Endogenous opioids and feeding in the male rat, a learning approach
by Gregory Lee Burns

A thesis submitted in partial fulfillment of the requirements for the degree . of Master of Science in Psychology
Montana State University

Abstract:
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A thesis submitted in partial fulfillment
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6/12/87
Date

Wesley C Lynch
Chairperson, Graduate Committee

Approved for the Major Department

6-18-87
Date

Richard A. Black
Head, Major Department

Approved for the College of Graduate Studies

6-23-87
Date

M P Malone
Graduate Dean

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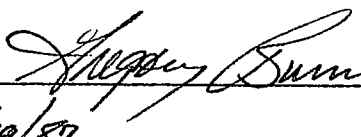
Signature 

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ABSTRACT

The role of endogenous opioid peptides (EOP's) in the ingestion of highly palatable solutions in the male rat was investigated in three experiments. In Experiment 1 acquisition of taste preference for sucrose or saccharin was blocked by the opiate antagonist naloxone. Following removal of the drug, preference quickly recovered, with intake levels surpassing that of controls. In Experiment 2 preference for a sucrose solution was enhanced with a low dose of the kappa-opiate receptor agonist U-50,488H. The high level of intake for this group was maintained for five days following removal of the drug. In Experiment 3 the release of EOP's in response to actual, or expected, intake of a palatable solution was assessed with an analgesia test. No decrease in pain sensitivity was observed when a delay was included between ingestion (or expected ingestion) and the analgesia test. However, testing without delay demonstrated significant analgesia effects following intake. These effects were reversible by administration of naloxone, suggesting that the analgesia was the result of EOP release. Taken together, the results of these experiments suggest that one of the functions of EOP's is the reinforcement of preference learning.

INTRODUCTION

Morphine (a derivative of the opium poppy) has long been known to possess analgesic and euphoric properties and has been used medicinally for more than a century. A number of opioid compounds have been developed that either mimic or block the effects of morphine; this has led to the proposal that specific opioid receptors exist within the nervous system that are the site of action for the opiate drugs (Reid, 1985). In a classic study, Pert and Snyder (1973) demonstrated the existence of specific opioid receptors in the nervous system, leading to the conclusion that there might be naturally produced neurotransmitters within the brain which bind with these receptors. In the last 15 years efforts have been directed towards identifying these substances (e.g., Goldstein, 1976) with a number of researchers reporting successful identification of endogenous peptides that possess opiate activity (Hughes, Smith, Morgan, & Fothergill, 1975; Terenius, & Wahlstrom, 1975; Pasternak, Goodman, & Snyder, 1975). For example, Hughes, Smith, Kosterlitz, Fothergill, Morgan, and Morris (1975) isolated and described two pentapeptides (enkephalins) which possessed opiate activity, such activity being blocked by the effects of the opiate antagonist naloxone. The discovery of these and other endogenous opioid peptides (EOP's) was important since such peptides have been implicated in the regulation of certain behaviors, such as ingestion.

In the present study, the role of the endogenous opioid peptides in ingestion is further investigated.

One approach for studying the effects of endogenous opioids on ingestion is to administer opioid antagonists (which occupy the receptors, thus blocking the action of EOP's) and then measure consequent intake of various substances. This approach has clearly implicated the opioid system in ingestion although the mechanism of the regulation of ingestion by opioids is unclear (Reid, 1985). Since the pioneering work of Holtzman (1975), in which decreases in ingestion were reported following single exposures to the opiate antagonists naloxone (NAL) and naltrexone (NALT), there have been numerous reports that opioid antagonists decrease the intake of food and water (e.g., Levine, Morley, Gosnell, Billington, & Bartness, 1985; Reid, 1985). These effects are both robust and reliable. For example, antagonists effectively inhibit intake of food and water when administered peripherally (Hemmer, Olson, Kastin, McLean, & Olson, 1982), when injected directly into specific brain regions (Siviy, Bermudez-Rattoni, Dargie, & Reid, 1981), and under stressful conditions such as mild deprivation (Jalowiec, Panskepp, Zolovick, Najam, & Herman, 1981). McCarthy, Dettmar, Lynn and Sanger (1981) demonstrated that these effects are not restricted to rats: NAL will also disrupt intake in cats and rabbits.

Yet the most intriguing findings point to the antagonists' abilities to affect the intake of preferred (e.g., sweet) substances. Reliable antagonist effects include the decrease in intake of sweetened water (Levine, Murray, Kneip, Grace, & Morley, 1982) and

the blocking of the normal development of preference for saccharin solutions (Lynch, 1986). The suppression of the ingestion of a highly palatable cafeteria diet has also been reported (Apfelbaum & Mandenoff, 1981). Interestingly, naloxone's effect on the intake of a 20% sucrose solution decreases over time (Olson, Delatte, Kastin, McLean, Phillpott, & Olson, 1985), an effect that has been interpreted as due to the development of tolerance to the NAL.

Since the effects of the EOP's are more pronounced for preferred ingestables than for standard laboratory chow and water, it seems that EOP's may function to regulate taste-motivated behavior. That is, the EOP's seem particularly involved when animals are feeding because they prefer the taste of a substance, rather than because they are hungry. This idea originated with a study by Rockwood and Reid (1982) in which rats with open gastric fistulas significantly reduced intake of a sucrose solution following NAL injections. This was a very important discovery since the fistulas drained off the sucrose before it could be absorbed in the digestive tract, thus the primary feedback the animals received regarding the sucrose was gustatory. This finding implies that the change in the drinking as a result of NAL administration was due to some modification of the taste by the drug. The implication of these results is that NAL may disrupt eating or drinking by modifying the palatability of foods.

In support of this conjecture is the finding that genetically obese rats who increase their intake of preferred foods to a greater degree than their lean counterparts (presumably because the obese rats find the food more palatable than the lean rats) are even more

sensitive to the suppressant effects of NAL (Cooper, Jackson, Morgan, & Carter, 1985). In a related study, Lynch and Libby (1983) found that NAL suppresses intake of a wider range of saccharin solutions when rats are deprived than when they are sated. Here, one interpretation is that since deprived rats find a wider range of substances palatable, a wider range of substances will be affected by the antagonists in deprived rats than in nondeprived rats.

If the EOP's are involved in taste preferences then the administration of opiate agonists should increase the intake of some substances. Indeed, such facilitation of intake has been reported when morphine is injected into the hypothalamus (Tepperman, Hirst, & Gowdey, 1981), and when dynorphin (an endogenous kappa agonist) is injected into the ventricles of rats' brains (Morley & Levine, 1981). These effects are less robust, however, than those found with the antagonists (Levine, et al., 1985). Reid (1985) noted that many variables may influence the responses to opiate agonists, including type of agonist, dose, time of test, and the nature of the test environment.

These variable effects can be partially explained by the fact that there exist at least three types of opioid receptors (Cooper & Sanger, 1984) which probably bind different opioid peptides. In support of this are studies investigating the effects of morphine (a putative mu-receptor agonist) on ingestion that have reported inconsistent changes in intake (Jalowiec et al., 1981; Morley, Levine, Grace, & Kneip, 1982), and those which have found more consistent increases in intake when kappa-receptor agonists are

employed. For instance, Morley and associates (Morley, Elson, & Levine, 1982; Morley, Levine, Grace, Kneip, & Zeugner, 1983; Morley & Levine, 1983) have demonstrated that kappa agonists, such as the highly selective U-50,488H and the kappa benzodiazepine, tifluadom, consistently stimulate intake of standard laboratory chow. Thus, the kappa receptor subtype may be more important than other opioid receptors in the regulation of normal feeding patterns (Cooper, Jackson, & Kirkham, 1985).

As with the antagonist effects, there is currently a great deal of interest regarding the effects of kappa agonists on the intake of preferred substances. In one study, Lynch (1983) reported that the mixed kappa/mu agonist ketocyclazocine stimulated intake of a saccharin solution in comparison to a control (saline) group in nondeprived rats, while Jackson and Cooper (1985) and Cooper et al. (1985) found that the kappa agonist U-50,488H significantly heightened rat intake of highly palatable solid food mixtures. These studies and others (e.g., Leander & Hynes, 1983; Kavaliers, Teskey, & Hirst, 1985) suggest that the kappa opioid receptor system is important for the expression of taste preferences in addition to the more general regulation of feeding patterns. Thus, the effects of both mu and kappa agonists on the intake of preferred sweet solutions in nondeprived animals should be investigated. Such studies would provide evidence concerning the differential effectiveness of agonists which bind with these receptor subtypes.

In order to understand the functions of the opioid system in the regulation of feeding and drinking, it is important to elucidate the

cause and timing of the release of the opioids. One approach for investigating these questions has been to assess actual changes in brain opioid levels in response to various stimulus events through the use of radioimmunoassay techniques. For example, Morley, Elson, Levine, and Shafer (1982) found increased cortical levels of dynorphin-like immunoreactivity in response to stressful events like deprivation and tail-pinch. In another study (Vaswani and Tejwani, 1986) it was reported that food deprivation results in increased and/or decreased levels of B-endorphin in different brain regions, which return to normal following intake. A related study looked at the locations of kappa receptors in nonstressed, nondeprived rats and found high concentrations in a number of gustatory and feeding sites (Lynch, Watt, Krall, & Paden, 1985). Of particular importance is the finding by Dum, Gramsch, and Herz (1983) that ingestion of sweet foods results in higher levels of B-endorphin (an endogenous opioid) in the hypothalamus.

Other researchers have provided less direct evidence for release of opioid peptides by showing that increased feeding behavior resulting from stress is blocked by the administration of opiate antagonists. Lowy, Maickel, and Yim (1980), for example, reported that stressors such as deprivation and tail-pinch induced rats to eat and such feeding behavior was blocked by NAL. A more recent study (Bertiere, Sy, Baights, Mandenoff, and Apfelbaum, 1984) demonstrated that stress-induced feeding is blocked not only by NAL, but also by injections of B-endorphin (an opiate agonist) into the ventricles. The authors employed learning principles as a framework for

understanding this apparently contradictory finding: the antagonist interfered with the rewarding properties of the food while the agonist made ingestion unnecessary because it produced satiation.

A related series of studies by Lieblich and associates have shown that rats which are genetically selected for high rates of self-administered brain stimulation also tend to consume large amounts of saccharin. After repeated ingestion of saccharin they demonstrate decreased analgesic responses (i.e., tolerance) to morphine (Lieblich, Cohen, Ganchrow, Blass, & Bergmann, 1983; Cohen, Lieblich, & Bergmann, 1984; Bergmann, Cohen, & Lieblich, 1984). These researchers suggest that repeated saccharin consumption stimulates release of EOP's as the result of activation of sweet receptors in the gustatory system, leading to tolerance development.

The results cited above are poorly integrated and it remains unclear what events (either physiological or environmental) are associated with endorphin release. One explanation for the role of the opiates in the regulation of feeding and drinking is derived from drive theory. This theory suggests that EOP release directly motivates, or "drives", the organism to ingest nutrient-rich materials (Leibowitz, 1985). This idea fails, however, to explain why agonist drugs enhance ingestion only under certain circumstances or how antagonists reduce intake which is motivated purely by taste (Lynch, 1983; Jackson & Cooper, 1985).

An alternative explanation is that certain behaviors are reinforced by the release of endorphins and it is this property that accounts for the establishment and/or maintenance of taste

preferences. In support of this view, Mucha and Iversen (1984) have shown via a conditioned place preference paradigm that morphine is rewarding and NAL is aversive (since it leads to conditioned place aversion). Additional support for the idea that opiates are rewarding comes from studies demonstrating that animals will self-administer opiates (eg. Belluzi & Stein, 1977). Finally, although opioid agonists sometimes stimulate intake, they do so for only certain concentrations (Lynch and Libby, 1983), a result which suggests that opiate receptor stimulation does not directly induce intake.

The involvement of the opioid receptor system in taste preferences is particularly well suited to a conditioning explanation since preference can be conceptualized as a learned behavior. For instance, if an animal has no physiological need to ingest, but does so nonetheless, it may be concluded that the animal is being reinforced in some manner for engaging in the ingestive behavior. Since EOP's appear to be involved in the acquisition of taste preferences, it seems reasonable to suggest that these peptides might normally act as reinforcers for the learning of preferences. To test this idea, the EOP's should be studied within an operant conditioning paradigm so as to investigate whether the relationship between these endogenous peptides and preference acquisition is one involving direct motivation, inspired by a drive, or instead involves reinforcement for learning a preference.

Support for this notion of learned preferences comes from a study by Dum and Herz (1984) which provided evidence for the release

of EOP's in response to the anticipation of receiving highly palatable substances. In this experiment, rats which had been taught to expect a sweet substance apparently released opioids since these animals demonstrated a NAL-reversible decrease in sensitivity to painful stimuli as compared to control animals.

To summarize, the opioid antagonists decrease intake and agonists sometimes increase intake. These effects, however, do not demonstrate a degree of reliability across situations that would be expected if there were a direct connection between receptor stimulation and ingestion. Since agonists do not directly stimulate intake it may be that they act indirectly by reinforcing behaviors which are conducive to ingestion. In other words, EOP's may be released in response to environmental cues that indicate the presence of appropriate foods, and this release may signal an organism to increase intake. If opioids are inherently rewarding, and if they encourage ingestion in the absence of physiological need, then it may be that the mechanism of opiate action involves operant conditioning rather than strictly motivation (i.e., drive).

In order to investigate that possibility, the timing and extent of opioid release should be examined. This could be accomplished by examining the events which cause the release of EOP's and the temporal pattern of such release. Specifically, it would be important to assess whether gustatory stimulation and/or the environmental cues that predict such stimulation (i.e., expectancy) result in maximal EOP release.

In addition to the operant conditioning idea outlined above, there are several subsidiary issues that deserve attention. For instance, the role of EOP's in regulating ingestion may be different for nutritive vs non-nutritive substances. This idea is indirectly supported by the results of studies showing that the blockade of preference acquisition by NAL does not decrease over time for saccharin (Lynch, 1986) but that it does decrease over time for sucrose (Olson et al. 1985). It is, therefore, also important to directly investigate both saccharin and sucrose within the same study to see if such a difference does exist.

Another important question concerns the permanence of the NAL effects. Lynch, Krall, Fernandez, and Paden (1985) showed, using autoradiographic receptor assays, that chronic blockade of opioid receptors caused an increase in the number of these receptors (upregulation). An upregulation of receptors could result in long-lasting alterations in taste preference. A logical next step is to investigate the effects of repeated exposure to NAL by first blocking the normal preference acquisition, then measuring the recovery of preference following removal of the antagonist. This type of study would provide additional data suggesting how the EOP's may be involved in the acquisition and maintenance of taste preferences.

The following experiments were designed to provide answers to the questions raised above. In Experiment 1 the intake of animals who were drinking for taste was blocked by administration of the opiate antagonist naloxone. Such blockade has been reported previously. The purpose of Experiment 1 was to extend these results

by reversing the order of the conditions so as to measure the recovery of preference in the NAL-treated animals and to investigate the effect NAL would have on animals which had already acquired a taste preference. It was expected that the antagonist would have a dramatic effect on the latter group, but that their drinking behavior would not be totally eliminated because of the strength of the learned response. In addition, it was thought that the animals which started out receiving NAL injections would, consequently, acquire preferences at an elevated rate based on reports that antagonist treatments cause receptor upregulation which would, presumably, result in extreme sensitivity of the rats to any subsequent taste-motivated EOP release.

In Experiment 2, the differential effects of mu and kappa-receptor agonists on intake of a sucrose solution was investigated. Based on previous reports that kappa agonists generally yield more reliable effects than agonists of other opioid receptor subtypes, it was expected that the kappa agonist would increase intake to a greater degree than would the mu agonist. What was new in this design was the addition of a recovery period during which all drugs were removed while the animals were allowed to continue their drinking of the sweet solution. It was predicted that, if any of the groups showed elevated intake, these levels would consequently decrease and approach that of the control group once the agonist drug was removed.

In Experiment 3, the idea that endogenous opioids may serve to reinforce ingestive behavior was investigated by attempting to

determine the time course of release of these compounds in response to feeding behavior. In this experiment, a NAL-reversible increase in analgesia (i.e., increased latency to tail-flick in response to a heat stimulus) was taken as an indication that an increase in the release of EOP's had occurred within the brain. The magnitude of the analgesic response is presumably proportional to the amount of the release. It was expected that both the actual ingestion of a sweet solution and the learned anticipation of such intake would result in opioid release, as indicated by increased tail-flick latency. This hypothesis is based on reports in the literature which indicate that both ingestion of preferred substances and the anticipation of ingesting preferred foods cause EOP release (Dum & Herz, 1984).

Taken together, these experiments should provide additional evidence concerning the role of endogenous opioids in the regulation of ingestion. One function of the EOP's may be to reinforce anticipatory as well as ingestive behaviors. Since these anticipations would be learned responses reinforced by the release of EOP's, a learning approach is an appropriate framework for investigating the relationship between ingestive behavior and these endogenous compounds.

EXPERIMENT 1

The purpose of Experiment 1 was to replicate and extend the work of Lynch (1986), who found that NAL interferes with the acquisition of taste prferences, by comparing the intake of sucrose or saccharin solutions in rats given NAL for 10 days to rats who received saline. After this period the antagonist was removed and all animals received injections of saline in order to investigate the long term effects of opioid receptor blockade (see Table 1). Both sucrose and saccharin were used to see if NAL has differential effects on these two sweeteners.

Table 1. Treatment Conditions for Experiment 1

		Drug Treatments		
Group	Solution	Days 1-10	Days 11-15	Days 16-20
1	sucrose	NAL	SAL	SAL
2	saccharin	NAL	SAL	SAL
3	sucrose	SAL	SAL	NAL
4	saccharin	SAL	SAL	NAL

Method

Subjects

Forty male Holtzmann Sprague Dawley albino rats, each weighing approximately 150-200g upon arrival, were purchased from Sasco Inc. for use in this experiment. The animals were housed in groups of five and had free access to food and water except during testing.

Apparatus

Intake tests occurred in ten individual test cages each of which was fitted with two standard 100 ml volumetric drinking bottles. One of these bottles contained water and the other either 20% sucrose (w/v) or 0.1% sodium saccharin (w/v) dissolved in water.

Procedure

The animals were randomly assigned to the four treatment conditions. Each day the animals were brought into the experimental room and given subcutaneous injections of either NAL (1mg/kg) or SAL (1ml/kg). Twenty minutes after injection, each animal was placed into a test cage where it was presented with two bottles, one containing water and the other the sweet solution. The rat was then allowed to drink ad lib. for a period of 30 minutes, after which it was returned to the home cage and the total volume of water and sweet ingested was recorded.

The experiment was run for a total of 20 days. During the initial preference acquisition phase (days 1-10) the intake of all animals was monitored following daily injections of either naloxone or saline. This phase was followed by five days of recovery (days 11-15) during which the NAL was removed and all animals received SAL injections while still experiencing the same procedures outlined above. The final five days of the experiment (days 16-20) involved a crossover of the initial drug treatments such that the animals who began the experiment with NAL (but had been receiving SAL for five days) continued to be injected with SAL, while the others (which had

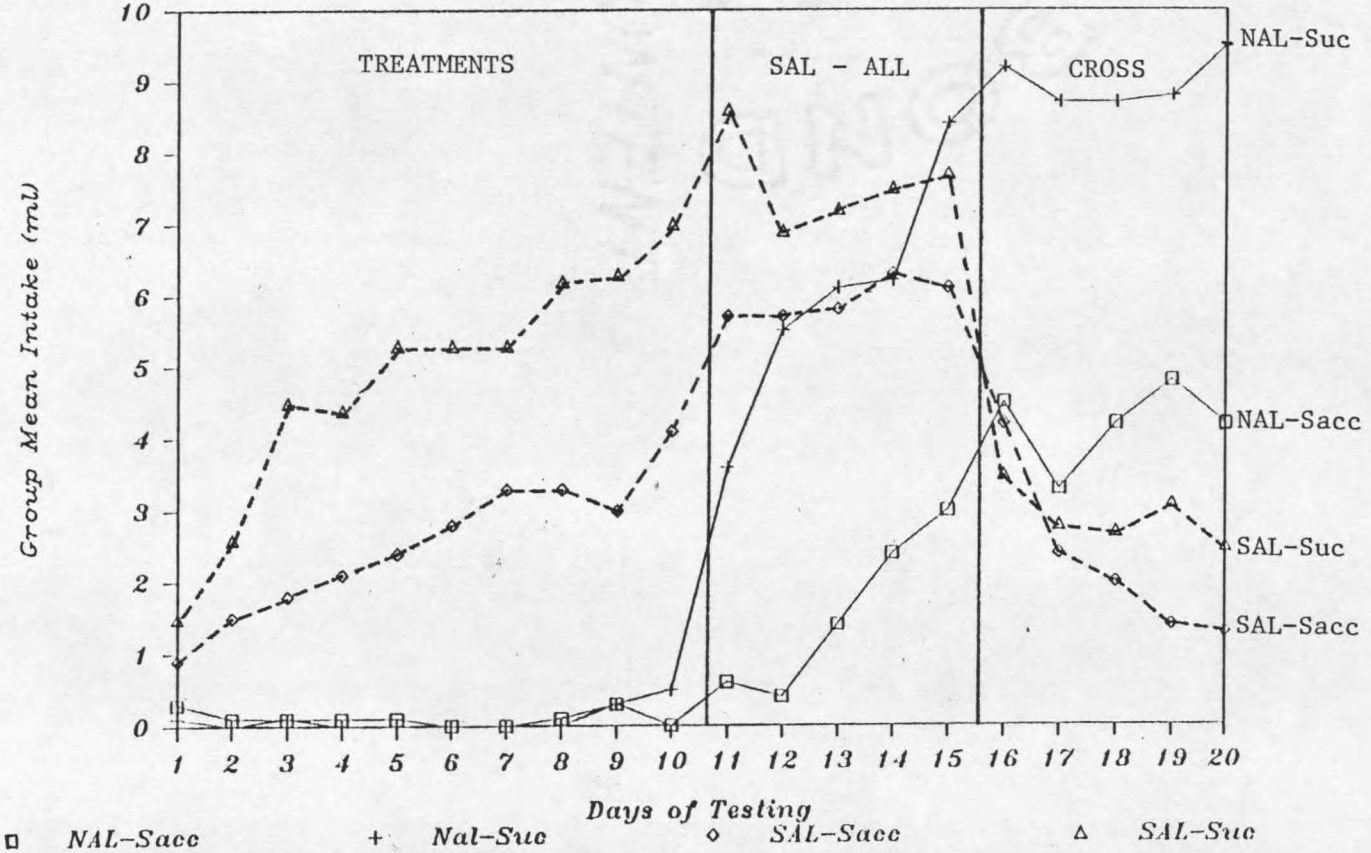
been injected with SAL up to this point) received NAL (1 mg/kg, sc).

Results

Figure 1 illustrates the effect of NAL on group mean intake volume over the 20 days of testing. It is apparent that the antagonist had a strong effect on the intake levels of the animals, in that naloxone suppressed intake of both sucrose and saccharin regardless of whether it was administered before, or after, a taste preference had been learned. Also of interest is the elevated intake of the group which originally was treated with NAL and offered sucrose, at day 20 of the experiment.

A 2 x 2 x 10 three-way Analysis of Variance (ANOVA) for split-plot designs was calculated on the data for days 1-10 (the acquisition period, see Table 2). The analysis revealed that drug type did reliably affect intake, $F(1, 36) = 74.22$, $p < .001$. Here, NAL reliably blocked ingestion. The analysis also revealed that solution type reliably affected intake, $F(1, 36) = 7.75$, $p < .01$. Here sucrose was ingested in larger amounts than saccharin. A significant interaction between drug and solution type was found to be present, $F(1, 36) = 7.88$, $p < .01$. This indicated that the animals which were injected with SAL consumed more sucrose than saccharin while the NAL-treated animals drank very little of either solution. The analysis also revealed that days reliably affected intake, $F(9, 324) = 15.45$, $p < .001$. Here, the amount of sweet ingested by the SAL groups increased over days. Also there was a

FIGURE 1. *Naloxone Effect on Sweet Intake*



significant interaction of days and drug, $F(9, 324) = 14.79$, $p < .001$. This confirms that the SAL-treated animals increased consumption over days while the NAL-treated animals did not. There was also a significant interaction of days and solution, $F(9, 324) = 2.03$, $p < .05$. This finding reveals that the SAL-treated animals ingested more sucrose than saccharin over days.

Table 2. Analysis of Variance for Experiment 1, Days 1-10.

Source	df	SS	MS	F
Between	39	2167.08	55.57	-
Drug	1	1278.06	1278.06	74.22***
Solution	1	133.40	133.40	7.75**
Drug X Solution	1	135.72	135.72	7.88**
error	36	619.89	17.22	-
Within	360	556.85	1.55	-
Days	9	158.08	17.61	15.45***
Days X Drug	9	151.71	16.86	14.79***
Days X Solution	9	20.77	2.31	2.03*
Days X Drug X Solution	9	14.35	1.59	1.39
error	324	370.02	1.14	-

* $p < .05$
** $p < .01$
*** $p < .001$

A 2 x 2 x 6 three-way ANOVA for split-plot designs (see Table 3) was performed on the intake volume data from days 10-15 (the recovery period). This analysis revealed a reliable overall difference between groups due to drug history, $F(1, 36) = 28.15$, $p < .001$. This indicates that the groups which had received SAL all along continued to ingest more sweet than the groups which began the experiment with NAL injections. The analysis also revealed a significant effect of solution type, $F(1, 36) = 19.49$, $p < 0.001$. Here, sucrose was ingested in greater amounts than saccharin in all groups. Again, as

in the previous analysis, there was a significant effect of days, $F(5, 181) = 22.33$, $p < .001$. This indicates that the amount ingested increased over the five days. A significant interaction was revealed between days and drug history, $F(5, 181) = 10.28$, $p < .001$. This indicates that the NAL-treated animals (now treated with SAL) increased intake over days while the intake of the SAL-treated animals changed very little. Finally, a significant interaction of days, drug and solution was revealed, $F(5, 181) = 6.68$, $p < .001$. This indicates that the groups which were changed from NAL to SAL increased intake and drank progressively more sucrose than saccharin over days.

Table 3. Analysis of Variance for Experiment 1, Days 10-15.

Source	df	SS	MS	F
Between	39	2083.96	53.43	-
Drug	1	683.44	683.44	28.15*
Solution	1	473.20	473.20	19.49*
Drug X Solution	1	53.20	53.20	2.19
error	36	874.12	24.28	-
Within	201	652.76	3.25	-
Days	5	265.74	53.15	22.33*
Days X Drug	5	122.34	24.47	10.28*
Days X Solution	5	19.97	3.99	1.68
Days X Drug X Solution	5	79.47	15.89	6.68*
error	181	430.98	2.38	-

* $p < .001$

A 2x2x6 three-way ANOVA for split-plot designs, was calculated on the intake volume data for days 15-20 (the crossover period). This analysis revealed that reversal of the drug treatments reversed the drinking patterns of the groups (see Table 4). Specifically, the former NAL-treated group (now treated with SAL) drank significantly more than the former SAL group (now treated with NAL), $F(1, 36) =$

37.05, $p < .001$. As in the previous analyses, there was a significant effect of solution, $F(1, 36) = 30.65$, $p < .001$. Here, all groups ingested more sucrose than saccharin. The analysis also revealed a reliable drug and solution interaction, $F(1, 36) = 15.90$, $p < .001$. This result indicates that the enhanced intake of sucrose, as compared to saccharin, was even more pronounced for the animals which had begun the experiment with NAL treatments than it was for those animals which began with SAL but later were treated with NAL.

Table 4. Analysis of Variance for Experiment 1, Days 15-20.

Source	df	SS	MS	F
Between	39	1896.92	48.64	-
Drug	1	589.07	589.07	37.05*
Solution	1	487.35	487.35	30.65*
Drug X Solution	1	248.07	248.07	15.60*
error	36	572.43	15.90	-
Within	201	543.93	2.71	-
Days	5	127.40	25.48	17.45*
Days X Drug	5	251.08	50.22	34.40*
Days X solution	5	13.70	2.74	1.88
Days X Drug X Solution	5	14.78	2.96	2.02
error	181	264.37	1.46	-

* $p < .001$

During this crossover period there was a reliable effect of days, $F(5, 181) = 17.45$, $p < .001$. This indicates that intake varied across days. A significant interaction between days and drug $F(5, 181) = 34.40$, $p < .001$ was also found. This reveals that the animals that had begun the experiment with SAL treatments (now treated with NAL) decreased intake over days while the NAL animals (now treated with SAL) increased intake.

To determine whether pretreatment with NAL results in different

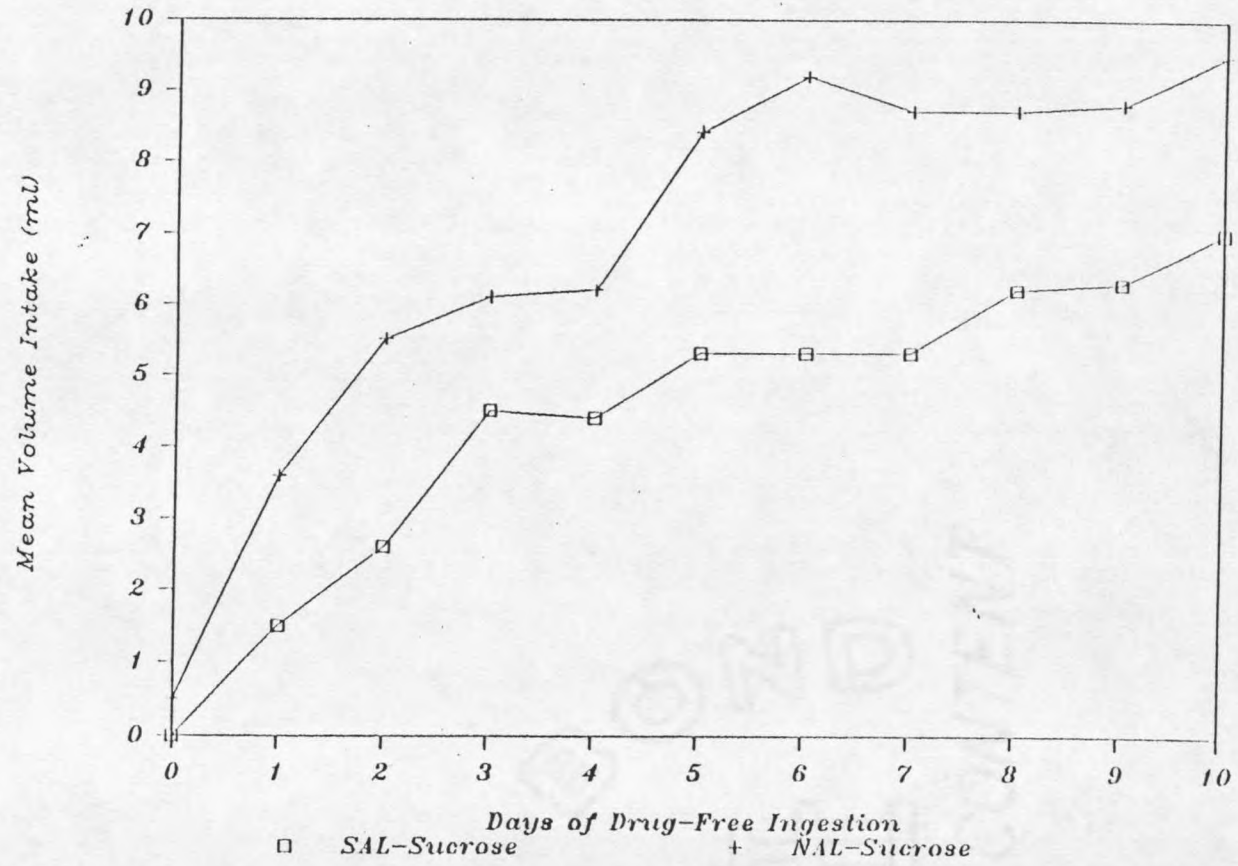
acquisition rates, a post hoc comparison was made between the level of sucrose intake on the fifth day of ingestion for the SAL control groups (day 5 of the experiment) and the fifth day of ingestion for the NAL groups (day 15 of the experiment). This analysis revealed a significant difference between controls and the animals previously treated with naloxone, $t(18) = 2.702$, $p = .015$, (see Figure 2). This result demonstrates that, after removal of the antagonist, the animals previously treated with NAL developed a stronger preference for sucrose within the same period of time as controls.

Discussion

This experiment demonstrates that sweet taste-motivated behavior can be disrupted by blockade of opioid receptors. The animals which received injections of NAL drank essentially no sweet solution while the control animals developed normal taste preferences for both sucrose and saccharin, although the sucrose preference was clearly stronger. Removal of the antagonist in the NAL-treated animals resulted in a recovery of taste preference. These animals consequently drank the sweet solutions in large amounts, and, as expected, they quickly developed preference for both palatable liquids.

The finding that, once the NAL was removed, the NAL-treated animals attained higher levels of intake than the control animals (who had received no antagonist treatment) may indicate that the daily pretreatment with NAL for 10 days resulted in fundamental changes in the opioid receptor system. Interestingly, the animals

FIGURE 2. *Effect of Drug History on Preference*



which were offered sucrose drank consistently more than did their saccharin-consuming counterparts across all experimental conditions.

Also of interest is the finding that when NAL was administered to those animals which had already acquired preferences, their level of intake was not completely eliminated. This implies that the EOP's are somehow important during the learning of taste preferences, but that such preferences can later be maintained in the absence of the peptides. This maintenance of the drinking behavior in the absence of opioid receptor stimulation argues against a drive theory explanation of EOP regulation of feeding, since such an explanation requires the dependancy of ingestion on a fully functioning opioid receptor system.

EXPERIMENT 2

The purpose of Experiment 2 was to test the possibility that the rewarding effects of opioid agonists might facilitate the intake of a 20% sucrose solution by repeated association of intake with either the mu agonist morphine or the kappa agonist U-50,488H. Intake was measured under four treatment conditions: a low dose of U-50, a high dose of U-50, morphine, or saline (see Table 5). Sucrose was used instead of saccharin in this study, following a suggestion made by Cooper (personal communication) that U-50,488H might mediate an increased intake of sucrose but may not have the same effect on saccharin. Also, U-50,488H was employed along with morphine because it is quite specific in affinity for kappa receptors which have been implicated in taste motivated intake (Lynch, 1983; Jackson & Cooper, 1985).

Table 5. Treatment Conditions for Experiment 2

Group	Days 1-10	Days 11-15
1	U-50L	SAL
2	U-50H	SAL
3	MOR	SAL
4	SAL	SAL

Method

Subjects

Forty male rats of the same strain were purchased from the same supplier as in Experiment 1. As in the first experiment, the animals

were housed in groups of five and allowed ad lib. access to food and water, except during testing.

Apparatus

The same test cages, as described in Experiment 1, were employed for presentation of water and sweet solutions. Each test cage was fitted with one bottle containing tap water and another bottle containing a 20% (w/v) sucrose solution.

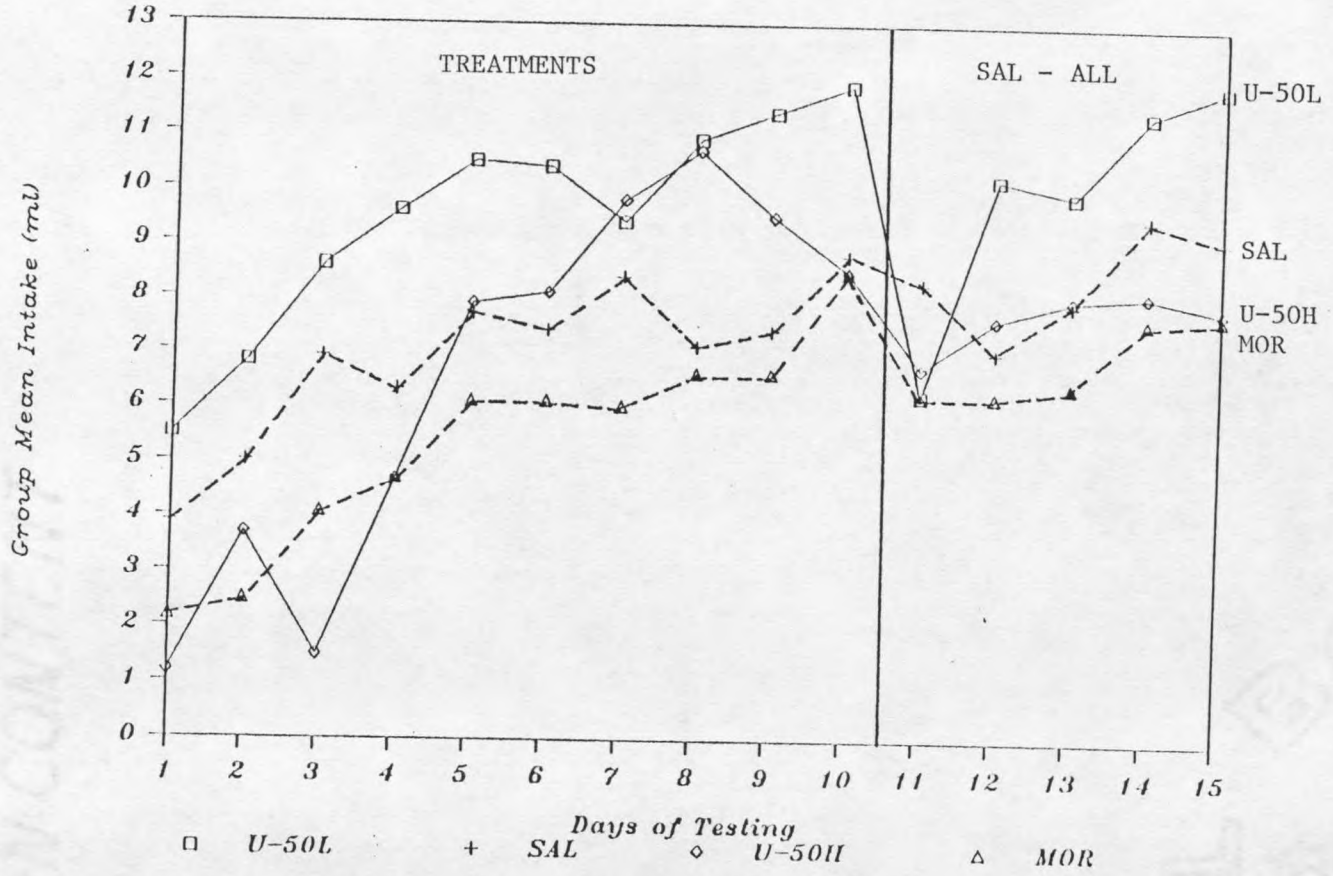
Procedure

The animals were randomly assigned to the four treatment conditions. Each day the animals were brought into the experimental room and administered one of four subcutaneous injections: 0.3 mg/kg U-50,488H (U-50L group); 1.0 mg/kg U-50,488H (U-50H group); 1.0 mg/kg morphine (MOR group); and 1.0 ml/kg saline (SAL group). 30 minutes after receiving the injection each animal was placed individually into a test cage for 30 minutes. After this intake period the animal was returned to the home cage and the intake volume was recorded. This occurred for 10 days and was followed by a 5 day recovery period during which the drug was removed from all animals and each received daily injections of SAL while their intake volumes continued to be recorded.

Results

Figure 3 illustrates the differential effects of agonists on intake volumes during 10 days of treatment and 5 days of recovery. It appears that the low dose of U-50,488H elevated intake

FIGURE 3. *Agonist Effects on Sucrose Intake*



consistently over the 10 days of treatment as compared to saline, and this higher level was maintained 5 days after the drug treatments ended. Neither of the other drug groups differed from the saline control group in intake volumes either before, or after, drug treatment.

A 2 x 2 x 10 three-way ANOVA for split-plot designs (see Table 6) calculated on the intake volume data for the first ten days revealed a significant main effect of drug treatment, $F(3, 36) = 7.10$, $p < .001$. This indicates that the treatment groups differed in the amount of sucrose intake. The analysis also revealed a significant effect of days, $F(9, 324) = 40.09$, $p < .001$. This result indicates that the volumes changed over days. A reliable interaction between days and drug, $F(27, 324)$, $p < .001$, was also revealed by the analysis. This demonstrates that the drug effects changed over days. A post hoc comparison, to determine if the U-50L group ingested more sucrose on day 10 than did the SAL group, was significant, $t(18) = 2.963$, $p = .0083$.

Table 6. Analysis of Variance for Experiment 2, Days 1-10.

Source	df	SS	MS	F
Between	39	2469.20	63.31	-
Drug	3	919.03	306.03	7.10*
error	36	1550.17	43.06	-
Within	360	1856.98	5.16	-
Days	9	1612.72	179.19	40.09*
Days X Drug	27	408.05	15.11	3.38*
error	324	1448.93	4.47	-

* $p < .001$

A 2 x 2 x 6 three-way ANOVA for split-plot designs (see Table 7)

calculated on the recovery data (days 10-15) revealed a significant effect of drug history, $F(3, 36) = 3.01$, $p < .05$. This indicates a difference in intake between the treatment groups. The analysis also revealed a significant interaction between days and drug history, $F(15, 181) = 1.91$, $p < .05$. This result indicates that the different intakes between groups changed over days, with the U-50L group maintaining intake levels above the SAL group.

Table 7. Analysis of Variance for Experiment 2, Days 10-15.

Source	df	SS	MS	F
Between	39	1817.96	46.61	-
Drug	3	364.08	121.36	3.01*
error	36	1453.88	40.38	-
Within	201	837.16	4.16	-
Days	5	183.67	36.73	9.21**
Days X Drug	15	114.15	7.61	1.91*
error	181	723.01	3.99	-

* $p < .05$
** $p < .001$

Discussion

This experiment was designed to measure the effect of subcutaneous injections of opioid agonists on ingestion of preferred sucrose solutions. As expected, the kappa agonist U-50,488H increased intake, but only when the dose was a relatively small one.

Five days after removal of the drugs, the U-50H, MOR, and SAL groups were not consuming significantly different volumes of sweet, but the U-50L group did demonstrate a marginally significant increase in intake compared to the SAL control group. What is indicated by these results is that non-deprived animals which are

drinking purely for sweet taste will increase their intake if a low dose of a kappa agonist is presented concurrently with ingestion.

It remains unclear if this facilitation of preference learning is due to a modification of taste quality or to the strength of the learned response which may have been reinforced by the repeated administration of exogenous opioid agonists. Again, as in Experiment 1, the maintenance of the preference in the absence of the agonist argues against a drive mechanism of opioid regulation of feeding.

EXPERIMENT 3

The purpose of Experiment 3 was to investigate the effect of ingestion, and the anticipation of ingestion, of a highly palatable solution on the release of EOP's. The release of EOP's was assessed by measuring sensitivity to a painful stimulus, reasoning (as did Cohen et al., 1984; Bergmann et al., 1984; and Dum & Herz, 1984) that a NAL-suppressible decrease in sensitivity to pain indicates the release of endogenous opioids. The analgesic response measure used in this experiment was the latency to tail-flick in response to a high-intensity beam of light (see D'Amour and Smith, 1941).

Method

Subjects

Forty male rats of the same strain, and purchased from the same supplier, as in Experiments 1 and 2 were also used in this experiment. These were housed in groups of five, allowed ad lib. access to food (except during testing), and deprived of water for 18 hours each day immediately prior to testing.

Apparatus

The same test cages, as described in Experiments 1 and 2, were employed for presentation of the water and sweet solutions. The sweet solution used in this experiment was a mixture of 3% glucose and .125% saccharin which has been demonstrated to induce large

intakes in laboratory rats (Smith & Foster, 1980). The analgesic response was defined as the latency to tail-flick and was measured by a device designed and built in our lab (see Figure 4). This device directs a semi-focused beam of light from a 150 watt G. E. projector lamp (type EKL) onto a blackened spot 4 cm from the base of each rat's tail. A digital timer is activated simultaneously with the onset of the light and measures to within 10 msec the time it takes for the animal to flick its tail away from the heat source. A photoresistor under the tail detects the light after the tail has moved and automatically stops the timer and turns off the light source. The animals were lightly restrained by hand by the experimenter while in this device which left the tail free to move.

Procedure

All animals were deprived of water in their home cages for 18 hours immediately prior to each training and testing session.

Days 1-10 comprised the training portion of the experiment. Subjects in groups 1 and 2 (the sweet and water pre-intake groups, respectively) were brought into the experimental room, injected subcutaneously with 1ml/kg SAL, and 15 minutes later placed individually in the tail-flick apparatus for 15 seconds without the heat stimulus being engaged. The animals in group 1 were placed into test cages which were fitted with two bottles, one containing water and the other a highly preferred mixture of 3% glucose + .125% saccharin. The group 2 animals were placed in test cages which also contained two bottles, both of which contained tap water. The animals