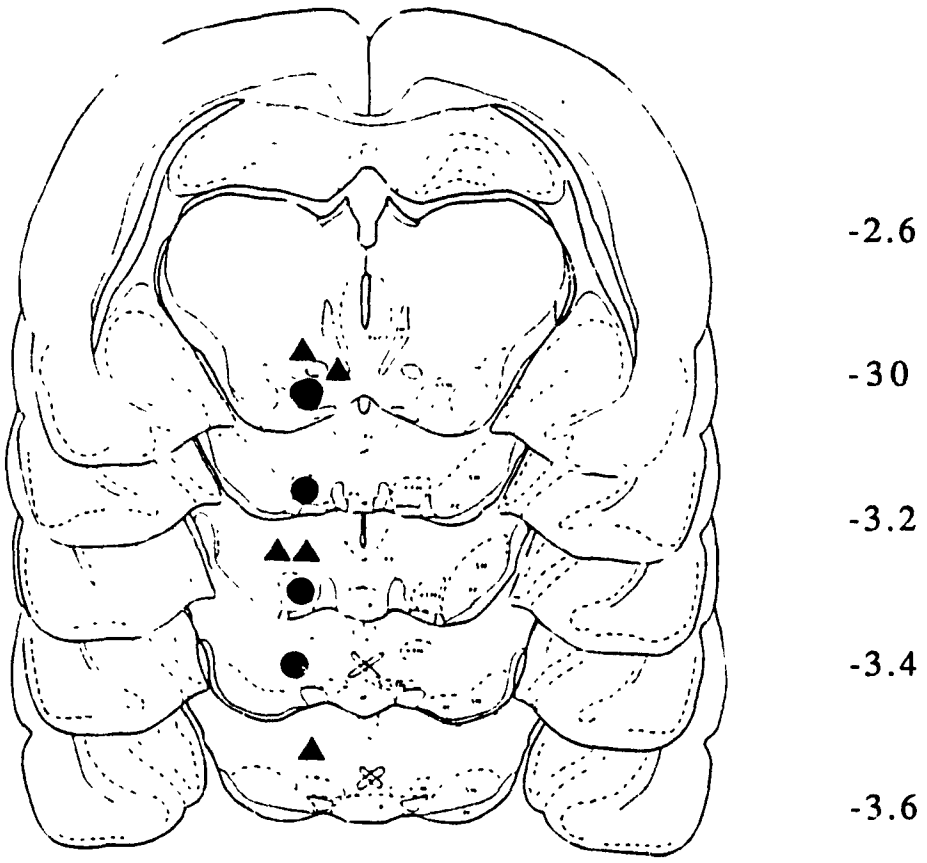

Figure 34. Histological placements for animals with injector tips located dorsal to the ventral tegmental area (triangles, n=5) and for animals with injector tips located in the ventral tegmental area (circles, n=6). Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice refers to the distance (in millimeters) posterior to bregma.

Experiment 5

Acute systemic injections of low doses of morphine stimulate locomotion. This activating effect of morphine is overshadowed by the drug's sedative effects when moderate or high doses are given (Babbini & Davis, 1972; Vasko & Domino, 1978). The effect of a high dose of morphine—sedation—dominates for an hour or two and the low dose effect—hyperlocomotion—is unmasked as the drug is metabolized to lower concentrations. The sedative effect of high doses of morphine undergoes tolerance with repeated intermittent administration, and the stimulant effect of low doses undergoes the reverse: sensitization (Martin et al., 1963; Babbini & Davis, 1972; Vasko & Domino, 1978; Eikelboom & Stewart, 1982).

Injections of morphine into the VTA cause stimulant effects, increasing locomotion and sensitizing the animals to subsequent injections of VTA morphine and also to systemic amphetamine. Moderate doses of morphine fail to cause any sedative effects when injected into the VTA (Joyce & Iversen, 1979; Vezina & Stewart, 1984; Kalivas & Duffy, 1987), but cause sedation without any stimulant effects when injected into sites caudal to the VTA (Broekkamp et al., 1976).

Like the locomotor effects of VTA morphine, the feeding effects of systemic opiates seem to be sensitized by repeated administration of the drug. Repeated systemic injections of moderate doses of morphine, ketocyclozacine, or N-allynormetazocine result in progressive enhancements in food intake (Morley & Levine, 1982; Gosnell et al., 1983; Thornhill & Saunders,

1983). The behavioral sensitization produced by systemic opiate administration is a robust effect and can persist up to eight months after opiate administration (Babbini, Gaiardi & Bartoletti, 1975; Vasko & Domino, 1978).

Many attempts have been made to explain sensitization to the stimulant effects of drugs. Some researchers have suggested that the progressive enhancement of the locomotor or feeding response is a secondary effect reflecting the development of tolerance to the sedative effects of opiates (Kumar, Mitchell & Stolerman, 1971; Morley & Levine, 1982). However, this explanation is not sufficient since repeated injections of low doses of systemic morphine or intra-VTA morphine produce sensitization without any obvious sedative effects. Others once suggested that systemic opiates act initially to inhibit dopamine release by acting on opioid receptors at dopamine terminals. It was suggested that this inhibition of dopamine release was followed by an increase in dopamine synthesis which gradually overcame the inhibitory effect and resulted in an increase in responding (Pollard et al., 1977; Pollard, Llorens-Cortes & Schwartz, 1977). However, it is unlikely that sensitization is due to the inhibitory effects of opiates on dopamine since low doses of systemic morphine produce only increases in locomotion and since administration of opiates to the VTA has only excitatory effects on mesolimbic dopamine (Gysling & Wang, 1982; Matthew & German, 1982).

Several lines of evidence suggest that opiate sensitization may depend on the ability of opiates to activate the mesolimbic dopamine system. First, the progressive enhancement in behavioral

activity that is produced with repeated injections of morphine is reminiscent of the sensitization of behavioral activation that is produced with repeated injections of the dopamine agonist amphetamine (Babbini & Davis, 1972; Joyce & Iversen, 1979; Robinson & Becker, 1986). Second, morphine-induced hyperactivity is enhanced in animals that have previously received injections of amphetamine. The converse has also been found (Kalivas & Weber, 1988; Vezina et al., 1989). Lastly, sensitization of opiate-induced locomotion that occurs with repeated injections of VTA opiates can be blocked by pretreating animals with dopamine antagonists (Joyce & Iversen, 1979; Vezina & Stewart, 1984; 1989). It is thought that morphine sensitization, like amphetamine sensitization, is dependent on underlying changes in the mesolimbic dopamine system.

The hypothesis that mesolimbic dopamine is involved in the development of opiate sensitization is supported by the finding that animals that have received repeated injections of opiates into the VTA show larger increases in nucleus accumbens dopamine metabolism following acute administration of VTA opiates than animals that have received repeated injections of saline into the VTA (Kalivas, 1985; Kalivas & Duffy, 1987).

The development of opiate sensitization seems to depend on opiate actions localized near the dopamine cell bodies. Sensitization of the locomotor response obtained following repeated injections of systemic morphine can be blocked by intra-VTA administration of naltrexone methobromide but not by nucleus accumbens injections of naltrexone methobromide (Kalivas & Duffy, 1987). In addition, while injections of opiates into either the VTA

or the nucleus accumbens increase locomotion, repeated injections of opiates into the VTA but not into the nucleus accumbens result in sensitization of opiate-induced forward locomotion (Kalivas, 1985; Kalivas, Taylor & Miller, 1985; Vezina, Kalivas & Stewart, 1987). This suggests that opiate sensitization depends on the actions of opiates near the VTA. Since mesolimbic dopamine fibers are activated by injections of VTA opiates but not by nucleus accumbens opiates it is possible that activation of mesolimbic dopamine plays an important role in the development of opiate sensitization.

The purpose of the present experiment was to determine if repeated injections of VTA opiates would cause progressively stronger (sensitization) or progressively weaker (tolerance) feeding responses. Because VTA administration of the kappa opiate, U-50,488H, had no effect on feeding this drug was not tested. Sensitization was investigated using only the selective mu (DAGO) and delta (DPDPE) opiates. Thus, one group of rats received repeated intermittent injections of DAGO and another group of animals received repeated intermittent injections of DPDPE into the VTA.

Method

Subjects and Surgery

Subjects were twelve naive adult male Long-Evans Old Colony rats individually housed and weighing between 340 and 400 grams at time of surgery. Each animal was implanted with a unilateral stainless steel cannula aimed at the ventral tegmental area.

Surgery, apparatus, and training procedure were the same as those of Experiment 1.

Drug and Injection Procedure

Five rats received injections of the selective mu agonist, DAGO, every second day for a total of six days. The dose of DAGO was 0.05 nmole. Seven rats received intracranial injections of the selective delta agonist DPDPE every second day for a total of six days. The dose of DPDPE was 0.5 nmole. Saline was administered on non-drug days. The injection procedure was identical to that of Experiment 1. The daily testing procedure was the same as that outlined for Experiment 1.

Results

The first injection of 0.05 nmole of DAGO into the VTA had no significant effect on feeding but the duration of time required to complete meal segments progressively decreased with each successive administration of the drug [$F(5,20)=5.99$, $p<0.0015$; Fig. 35]. The fourth, fifth and sixth injections of DAGO produced significantly shorter durations than the first injection of DAGO ($p< 0.05$). Following the first injection of saline, each successive injection of saline produced progressive decreases in the duration of time required to complete meal segments in the animals that received repeated injections of DAGO [$F(5,20)=3.23$, $p<0.0267$; Fig. 37]. However, post hoc tests failed to reveal any differences between any of the saline injection days.

Latency scores were not affected by either the first injection of DAGO or by repeated injections of DAGO into the VTA [$F(5,20)= 1.42$, $p<0.2311$; Fig. 36]. Latency scores were not altered by repeated injections of saline [$F(5,20)=1.13$, $p<0.2462$; Fig. 38].

The first injection of 0.5 nmole of the selective delta agonist DPDPE into the VTA had no affect on feeding behavior, and repeated injections DPDPE into the VTA failed to produce significant progressive decreases in the time required to complete meal segments [$F(5,30)=1.79$, $p<0.1441$; Fig. 39] or in the latency to initiate feeding [$F(5,30)=1.63$, $p<0.1562$; Fig 40] scores. Repeated VTA saline injections had no significant effect on the duration [$F,(5,30)=1.86$; $p<0.1142$; Fig. 41] or the latency [$F,(5,30)=1.72$; $p<0.1482$; Fig. 42] scores in animals that received repeated injections of DPDPE.

Neither the first injection nor repeated administration of DAGO or DPDPE produced any increase in freezing or startle responses. Such responses were rarely observed in animals administered either DAGO or DPDPE, and when seen they occurred equally during the first and last drug test. Instances where animals did not finish their food pellets were rare and were usually a result of feeding being interrupted by grooming or exploratory behavior. This happened equally in all drug and saline tests.

Location of injector tips aimed at the VTA for animals that received repeated injections of DAGO or DPDPE are illustrated in figure 43.

Discussion

Neither the first injection of 0.05 nmole of the selective mu opiate DAGO, nor 0.5 nmole of the selective delta opiate DPDPE, into the VTA had any significant effect on feeding. However, repeated intermittent injections of VTA DAGO, produced a progressive enhancement of opiate facilitation of feeding. There was no sensitization of the feeding effect with repeated injections of VTA DPDPE, despite the fact that a dose 10 times higher than the effective DAGO dose was used. This suggests that at least part of the sensitization of feeding produced by repeated systemic injections of opiates is due to the actions of systemic opiates at mu receptors.

Repeated intra-VTA injections of DAGO have been found to produce progressive enhancements in forward locomotion (Vezina, Kalivas & Stewart,1987) as have morphine and DALA (Vezina & Stewart, 1984; Kalivas, 1985; Kalivas, Taylor & Miller, 1985). The observation that each of these opiates binds preferentially to mu receptors (Goldstein & Naidu, 1989) in conjunction with the finding that repeated VTA injections of 0.5 nmole of DPDPE do not produce sensitization of feeding suggests that activation of mu opioid receptors is sufficient for the development of opiate sensitization.

Intra-VTA injections of either DAGO or DPDPE produce locomotion (Jenck et al., 1987; Vezina, Kalivas & Stewart, 1987) and rats will self-administer either drug directly into the VTA (Devine & Wise, 1990). Injections of DPDPE or DAGO into the VTA increase dopamine release from the nucleus accumbens (Devine et al., 1991a; 1991b; Spanagel et al., 1991) and activation of mu or delta opioid

receptors have the same effect on neuronal membranes; both increase potassium conductance (North, 1986). These findings demonstrate that DAGO and DPDPE have similar qualitative effects. Given these findings it was unexpected that repeated VTA injections of DAGO but not DPDPE produced a progressive enhancement of feeding.

The most reasonable explanation for why sensitization of feeding was obtained with repeated injections of DAGO but not DPDPE is that DAGO and DPDPE are quantitatively rather than qualitatively different from one another. This is supported by the finding that DAGO is about 100 times more potent than DPDPE (Latimer, Duffy & Kalivas, 1987; Devine et al., 1991a). The dose of DPDPE that was administered may have been too small to obtain sensitization. The dose of DPDPE that was administered should have been 100 times larger, instead of 10 times larger, than the dose of DAGO that was used. Thus, it is possible that repeated injections of DPDPE that are 100 times larger than the dose of DAGO that was used would result in sensitization of feeding.

Of course it is possible that mu opioids activate the mesolimbic dopamine system through a different mechanism than delta opioids. Two lines of evidence support this possibility. First, damage produced by quinolinic acid to non-dopaminergic perikarya in the A10 region produces a 50% reduction in mu opioid receptors but fails to significantly alter delta receptors. Destruction of mesolimbic dopamine fails to alter either mu or delta binding in the A10 region. (Dilts & Kalivas, 1989; 1990). This suggests that mu opioid receptors are on non-dopaminergic neurons intrinsic to the

A10 region. Delta receptors, on the other hand, appear to be on neither A10 dopamine neurons nor on the same population of non-dopaminergic neurons that has the mu receptors in the A10 region.

Second, DAGO but not DPDPE appears to be capable of modulating activity of non-dopaminergic neurons. Two types of neurons have been identified in the ventral mescencephalon. These have been called principal neurons (believed to be dopamine neurons) and secondary neurons. Secondary neurons may be $GABA_B$ receptors (Lacey, Mercuri & North, 1989). DAGO, but not DPDPE, can decrease the firing rate of these secondary neurons. DAGO can also cause membrane hyperpolarization while DPDPE has no effect on membrane polarity of these neurons. While both DAGO and DPDPE increase mesolimbic dopamine the activity of dopamine (principal) neurons is not affected by the direct administration of either of these drugs (Lacey, Mercuri & North, 1989). It has been suggested that the increase in mesolimbic dopamine that occurs following administration of mu opiates may be mediated by the disinhibition of GABA interneurons by mu opiates (Kalivas et al., 1988; Lacey, Mercuri & North, 1989). At present it is not clear how delta opiates activate mesolimbic dopamine. These findings suggest that mu and delta opioids may have different mechanisms.

In conclusion, mu and delta but not kappa opiates facilitate feeding when injected into the VTA. Mu opiates facilitate feeding at smaller doses and produce a larger facilitation of feeding than do delta opiates. This suggests that mu opioid receptors play a greater role in the feeding induced by VTA and systemic morphine than delta opioid receptors. Further, repeated VTA injections of 0.05 nmole of

DAGO but not 0.5 nmole of DPDPE produce a progressive enhancement of feeding. It is possible that mu receptors play a more active role in the development of sensitization to repeated injections of systemic opiates than delta receptors, however, until larger doses of delta opiates are tested it is not clear whether progressive enhancement of feeding can be obtained with repeated injections of delta opiates. Finally, changes in the mesolimbic dopamine system appear to underlie both the potentiation of feeding and the potentiation of forward locomotion by opiates as well as the sensitization of these behaviors that occurs following repeated opiate administration. This suggests that a common mechanism may mediate both opiate-induced feeding and opiate-induced locomotor activity.

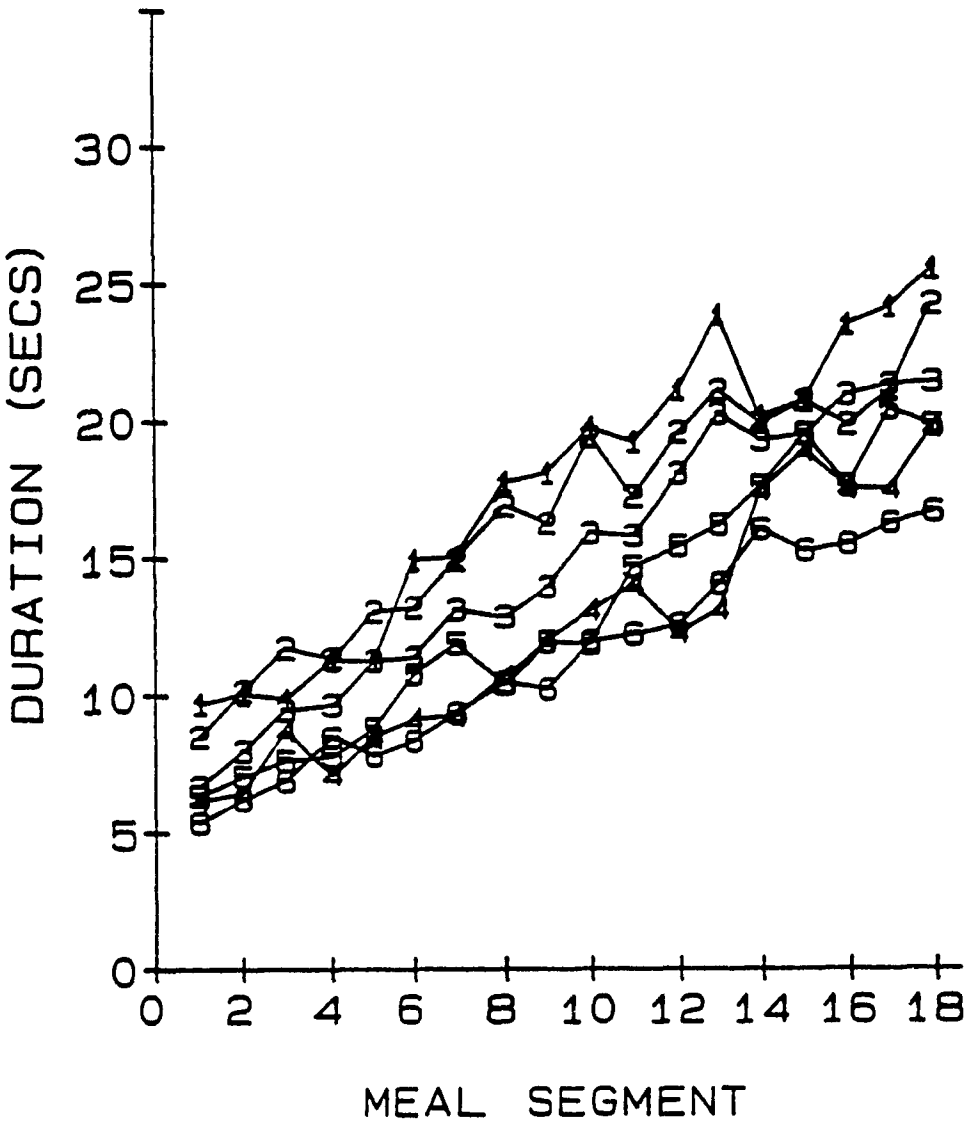

Figure 35. The mean time required to complete meal segments following repeated administration of 0.05 nmole of DAGO into the ventral tegmental area (n=5). Curves are numbered from 1 to 6 and correspond to the day of drug injection.

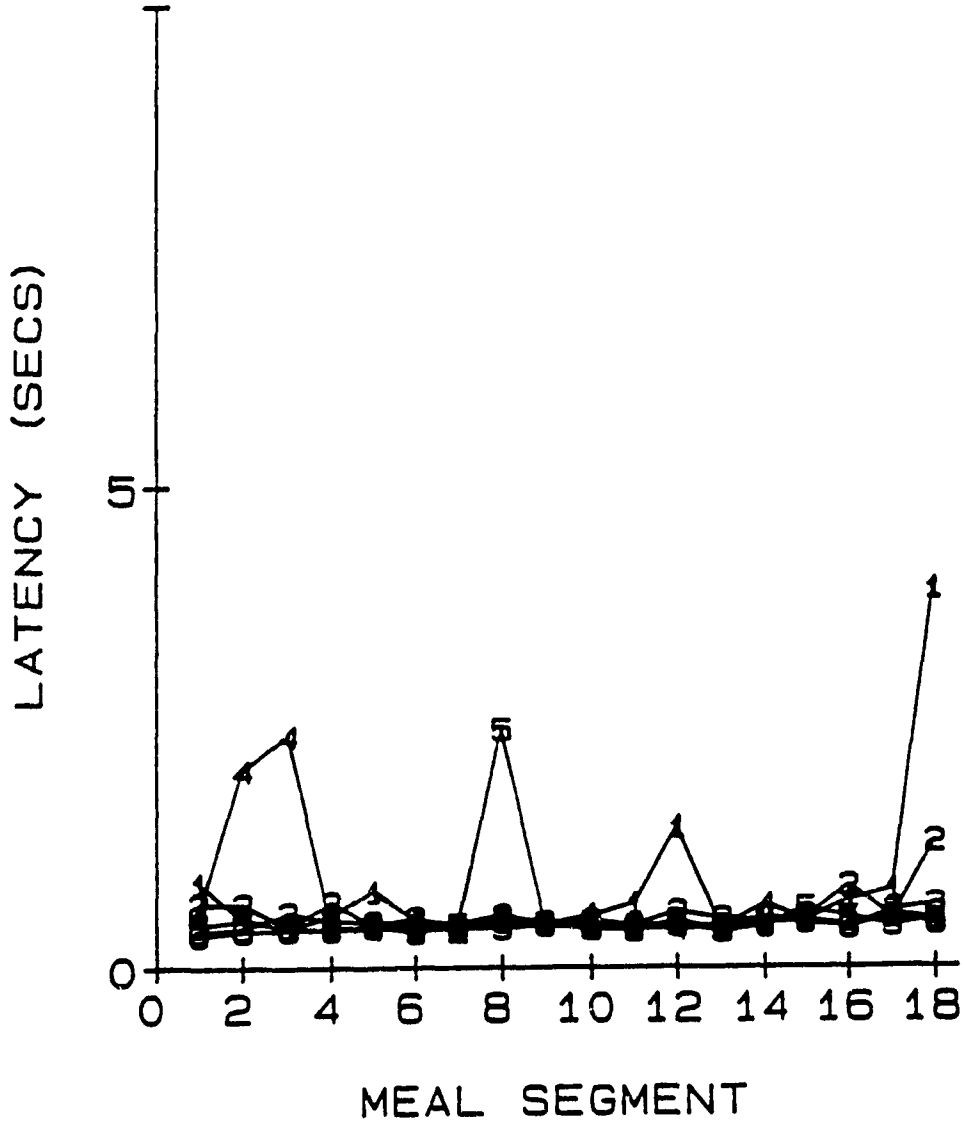

Figure 36. The mean latency to initiate feeding for each meal segment following repeated administration of 0.05 nmole of DAGO into the ventral tegmental area (n=5). Curves are numbered from 1 to 6 and correspond to the day of drug injection.

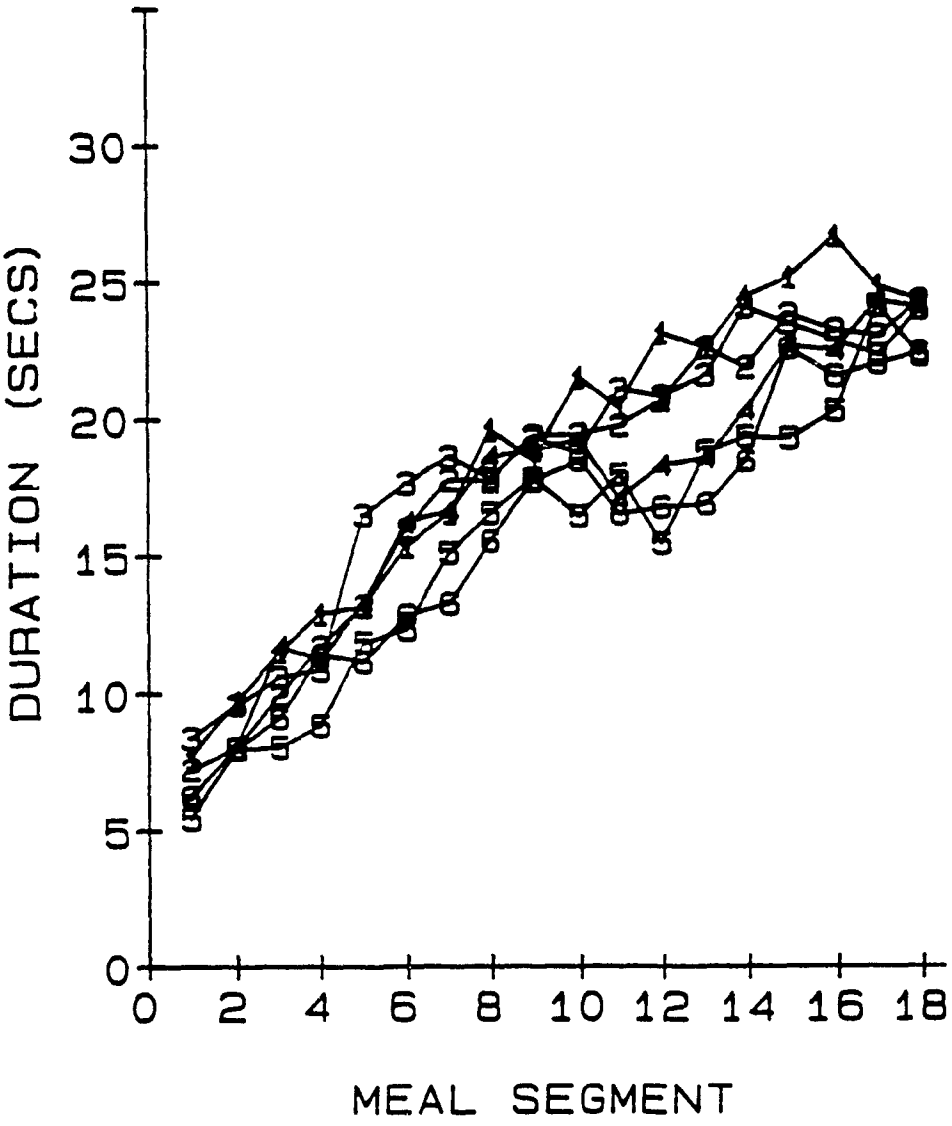

Figure 37. The mean time required to complete meal segments following repeated administration of saline into the ventral tegmental area of rats that were receiving repeated injections of DAGO (n=5). Curves are numbered from 1 to 6 and correspond to the day of saline injection.

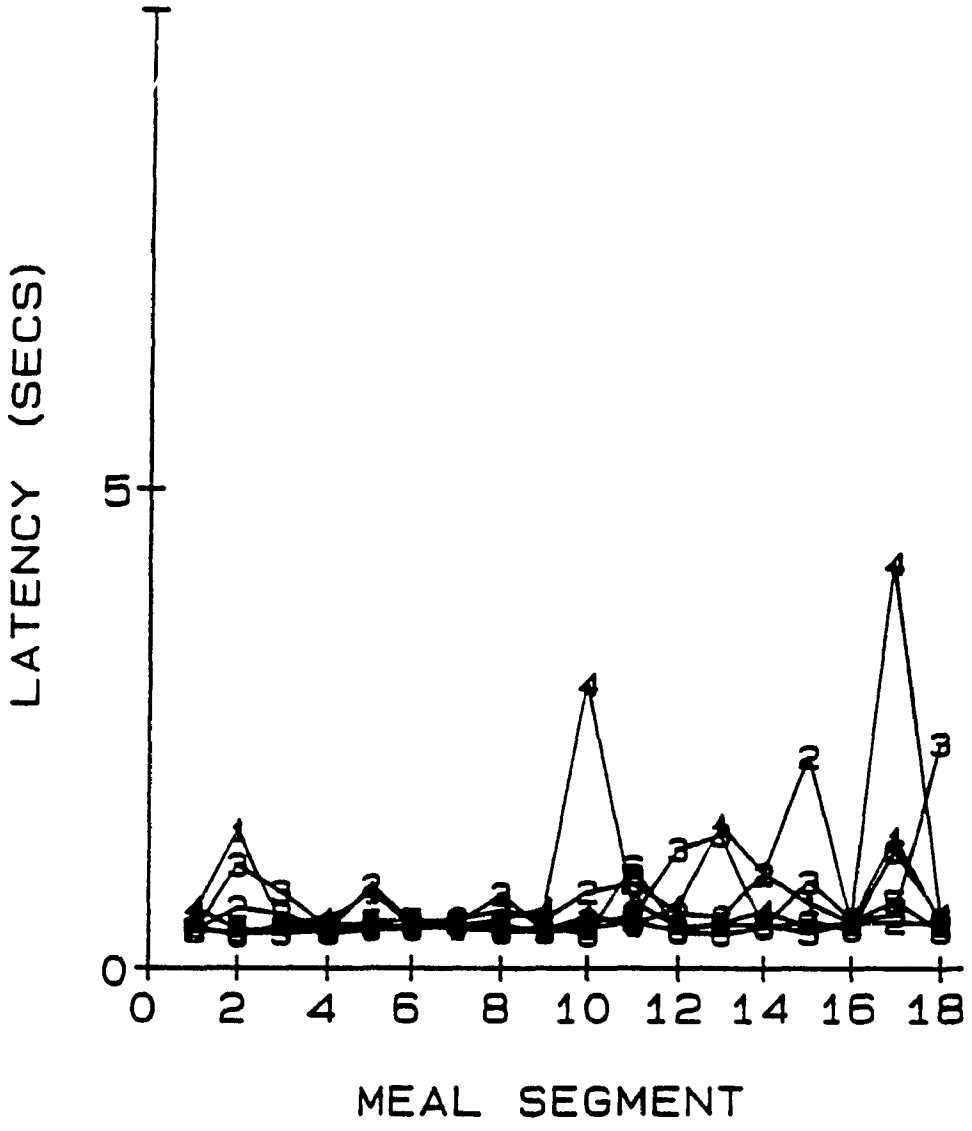

Figure 38. The mean latency to initiate feeding for each meal segment following repeated administration of saline into the ventral tegmental area of rats that were receiving repeated injections of DAGO (n=5). Curves are numbered from1 to 6 and correspond to the day of saline injection.

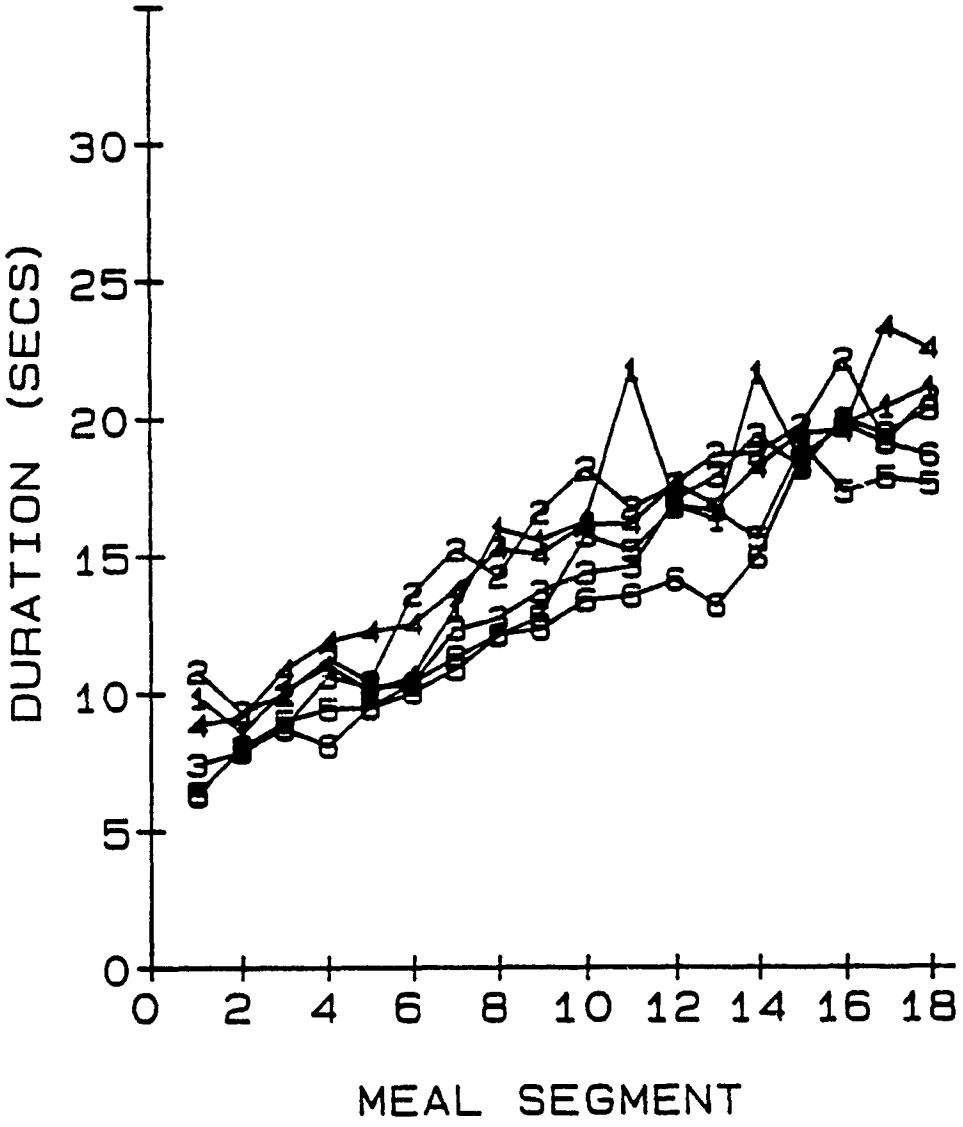

Figure 39. The mean time required to complete meal segments following repeated administration of 0.5 nmole of DPDPE into the ventral tegmental area (n=7). Curves are numbered from 1 to 6 and correspond to the day of drug injection.

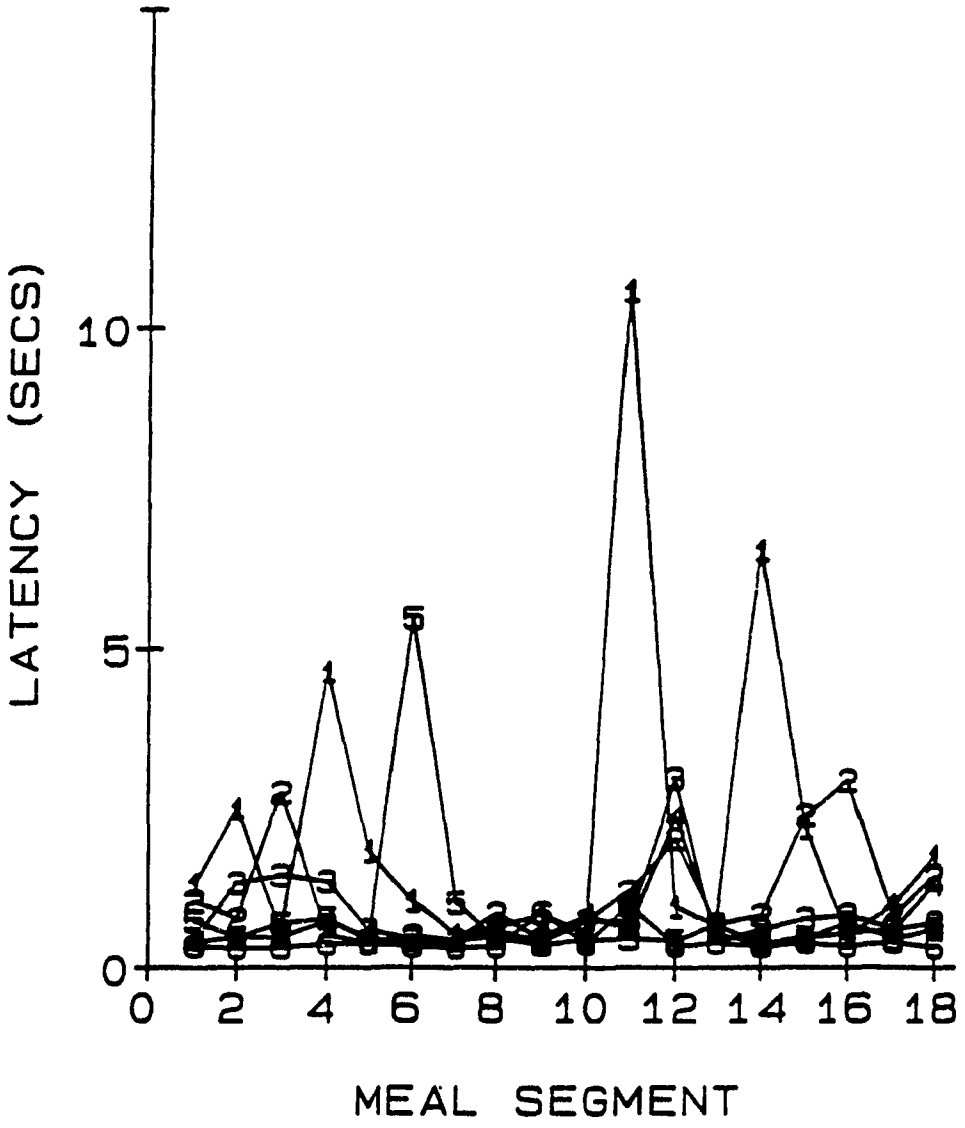

Figure 40. The mean latency to initiate each meal segment following repeated administration of 0.5 nmole of DPDPE into the ventral tegmental area (n=7). Curves are numbered from 1 to 6 and correspond to the day of drug injection.

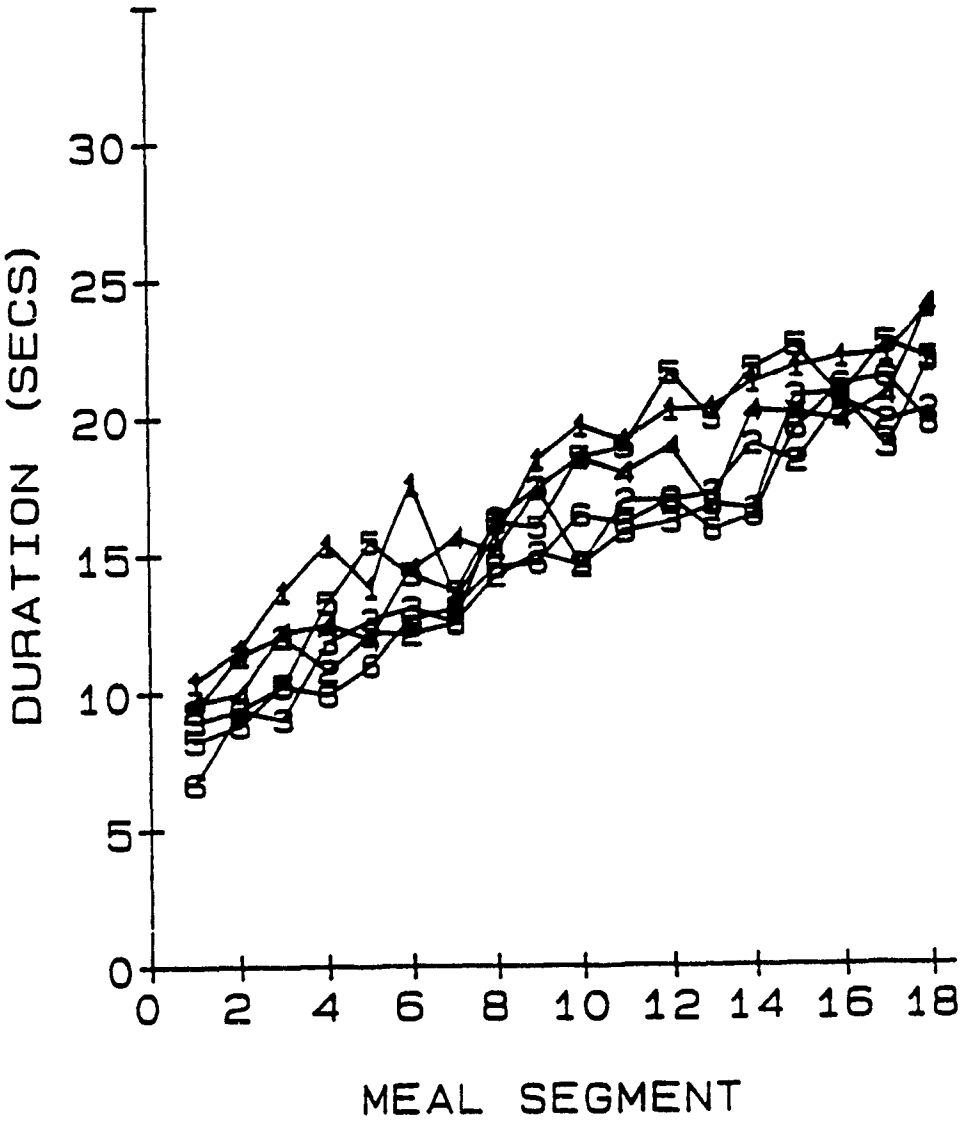

Figure 41. The mean time required to complete meal segments following repeated administration of saline into the ventral tegmental area of rats that were receiving repeated injections of DPDPE (n=7). Curves are numbered from 1 to 6 and correspond to the day of saline injection.

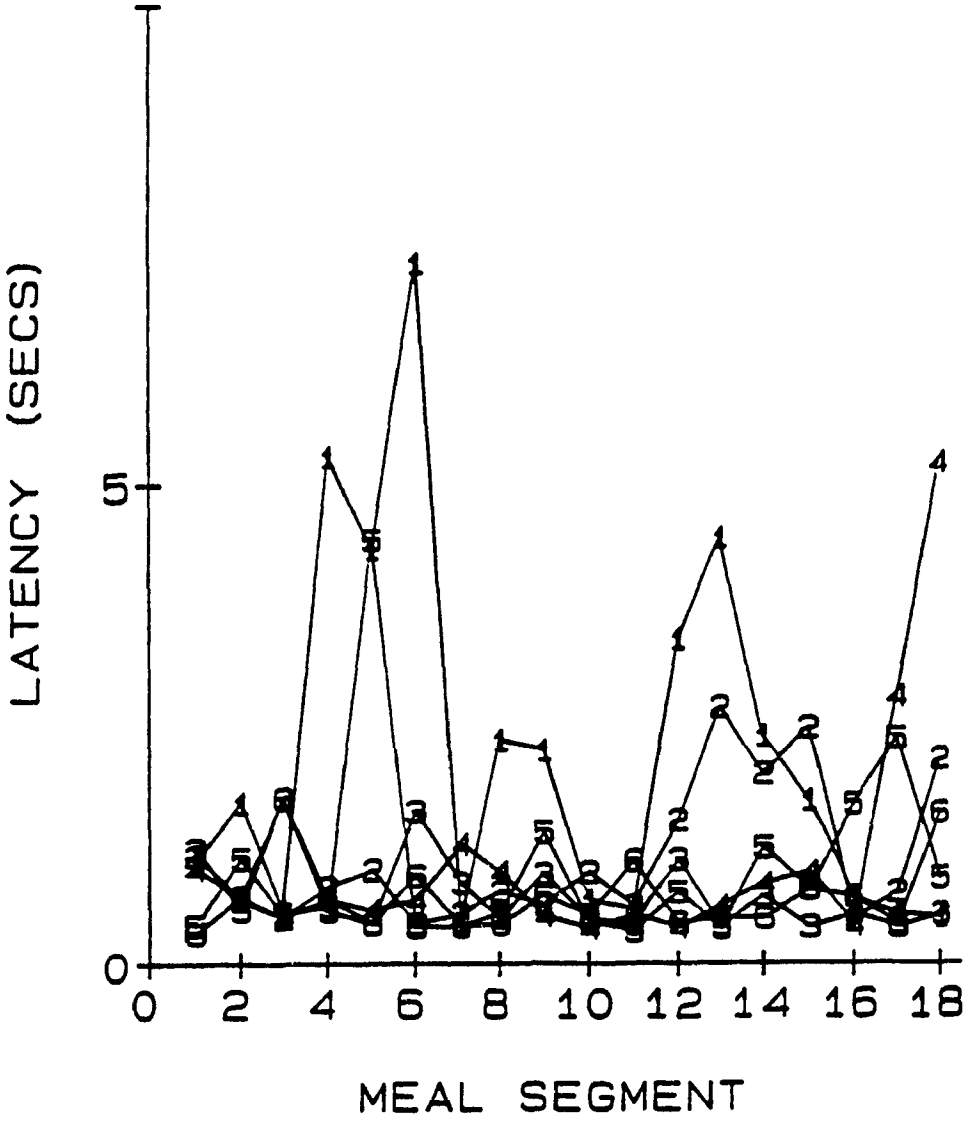

Figure 42. The mean latency to initiate each meal segment following repeated administration of saline into the ventral tegmental area of rats that were receiving repeated injections of DPDPE (n=7). Curves are numbered from 1 to 6 and correspond to the day of saline injection.

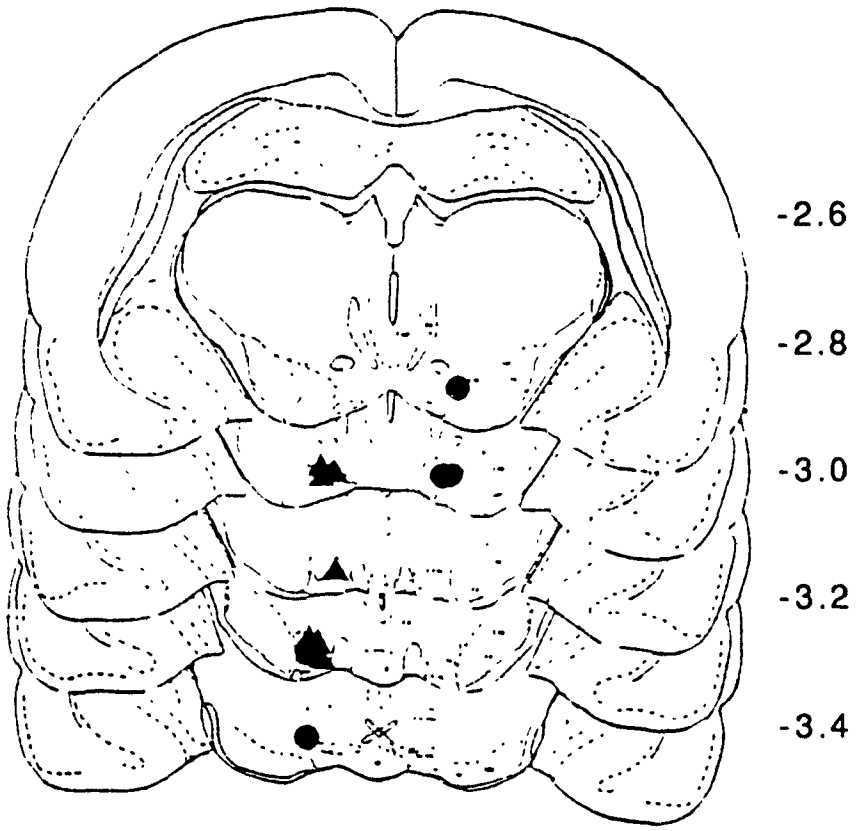

Figure 43. Histological placements for animals that received DAGO into the ventral tegmental area (triangles, n=5) and for animals that received DPDPE into the ventral tegmental area (circles, n=7). Reconstructions are based on the stereotaxic atlas of Pellegrino, Pellegrino, and Cushman (1979). The number beside each brain slice refers to the distance (in millimeters) posterior to bregma.

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