INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

UMI®

ROLE OF THE NUCLEUS ACCUMBENS IN THE MEDIATION OF OPIOID-INDUCED FEEDING IN RATS.

by

ANDRÉ K. RAGNAUTH

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, City University of New York.

2000

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

9/21/2000
Date

Richard J. Bodnar, Ph. D
Chair of Examining Committee

9/21/2000
Date

Joe Glick
Executive Officer

Benjamin Kest, Ph. D.

Kenneth D. Carr, Ph. D.

Thomas E. Frumkes, Ph. D.

Donald W. Pfaff, Ph. D.

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK

ABSTRACT

The opioid system has been shown to be one of the most important neurochemical systems involved in centrally mediating ingestive behaviors. Following peripheral, systemic and site-specific injections, opioid agonists and antagonists respectively induce and reduce feeding in rats. Because of the existence of multiple opioid receptor subtypes (μ, δ and κ), an obvious question is what is the nature of the interactions between the receptor subtypes and their various agonists and antagonists *vis a vis* the induction or attenuation of feeding. The nucleus accumbens is a forebrain structure implicated in the mediation of motivated behaviors, including food intake. Whereas its core region appears to share commonalities with the neighboring neostriatal motor system, its shell region appears similar to the extended amygdala and limbic system in terms of anatomical connections, histology and cytology. The ventral tegmental area is the primary source of dopaminergic projections to the nucleus accumbens, and in turn receives reciprocal projections. Likewise, it has been implicated in affecting motivated behaviors through dopamine input to the nucleus accumbens where it interacts with opioids in the shell region.

The first aim of this dissertation examined the role of general (naltrexone), μ (β-funaltrexamine), μ_1 (naloxonazine) or κ (nor-binaltorphamine) opioid receptor subtype antagonists in mediating feeding induced by deprivation, glucoprivation and palatable conditions following intracerebral injections into the shell region of the nucleus accumbens. Data indicate that general, μ and κ, but not μ_1 opioid antagonists in the NAcc each significantly decreased deprivation-induced intake. Further, general, μ and κ opioid antagonists in the NAcc each significantly reduced 2-deoxy-D-glucose (2DG)-induced

feeding. Finally, while general and μ opioid antagonists in the NAcc significantly decreased sucrose intake, the κ antagonist was ineffective. These data established the NAcc as an integral structure in mediating different forms of feeding controlled by opioid receptor blockade.

The second aim of the dissertation was to examine whether the ventral tegmental area (VTA) shared similarities with the NAcc by evaluating effects upon feeding under deprivation, glucoprivic and palatable conditions following general (naltrexone), μ (β-funaltrexamine), κ (nor-binaltorphamine), δ_1 (DALCE) or δ_2 (naltrindole isothiocyanate) opioid receptor subtype antagonist microinjections. The data from the second series of studies clearly showed that opioid antagonists effects in the VTA were far less effective than in the NAcc. Only general and δ_2 opioid antagonism in the VTA significantly decreased deprivation-induced intake at high doses; μ, κ_1 and δ_1 opioid antagonists in the VTA were ineffective. An identical pattern of antagonist results was observed for the VTA in mediating 2DG-induced intake. Whereas sucrose intake was significantly decreased by VTA microinjections of general and δ_2 antagonists, μ, κ_1 and δ_1 opioid antagonists were again ineffective.

Analgesic studies strongly suggest receptor subtype specificity between opioid agonists and antagonists, while feeding studies have shown that feeding induced by selective opioid agonists can be mediated through multiple (and probably trans-synaptic) receptors. This issue was explored in the third aim by challenging opioid agonist-induced feeding by selective μ (DAMGO), δ_1 (DPDPE) and δ_2 (deltorphin) agonists microinjected into the shell region of the NAcc following intracerebral pretreatment with general, μ, μ_1, κ_1, δ_1 and δ_2 opioid antagonists. Pretreatment with selective μ, but not μ_1 opioid receptor subtype

antagonists reduced DAMGO-induced feeding. Additionally, δ_2 and κ_1, but not δ_1 opioid receptor subtype antagonists also reduced NAcc DAMGO-induced feeding. Therefore, it appears that the multiple μ, δ_2 and κ_1 opioid receptors antagonists, but not μ_1 or δ_1 opioid receptor antagonists administered into the NAcc block feeding induced by the μ opioid agonist, DAMGO. A somewhat similar pattern of effects was observed for antagonist effects upon DPDPE-induced feeding in the NAcc with μ, δ_1, δ_2 and κ_1, but not μ_1 opioid receptor antagonists effective in blocking this ingestive response. An important exception to this pattern was observed for deltorphin-induced feeding in the NAcc. Whereas δ_2 opioid antagonism failed to alter this ingestive response, both μ and κ opioid antagonists augmented NAcc deltorphin-induced feeding. Thus, multiple opioid receptors in the NAcc mediate feeding induced by individual opioid receptor subtype agonists with the pattern of antagonist effects dependent upon the selective agonist employed.

Given that a major modulatory input to the NAcc is dopaminergic innervation from the VTA, the fourth and final group of experiments ascertained whether feeding induced by opioid agonists in the shell region of the NAcc was altered by intracerebral pretreatment with selective dopamine D_1 or D_2 receptor antagonists. The selective D_1 antagonist, SCH23390 dose-dependently and significantly reduced NAcc DAMGO-induced feeding at higher doses with the selective D_2 antagonist, raclopride, producing less consistent effects. In contrast, neither SCH23390 nor raclopride produced any consistent inhibition of deltorphin-induced feeding in the NAcc. These data suggest that dopamine, considered to be a major modulatory influence upon feeding and other motivated behaviors in the shell region of the NAcc plays only a minor role in mediating opioid-induced feeding in this nucleus.

This dissertation firmly established the NAcc, and secondarily the VTA, as putative sites of action at which opioid peptides and their receptors act to modulate feeding under a wide variety of conditions.

Acknowledgments:

I would like to thank all my committee members and outside readers: Drs. Ken Carr, Tom Frumkes, Ben Kest and Don Pfaff for their advice and suggestions. I'm particularly grateful to Dr. Don Pfaff for the opportunity given to me to work in his laboratory. I would also like to thank the following individuals for their discussions on neuroscience over the years (Marcello Spinella, Dr. Frummer, Michael Schmidt, Malgorzata Moroz, Kurt Kreiter, Liza Leventhal, Maria Hadjimarkou and Rob Silva), their collaborations in the experiments comprising my dissertation (Michael Glass, Malgorzata Moroz, Vladimir Znamensky, Madeline Cooper and Hildegard Ruegg) and for their assistance and advice during the preparation of the dissertation (Maria Hadjimarkou).

I am forever indebted to Dr. Richard Bodnar, my mentor, for the privilege of working in his lab and for imbuing me with his wisdom, friendship and love of science.

Dr. B made me into a scientist. Thank you sir.

This dissertation is dedicated to my late mom, Farida, my pops, Kumar, my sis, Elizabeth and my bro, Charles. Thanks for always being there. Without all of you, it would not have been possible. Thanks for putting up with me.

Mom and Dad, here's to you.

Table of Contents

ABSTRACT		**iv**
ACKNOWLEDGMENTS		**viii**
LIST OF FIGURES		**xiii**
LIST OF ABBREVIATIONS		**xx**
CHAPTER 1. INTRODUCTION.		**1**
	SPECIFIC AIMS	
I.	**Opioid peptides**	**4**
	A. Proopiomelanocortin (POMC)	**5**
	B. Pro-enkephalin	**6**
	C. Pro-dynorphin	**6**
	D. Pro-orphanin/Pro-nociceptin	**7**
	E. Endomorphins	**8**
II.	**Opioid receptors and selective agonists and antagonists**	**8**
	A. μ opioid receptors	**9**
	B. δ opioid receptors	**10**
	C. κ opioid receptors	**11**
	D. Pitfalls of Selective Agonists and Antagonists	**11**
III.	**Opioid receptor clones**	**12**
IV.	**Systemic and Ventricular Opioids and Ingestion**	**13**
	A. Endogenous opioid peptides and ingestion	**14**
	B. Opioid receptor subtype agonists and ingestive behavior	**15**

1. μ receptor compounds and food intake 15

2. δ receptor compounds and food intake 17

3. κ receptor compounds and food intake 18

V. **Central sites of action of opioid agonists and antagonists upon feeding** 19

VI. **Opioid-induced feeding along the VTA-NAcc Axis** 21

VII. **Anatomy and Projections of the NAcc** 24

A. Morphological distinctions of NAcc regions 24

B. Immunohistological distinctions of NAcc regions 26

VIII. **Rationale** 29

A. The Use of Multiple Feeding Models. 29

B. The PVN as a model system for the study of differential opioid antagonist effects upon different forms of ingestive behavior. 31

C. Specific Aim 1: 34

Discerning the role of the NAcc in mediating selective opioid receptor subtype antagonist effects upon different forms of feeding.

D. Specific Aim 2: 36

Discerning the role of the VTA in mediating selective opioid receptor subtype antagonist effects upon different forms of feeding.

E. Specific Aim 3: 38

Defining which opioid receptor subtypes mediate feeding induced by selective μ and δ opioid agonists in the NAcc.

F. Specific Aim 4: 43

Defining whether dopamine receptor subtype antagonists mediate feeding induced by selective μ and δ opioid agonists in the NAcc.

CHAPTER 2. GENERAL METHODS. 46

Subjects, Surgery and Histology 46

Statistical Analyses for Specific Aims 1 and 2 46

Statistical Analyses for Specific Aims 3 and 4 47

CHAPTER 3: EXPERIMENT 1. 48

Opioid Receptor Subtype Antagonists effects in the Nucleus Accumbens in Mediating Feeding Elicited by Food Deprivation, 2DG Glucoprivation and Exposure to Sucrose Solutions.

Methods: Experiment 1 48

Results: Experiment 1 50

Discussion: Experiment 1 60

CHAPTER 4: EXPERIMENT 2. 65

Role of Opioid Receptor Subtype Antagonists in the Ventral Tegmental Area in Mediating Feeding Elicited by Food Deprivation, 2DG Glucoprivation and Exposure to Sucrose Solutions.

Methods: Experiment 2 65

Results: Experiment 2 67

Discussion: Experiment 2 78

CHAPTER 5: EXPERIMENT 3. 86

Role of Opioid Receptor Subtype Antagonists in the Nucleus Accumbens Shell in Mediating Feeding Elicited by Opioid Receptor Subtype Agonists in the Nucleus Accumbens Shell.

Methods: Experiment 3 86

Results: Experiment 3 89

Discussion: Experiment 3 104

CHAPTER 6: EXPERIMENT 4. 111

Role of Dopamine Receptor Subtype Antagonists in the Nucleus Accumbens Shell in Mediating Feeding Elicited by Opioid Receptor Subtype Agonists in the Nucleus Accumbens Shell.

Methods: Experiment 4 111

Results: Experiment 4 113

Discussion: Experiment 4 119

CHAPTER 7: GENERAL DISCUSSION. 123

REFERENCES. 133

List of Figures:

Page

FIGURE 1. 52

Histological verification of cannula placements (asterisks) in the NAcc. Note that virtually all placements are found in the core region, and occupy the rostral two-thirds of the nucleus. The number adjacent to the placement refers to the number of animals in the three paradigms with placements at that location.

FIGURE 2. 54

Alterations in cumulative intakes (g, ±S.E.M.) following micro-injections of either naltrexone (Panel A), β-funaltrexamine (B, Panel B), naloxonazine (N, Panel B) or nor-binaltorphamine (Panel C) into the NAcc in rats deprived of food for 24 h. The daggers in this and subsequent figures denote significantly reduced intake relative to vehicle treatment (Tukey comparisons).

FIGURE 3. 56

Alterations in cumulative intakes (g, ±S.E.M.) following micro-injections of either naltrexone (N, Panel A), β-funaltrexamine (B, Panel B) or nor-binaltorphamine (N, Panel C) into the NAcc in rats receiving 2-deoxy-D-glucose (2DG).

FIGURE 4. 58

Alterations in cumulative sucrose (10%) intakes (g, ±S.E.M.) following

microinjections of either naltrexone (Panel A), β-funaltrexamine (B, Panel B) or nor-binaltorphamine (N, Panel B) into the NAcc.

FIGURE 5. 70

Histological verification of cannula placements in the VTA. Note that 24 of the animals had bilateral (n=48) cannula placements in the VTA. Seven animals had a unilateral (n=7) cannula placement in the VTA and the second (n=7) cannula placement in the medial aspect of the substantia nigra (SN), pars compacta. The following numbers of cannula placements were in the depicted areas: a (n=3), b (n=2), c (n=11), d (n=14), e (n=3), f (n=14), g (n=11) and h (n=4). Rats with bilateral VTA placements or VTA/SN combination placements did not differ in their antagonist-induced effects, and their data were pooled. The remaining seven animals had misplaced cannulae dorsal and/or lateral to the VTA, and were not included in the data analysis. They failed to display antagonist-induced effects.

FIGURE 6. 72

Alterations (Mean, ±SEM) in food intake (4 h) following microinjections (μg) of either vehicle (n=11), the general opioid antagonist, naltrexone (NTX) at doses of 10 (n=8), 20 (n=7) and 40 (n=7) μg, the μ opioid antagonist, β-funaltrexamine (β-FNA, n=8), the κ_1 opioid antagonist, nor-binaltorphamine (Nor-BNI, n=8), the δ_1 opioid antagonist, DALCE (n=5) or the δ_2 opioid antagonist, naltrindole isothiocyanate (NTII, n=5) into the VTA in rats deprived of food for 24 h. The daggers denote significantly

reduced intake relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$).

FIGURE 7. 74

Alterations (Mean, ±SEM) in food intake (4 h) following microinjections (μg) of either vehicle (n=10), naltrexone (NTX, upper panel) at doses of 20 (n=4) and 50 (n=5) μg, β-funaltrexamine (β-FNA, n=5, upper panel), nor-binaltorphamine (Nor-BNI, lower panel) at doses of 1 (n=4) and 4 (n=4) μg, DALCE (n=6, lower panel) or naltrindole isothiocyanate (NTII, lower panel) at doses of 1 (n=4) and 4 (n=5) μg into the VTA in rats receiving 2-deoxy-D-glucose (2DG: 500 mg/kg, ip). The stars denote significant increases in food intake in groups receiving 2DG relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$). The daggers denote significantly reduced 2DG-induced hyperphagia relative to VEH-2DG treatment (Tukey comparisons, $p<.01$).

FIGURE 8. 76

Alterations (Mean, ±SEM) in sucrose (10%) intake (1 h) following microinjections (μg) of either vehicle (n=10), naltrexone (NTX) at doses of 20 (n=6) and 50 (n=6) μg, β-funaltrexamine (β-FNA, n=6), nor-binaltorphamine (Nor-BNI, n=7), DALCE (n=5) or naltrindole isothiocyanate (NTII) at doses of 1 (n=7) or 4 (n=7) μg into the VTA. The daggers denote significantly reduced intake relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$).

FIGURE 9. 92

Schematic depiction of directions of bilateral guide cannulae placements positioned towards the shell region of the NAcc (AcbSh). The majority of the tips of the confirmed cannula placements were found in the shaded area. All placements were found medial to the core region of the NAcc (AcbC) and lateral to the ventral diagonal band (VNB) area. aca: anterior commissure. This schematic was based upon the Paxinos and Watson Brain Atlas (42).

FIGURE 10. 94

Alterations in spontaneous food intake (g, ± SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the NAcc shell relative to Control treatment. The upper and lower panels depict effects upon DAMGO-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective μ-opioid antagonist, β-funaltrexamine (β-FNA) or the selective μ_1-opioid antagonist, naloxonazine (NAZ) respectively. The asterisks (*) in this and subsequent figures indicate significant increases in food intake following opioid agonist treatment relative to Control treatment (Tukey comparisons, $p<0.05$). The crosses (+) in this and subsequent figures indicate significant alterations in food intake following opioid antagonist pretreatment relative to opioid agonist treatment alone (Tukey comparisons, $p<0.05$).

FIGURE 11. 96

Alterations in spontaneous food intake (g, ± SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon DAMGO-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective δ_1-opioid antagonist, DALCE or the selective δ_2-opioid antagonist, naltrindole isothiocyanate (NTII) respectively. The lower panel depicts effects upon DAMGO-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).

FIGURE 12. **98**

Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_1-selective opioid agonist, DPDPE (DPD) bilaterally in the NAcc shell relative to Control treatment. The upper and lower panels depict effects upon DPDPE-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective μ-opioid antagonist, β-funaltrexamine (β-FNA) or the selective μ_1-opioid antagonist, naloxonazine (NAZ) respectively.

FIGURE 13. **100**

Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_1-selective opioid agonist, DPDPE (DPD) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon DPDPE-induced feeding following pretreatment (24 h) with different

doses (μg) of either the selective δ_1-opioid antagonist, DALCE or the selective δ_2-opioid antagonist, naltrindole isothiocyanate (NTII) respectively. The lower panel depicts effects upon DPDPE-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).

FIGURE 14. 102

Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_2-selective opioid agonist, Deltorphin (Delt) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon Delt-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective δ_2-opioid antagonist, NTII or the selective μ-opioid antagonist, β-FNA respectively. The lower panel depicts effects upon DAMGO-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).

FIGURE 15. 115

Alterations in spontaneous food intake (g, +SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the shell region of the NAcc relative to Control treatment. The upper and lower panels depict effects upon DAMGO-induced feeding following pretreatment (20 min) with different doses (μg) of either a D_1-selective (SCH23390) or D_2-selective (raclopride) antagonist respectively. The asterisks (*) in this and

the subsequent figure indicate significant increases in food intake following opioid agonist treatment relative to Control treatment (Tukey comparisons, $p<0.05$). The crosses (+) in this and the subsequent figure indicate significant decreases in food intake following dopaminergic antagonist pretreatment relative to opioid agonist treatment alone (Tukey comparisons, $p<0.05$).

FIGURE 16. 117

Alterations in spontaneous food intake (g, +SEM) following a 5 μg dose of the δ_2-selective opioid agonist, Deltorphin (DELT) bilaterally in the shell region of the NAcc relative to Control treatment. The upper and lower panels depict effects upon Deltorphin-induced feeding following pretreatment (20 min) with different doses (μg) of either a D_1-selective (SCH23390) or D_2-selective (raclopride) antagonist respectively.

List of Abbreviations:

aca: anterior commissure

AcbC: nucleus accumbens, core region

AcbSH: nucleus accumbens, shell region

ACTH: adrenocorticotrophic hormone, endogenous peptide

AS ODN: antisense oligonucleotides

β-FNA: β-funaltrexamine, general μ receptor antagonist

CNA: central nucleus of the amygdala

CTOP: Cys^2-Try^3-Orn^5-Pen^7, general μ receptor antagonist

DA: dopamine

DADL: D-Ala^2, D-Leu^5-enkephalin, general δ receptor antagonist

DALCE: D-Ala^2, Leu5, Cys^6-enkephalin, selective δ_1 receptor antagonist

DAMGO: D-Ala^2, Met-Phe^4, $Gly(ol)^5$-enkephalin, selective μ receptor agonist

DAT: dopamine transporter protein, facilitates presynaptic reuptake of dopamine

DOR-1: δ opioid receptor clone

DPDPE: D-Pen^2, D-Pen^5-enkephalin, selective δ1 receptor agonist

DSLET: D-Ser^2, Leu^5-enkephalin-Thr6, general δ receptor agonist

DTLET: D-Thr^2, Leu^5-enkephalin-Thr6, general δ receptor agonist

icv: intracerebroventricular

KOR1: κ opioid receptor clone

KOR3: κ_3 opioid receptor clone

M6G: morphine-6-β-glucunoride

MOR1: μ opioid receptor clone

NAcc: nucleus accumbens

NalBzOH: naloxone benzolhydrazone, selective κ_3 receptor agonist

NAZ: naloxonazine, selective μ_1 opioid receptor antagonist

NRGC: nucleus reticularis giganto cellularis

NRM: nucleus raphe magnus

NTII: naltrindole-5'-isothiocynate, selective δ_2 receptor antagonist

NTS: nucleus tractus solitarius

NTX: naltrexone, general opioid receptor antagonist

Nor-BNI: nor-binaltorphamine, selective κ_1 receptor antagonist

ORL-1: orphan opioid receptor clone

OFQ/N: orphanin/ nociceptin, endogenous opioid peptide

PVN: paraventricular nucleus of the hypothalamus

PAG: periaqueductal gray

POMC: pro-opiomelanocortin, endogenous opioid peptide

SNR: substantia nigra, pars reticulata

2DG: 2-deoxy-D-glucose, anti-metabolic agent

VDB: ventral diagonal band

VTA: ventral tegmental area

SPECIFIC AIMS

The underlying neurochemical control of food intake depends upon the interplay of multiple neurotransmitter and neuropeptide systems in multiple sites in the brain. Whereas some of these systems are primarily involved in the cessation of intake through regulatory or satiety mechanisms, other systems are primarily involved in the elicitation of ingestive behavior. Substantial research over the past 20 years has identified the endogenous opioid system as involved in the elicitation of feeding behavior such that opioid antagonists typically reduce food intake under a variety of situations, whereas opioid agonists typically stimulate food intake under a variety of situations (see reviews: Morley et al., 1983; Levine et al., 1985; Cooper et al., 1988; Gosnell and Levine, 1996; Bodnar, 1996).

The three classic opioid receptor subtypes (μ, δ, κ) and their opioid receptor clones (MOR-1, DOR-1 and KOR-1), as well as the more recently-identified ORL-1 clone each elicit feeding following agonist stimulation. Such activation occurs under a wide range of ingestive situations, including spontaneous intake as well as intake under conditions of food deprivation, glucoprivation, and exposure to palatable ingesta. The supraspinal sites where such activation takes place have been identified as well, and includes limbic and gustatory nuclei, including the hypothalamic paraventricular and ventromedial nuclei, the amygdala, the nucleus tractus solitarius, and the parabrachial region. One traditional pathway that has historically been implicated in reward and reinforcement processes in general, the connections between the ventral tegmental area (VTA) and the nucleus accumbens (NAcc), has also been intimately implicated in their ability to elicit feeding responses following opioid agonist microinjection. This pathway has come under intense scrutiny as a potential interface between

motivational, regulatory and motor processes for reinforcement mechanisms, including ingestive behavior.

The present proposal attempts to integrate the role of specific opioid receptor subtypes in mediating feeding behavior elicited by this pathway under a variety of ingestive situations. Given that selective μ and κ, and to a lesser degree δ opioid receptor subtype antagonists have been shown to differentially modulate feeding under deprivation, glucoprivic and palatable situations following ventricular administration, the **first specific aim** of this dissertation is to ascertain whether general (naltrexone), μ (β-funaltrexamine) or κ (nor-binaltorphamine) opioid receptor subtype antagonists significantly altered food intake under deprivation (24 h), glucoprivic (2-deoxy-D-glucose) or palatable (exposure to 10% sucrose solutions) conditions following antagonist microinjections into the NAcc.

Given that μ, δ and κ receptor agonists stimulate feeding in the VTA, and to assess potential antagonist similarities with the NAcc, the **second specific aim** of this dissertation is to ascertain whether general (naltrexone), μ (β-funaltrexamine), κ (nor-binaltorphamine), δ_1 (DALCE) or δ_2 (naltrindole isothiocyanate) opioid receptor subtype antagonists significantly altered food intake under deprivation, glucoprivic or palatable conditions following antagonist microinjections into the VTA.

Given that the shell region of the NAcc has been established as the site responsible for opioid-induced feeding elicited by μ and δ, but not κ agonists, and given the observation that multiple opioid receptors participate in modulating feeding elicited by specific agonists in other brain areas, **the third specific aim** of this dissertation examined whether pretreatment of selective opioid receptor subtype antagonists (μ, μ_1, κ, δ_1, δ_2) would differentially alter

spontaneous food intake elicited by selective μ (DAMGO), δ_1 (DPDPE) or δ_2 (deltorphin) opioid receptor agonists administered into the shell region of the NAcc.

Given the important modulatory role of dopaminergic input from the VTA to the NAcc upon other reinforcement mechanisms in the latter structure, **the fourth specific aim** of this dissertation examined whether pretreatment of selective dopamine receptor subtype antagonists (D_1, D_2) would differentially alter spontaneous food intake elicited by selective μ (DAMGO) or δ_2 (deltorphin) opioid receptor agonists administered into the shell region of the NAcc. The following sections of the introduction will provide background relevant to these specific aims according to the following organization: **I.** opioid peptides, **II.** opioid receptors, **III.** opioid receptor clones, **IV.** role of systemically-applied and ventricularly-applied opioids and ingestion, **V.** central sites of action of opioid agonist-induced feeding, **VI.** opioid-induced feeding along the VTA-NAcc axis, **VII.** anatomy and projections of the NAcc, and **VIII.** a rationale for the present experiments.

INTRODUCTION

I. Opioid peptides

For centuries extracts from the poppy seed, opium, have been used as analgesics and to induce a "dream-like" state of euphoria. Morphine, named after the Greek god of sleep and dreams, Morpheus, was identified early on as the most active constituent, with codeine and papaverine being isolated later. The identification of the opiate receptor came as the result of a search for the site of action of morphine (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). Martin and co-workers (Martin et al., 1976), using a chronic spinal dog preparation, soon recognized that there appeared to be three subtypes of opioid receptors based on the inability of prototypical drugs to show significant cross-tolerance for each other: morphine (μ), SKF 10047 (σ) and ketocyclazocine (κ). This was soon followed by the description of another (δ: deferens) opioid receptor subtype, discovered using the mouse vas deferens bioassay, while the μ receptor was further characterized in the guinea pig ileum assay where morphine was more potent than the enkephalins (Lord et al., 1977).

In 1975, the identification of met- and leu-enkephalin as endogenous opioid penta-peptides (Hughes et al., 1975) began a greater understanding of opioid pharmacology. Subsequently, it was found that opioid peptides are derived from one of five gene precursor molecules: a) pro-opiomelanocortin (POMC), b) pro-enkephalin, c) pro-dynorphin, d) pro-nociceptin and e) endomorphins. The first three opioid peptide precursors all share a common opiate-active pentapeptide core (Try-Gly-Gly-Phe), while the latter two peptide precursors vary from this classical opioid motif (see review: Sherman, Akil and Watson, 1989; Mansour et al., 1995; Meunier et al., 1995; Reinscheid et al., 1995; Zadina et al., 1997). Interestingly,

because invertebrate tissues contain mammalian-like proopiomelanocortin, proenkephalin and prodynorphin, exhibiting high sequence homology with their mammalian counterparts, and because opioid precursor processing is also similar to that described in mammals, it appears that the opioid precursors and their processing enzymes first evolved early in "simple" animals and since then have been maintained and embellished during the course of evolution, perhaps guided by conformational matching (see review: Stefano and Salzet, 1999; Zagon et al., 1995)

A. POMC. The C-terminus of POMC contains the 31-amino acid peptide β-endorphin, and its 91 amino acid precursor β-lipotropin which also gives rise to α- and γ-endorphin (Eipper and Mains, 1978; Mains et al., 1977; Roberts et al., 1979). POMC can also be cleaved into ACTH (18-39), α-melanotropin and corticotropin-like intermediate lobe protein. However, of all the POMC derived peptides, β-endorphin is the only opioid peptide (Mains et al., 1977). Whereas the pituitary is the major site of POMC synthesis, the brain contains two distinct POMC-derived cell groups (Khachaturian et al., 1985). The first cell group is in the arcuate nucleus and surrounding peri-arcuate nuclei of the hypothalamus (Watson et al., 1978). These cells project extensively throughout the brain (Khachaturian et al., 1985). Specifically, rostrally-projecting fibers course through periventricular, diencephalic and telencephalic areas, innervating many hypothalamic and limbic structures, including the pre-optic area, septum, and bed nucleus of the stria terminalis. Lateral projections extend through the medial-basal hypothalamic region to the temporal cortex and amygdala. Caudally-projecting fibers innervate the periventricular thalamus, the periaqueductal gray (PAG), the nucleus raphé magnus (NRM), the nucleus reticularis giganto cellularis (NRGC), the nucleus tractus solitarius (NTS) and the nuclei reticularis lateralis, parabrachialis, ambiguus as well as the dorsal motor nucleus of vagus.

The second cell group containing β-endorphin is located in the caudal region of the NTS which projects laterally to the lateral reticular nucleus (Khachaturian et al., 1985).

B. Pro-enkephalin. Pro-enkephalin contains several opioid peptides including leu--enkephalin, met-enkephalin, met-enkephalin-Arg-Phe and met-enkephalin-Arg-Gly-Leu (Kimura et al., 1980; Comb et al., 1982). Hughes was the first to isolate leu- and met-enkephalin from brain and to demonstrate their opioidergic activity (Hughes et al., 1975). Enkephalins are found as inter-neurons in many neuronal systems from the telencephalon to the spinal cord. Specifically. immunoreactive enkephalin perikarya are found in such telencephalic structures as the cerebral cortex, olfactory tubercle, amygdala, hippocampus, bed nucleus of the stria terminalis and pre-optic area, such diencephalic structures as the hypothalamus and periventricular and lateral geniculate nuclei of the thalamus, such mesencephalic structures as the superior and inferior colliculi, PAG and interpeduncular nucleus, and such metencephalic and myelencephalic structures as the parabrachial, dorsal tegmental, vestibular and raphé nuclei. NRM, NRGC, NTS, lateral reticular nucleus, spinal trigeminal nucleus and spinal cord dorsal gray (Hokfelt et al., 1977; Khachaturian et al., 1983, 1985; Sar et al., 1978). Extrinsic enkephalinergic pathways project from the central and medial nuclei of the amygdala to the PAG and adjacent dorsal raphé nucleus (Rizvi et al., 1991), and also project from the PAG to the NRM (Beitz. 1982).

C. Pro-dynorphin. Pro-dynorphin is cleaved into three leu-enkephalin-containing peptides: α and β-neoendorphin, dynorphin A and dynorphin B (Goldstein et al., 1981; Kangawa et al., 1981). There are several peptides synthesized from dynorphin A that are biologically active, including dynorphin A_{1-8}, dynorphin A_{1-11} and several other intermediate-

length peptides (Goldstein et al., 1981; Seizinger et al., 1981; Suda et al., 1982). Immuno-reactive dynorphin perikarya are located in telencephalic (cerebral cortex, hippocampus, striatum and amygdala), diencephalic (supra-optic, paraventricular [PVN], and arcuate nuclei of hypothalamus), mesencephalic (PAG) and metencephalic/myelencephalic (parabrachial and spinal trigeminal nucleus, NTS, lateral reticular nucleus) structures as well as the dorsal and ventral horns of the spinal cord. Further, most dynorphin perikarya in the PVN of the hypothalamus co-exist with vasopressin in the magnocellular nuclei (Watson et al., 1982).

D. Pro-orphanin/Pro-nociceptin. Orphanin (OFQ) is a recently discovered (Meunier et al., 1995; Reinscheid et al., 1995) heptadecapeptide which is structurally similar to dynorphin A. Unlike classical opioid peptides, OFQ does not have a Tyr-Gly-Gly-Phe core at the N-terminus, but rather has a Phe-Gly-Gly-Phe motif. Further, unlike traditional opioid peptides, it binds with very low affinity to classical opioid receptor subtypes. Like POMC, the pre-pro-orphanin/nociceptin gene contains additional pairs of basic amino acid residues that delineate two putative biologically-active peptides that are respectively 17 and 35 amino acids long immediately downstream of OFQ (Meunier et al., 1995; Reinscheid et al., 1995). The pre-pro-orphanin gene also contains a precursor peptide that is biologically active called nocistatin (Okuda-Ashitake et al., 1998). Further, OFQ contains two pairs of basic amino acids, raising the possibility that it may be subject to post-translational processing into either QFQ_{1-11}, or OFQ_{1-7} which may have biological activity (see review: Henderson and McKnight. 1997). Immunohistochemical and autoradiographic studies have identified OFQ in the bed nucleus of the stria terminalis, medial pre-optic area, lateral septum, amygdala and median eminence. A dense plexus of OFQ terminal fibers are also present in the superficial layer of the dorsal horn,

in the sensory trigeminal complex, raphé nuclei and PAG (Henderson and McKnight, 1997).

E. Endomorphins. Endomorphins are the most recent opioid peptides to be isolated from the brain (Zadina et al., 1997). Their N-terminus sequence differs from the classical opioid peptides: endomorphin-1 (Try-Pro-Trp-Phe-NH) and endomorphin-2 (Try-Pro-Phe-Phe-NH). Preliminary radioimmunoassay studies of endomorphin-1 suggest that it is found in the thalamus, hypothalamus, cortex and striatum (Zadina et al., 1997). Further, endomorphin-2-like immunoreactivity has been localized in the medulla and dorsal root and dorsal root ganglia of the spinal cord (Martin-Schild et al., 1997).

Summary: Thus, at least five classes of opioid peptide families exist. The following section will summarize the different opioid receptor subtypes and how they interact with endogenous opioid peptides.

II. Opioid receptors and selective agonists and antagonists

Although both endogenous opioid peptides and receptors have been localized, there is relatively poor anatomical correspondence between them (see review: Akil et al., 1984). Further, there appears to be cross-reactivity between opioid peptides and receptors in binding assays. β-endorphin selectively binds both μ and δ opioid receptors, but not κ opioid receptors. Enkephalins and dynorphins bind preferentially to δ and κ opioid receptors respectively *in vitro*. Moreover, all proenkephalin and pro-dynorphin peptides can bind to μ, κ and δ opioid receptors depending on the peptide product and species (Corhett et al., 1982; Quirion et al., 1983). Subsequent studies indicated that the σ receptor may not be an opioid receptor, since actions mediated by this receptor are not reversed by the general opioid antagonist, naloxone (Vaupel, 1983). Unlike classical opioid peptides, the newer peptides, OFQ and endomorphin,

demonstrate selective biochemical correspondence with their endogenous opioid receptors. Whereas OFQ shows little or no affinity for μ, κ and δ opioid receptors, it displays high affinity for the orphanin receptor (Meunier et al., 1995; Reinscheid et al., 1995). Endomorphin-1 and endomorphin-2 have a high affinity and selectivity for the μ receptor, and it has been suggested that they are the actual endogenous ligands for the μ receptor (Zadina et al., 1997). This section will briefly review the μ, δ and κ opioid receptors and their pharmacologically-identified subtypes.

A. μ opioid receptors. μ receptors are widely distributed throughout the forebrain, midbrain and hindbrain. Binding of the μ receptor is most dense in the neocortex, caudate-putamen, NAcc, thalamus, hippocampus, amygdala, inferior and superior colliculi, NTS, spinal trigeminal nucleus and dorsal horn. Moderate binding is observed in the PAG and raphé nuclei, and little binding is observed in the hypothalamus, pre-optic area and globus pallidus (Mansour et al., 1988). The μ receptor has been characterized pharmacologically using the μ-selective agonist (i.e., D-Ala^2, Met-Phe^4,Gly (ol)^5-enkephalin, DAMGO: Handa et al., 1981) and antagonists (i.e, β-funaltrexamine, β-FNA: Portoghese et al., 1980; Takemori et al., 1981) and (Cys^2-Tyr^3-Orn^5-Pen^7, CTOP: Gulya et al., 1986).

1. μ_1 and μ_2 opioid receptors. The μ receptor has been further classified into μ_1 and μ_2 receptor subtypes based on pharmacological assays in which naloxone and naloxonazine selectively antagonize μ receptor actions *in vitro* and *in vivo* (Hahn et al., 1982. Ling et al., 1986; Pasternak et al., 1980; Pick et al., 1991). The μ receptor binds opiates and most enkephalins with similar high affinity while the μ_2 receptor binds morphine more potently than enkephalins (see review: Pasternak et al., 1986). Autoradiographic studies revealed similar, but

not identical, distributions of μ and μ_2 receptors (Goodman et al., 1985; Moskowitz et al., 1985). μ binding is denser in the frontal cortex, striatum, ventral palladium, NAcc, medial thalamus, interpeduncular nucleus, median raphé and PAG. μ_2 binding is denser in the parietal, occipital and temporal cortices, hippocampus, amygdala, dorsal motor nucleus of vagus and NTS. Behavioral studies have also distinguished the actions of μ, and μ_2 receptors in spinal and supraspinal analgesia (Bodnar et al., 1988; Paul et al., 1989; Pick et al., 1991).

B. δ opioid receptors. Autoradiographic studies indicate that δ receptor binding is densest in the olfactory-related neural areas, neocortex, caudate-putamen, NAcc and amygdala. In contrast, little or no binding is observed in the thalamus, hypothalamus and brainstem (Mansour et al., 1988). Initial studies characterizing the δ receptor utilized enkephalin analogues as general δ agonists: D-Ser^2, Leu-enkephalin-Thr^6 (DSLET) and D-Ala^2, D-Leu^5-enkephalin (DADL) (Lord et al., 1977; Mosberg et al., 1983a), and general δ antagonists, ICI 174864 (Cotton et al., 1984) and naltrindole (Portoghese et al., 1988). Subsequently, more selective δ ligands were developed.

1. δ_1 and δ_2 opioid receptors. The development of selective δ receptor agonists and antagonists led to the classification of δ and δ_2 receptor subtypes. The δ receptor has been pharmacologically characterized by the agonist D-Pen^2, D-Pen^5-enkephalin (DPDPE: Mosberg et al., 1983b) and long-term actions of the antagonist D-Ala^2, Leu^5,Cys^6-enkephalin (DALCE: Bowen et al., 1987; Jiang et al., 1990a). The δ_2 receptor has been pharmacologically characterized by the agonist D-Ala^2, Glu^4-deltorphin (Jiang et al., 1991) and the antagonist naltrindole-5'-isothiocyanate (NTII) (Portoghese et al., 1990). Behavioral studies have also distinguished the actions of δ and δ_2 receptors in spinal and supraspinal analgesia (Jiang et al.,

1991; Mattia et al., 1992).

C. κ opioid receptors. κ receptor binding is densest in the caudate-putamen, NAcc, amygdala, hypothalamus, neural lobe of the pituitary, median eminence, and NTS and moderate in the PAG, raphé nuclei, spinal trigeminal nucleus and dorsal horn (Mansour et al., 1988).

1. κ_1 receptors. Selective agonists and antagonists have also distinguished multiple κ receptor subtypes. The κ_1 receptor subtype has been characterized using the agonist U50,488H (Van Voigtlander et al., 1983) and the antagonist nor-binaltorphamine (Nor-BNI: Portoghese et al., 1987). The κ_2 receptor has been demonstrated in biochemical assays as being U50,488H-insensitive but has not been demonstrated *in vivo* (Zukin et al., 1988).

2. κ_3 receptors. A κ_3 receptor has also been identified as a U50,488H-insensitive site which selectively binds the agonist naloxone benzoylhydrazone (NaIBzOH: Clark et al., 1989; Gistrak et al., 1989; Paul et al., 1990). Hyperphagia induced by centrally-administered NaIBzOH was also insensitive to Nor-BNI pretreatment (Koch et al., 1992).

D. Pitfalls of Selective Agonists and Antagonists. Many of the selective agonists and antagonists described above exhibit a high degree of selectivity and specificity. However, under certain conditions, the selectivity of some of the agonists and antagonists has been challenged. For example, repeated administration of the selective κ antagonist Nor-BNI equally blocked the analgesic actions of μ, δ and κ agonists (Spanagel et al., 1994). The characterization of the opioid receptor subtypes using selective agonists and antagonists has been aided by the recent cloning of the traditional opioid receptor subtypes. The isolation of the cDNA's encoding the opioid receptors has allowed for biochemical, molecular, and functional analysis confirming earlier distinctions made employing selective agonists and antagonists.

Summary: Thus, at least three classes of opioid receptor families exist. The following section will summarize the recently-cloned opioid receptors, and how they interact with endogenous opioid peptides.

III. Opioid receptor clones

The DNA sequence for the δ opioid receptor (DOR) was first identified simultaneously in 1992 by two groups (Evans et al., 1992; Kieffer et al., 1992) from cDNA libraries derived from the RNA of NG108-15 cells. The similarity of the cloned DOR to the somatostatin receptor enabled the cloning of the κ opioid receptor (KOR) in 1993 (Yasuda et al., 1993). Hybridization of cDNA probes for the DOR has also led to the cloning of the μ opioid receptor (MOR) from rat brain (Chen et al., 1993). As a result of the cloning of the DOR, two separate groups (Mollereau et al., 1994; Bunzow et al., 1994) were able to clone and identify yet another receptor (ORL1) with strong homology to the three previously cloned opioid receptors. This receptor shares between 49-50 % sequence homology with the previously cloned DOR, KOR and MOR. While there are many different subtypes of opioid receptors, μ_1, μ_2, κ_1, κ_3, δ_1 and δ_2, the cloned sequences are very specific for which receptors they code for. The DOR receptor appears to code for the δ_2 opioid receptor, the KOR receptor appears to code for the κ_1 opioid receptor and the MOR appears to code for the μ_1 opioid receptor. This may be explained on the grounds that the various subtypes of the receptors are alternate splice variants of the cloned receptors. Data obtained from the cloned receptors has confirmed much of the previous pharmacological and biochemical data obtained using opioid selective agonists and antagonists regarding affinity, specificity and localization.

Summary: Therefore, cloned opioid receptor subtypes have been identified that show

both similarities and differences from the receptor subtypes defined using pharmacological and biochemical techniques. The major goal of this dissertation is to establish specific brain sites in opioid mediation of ingestive behavior, and the roles of endogenous opioid peptides and their receptors in eliciting feeding responses are reviewed in the next section.

IV. Systemic and Ventricular Opioids and Ingestion

That opioids may be involved in ingestion was first noted by Flowers, et at., (Flowers, Dunham and Barbour, 1929) when it was observed that chronic morphine increased water intake while opiate withdrawal increased food intake in rats. Further, morphine increased basal metabolic rates in dogs, suggesting opioid involvement in both energy expenditure and intake (Barbour, Gregg and Hunter, 1930). Morphine-tolerant rats also ate large amounts of food following daily morphine treatment (Martin et al., 1963). The role of the central endogenous opioid system in opioid modulation of ingestion was first confirmed by the observation that β-endorphin microinjected into the ventromedial hypothalamus (VMH) increased food intake (Grandison and Guidotti, 1977), whereas general opioid antagonism decreased food and water intake in deprived rats (Holtzman, 1974). Later studies examined the role of various opioid agonists and antagonists on ingestion in a variety of brain sites across a number of intake conditions. Finally, recent results of studies examining the feeding responses of molluscs and arthropods treated with various opiate agonists and antagonists indicate that μ, δ and κ opioid systems differentially and selectively mediate various components of their natural feeding behavior, indicating that opioid influences on feeding may have been conserved through evolution (see review: Kavaliers and Hirst, 1987).

A. Endogenous opioid peptides and ingestion. Opioid effects on ingestion as a

consequence of specific brain site injections have been studied primarily by examining food intake during the light cycle, when rats normally eat very little. Food intake can be stimulated following β-endorphin microinjection into the lateral ventricle (icv) and PVN (Leibowitz and Hor, 1982), the VMH (Grandison and Guidotti, 1977) and the NAcc (Przewlocka et al., 1986). Both met- and leu-enkephalin into the VMH elicit feeding (Tepperman and Hirst, 1983) and dynorphin induces feeding in non-deprived rats (Morley and Levine, 1983). While dynorphin$_{1-17}$ increases feeding in the NAcc (Majeed et al., 1986), VMH and PVN (Gosnell et al., 1986), dynorphin$_{1-13}$ increases feeding when injected into the VTA (Hamilton and Bozarth, 1988). OFQ induces feeding when injected into the lateral ventricle (Pomonis et al., 1996), VMH and NAcc (Stratford et al., 1997). Ventricular administration of antisense probes directed against either exons 1, 2 or 3 of the ORL-1 clone each reduce feeding elicited by OFQ (Leventhal et al., 1998a). Finally, endomorphins, the most recently-discovered endogenous opioids for the MOR-1 clone (Gong et al., 1998), have orexigenic effects when given icv (Asakawa et al., 1998).

B. Opioid receptor subtype agonists and ingestive behavior. Opioid agonists for μ, δ and κ opioid receptors all induce eating, which in some cases is greater after repeated injections than after the first injection (Jalowiec et al., 1981). Systemic injections of both μ (morphine) and κ (bremazocine) agonists increase food intake (Morley and Levine, 1983; Sanger and McCarthy, 1980). In contrast, icv injection of μ, δ, κ and ORL-1 opioid receptor agonists all elicit feeding through central rather than peripheral sites of action (Morley and Levine, 1983; Jackson and Sewell, 1985; Gosnell et al., 1986). The following sections provide a brief synopsis of each opioid receptor subtype in eliciting feeding under different ingestive conditions.

1. μ receptor compounds and food intake. Chronic systemic administration of morphine, heroin, codeine and levorphanol each increased spontaneous food intake (Martin et al., 1963; Sanger and McCarthy, 1980; Thornhill et al., 1976). Central injections of the μ agonist DAMGO, also increased spontaneous food intake in rats (Gosnell et al., 1986). μ-agonists also alter intake of palatable foods, including sucrose, glucose, saccharin and sodium chloride in both intake (Bertino et al., 1988; Cooper, 1983; Czirr and Reid, 1986; Gosnell and Majchrzak, 1989, 1990; Ruegg et al., 1997); and operant (Gosnell and Patel, 1993) conditions. While some studies suggest that morphine selectively increases fat intake (Marks-Kaufman, 1982; Marks-Kaufman and Kanarek, 1990), others suggest selective increases in fat intake only in food-restricted rats (Shor-Posner et al., 1986). Indeed, morphine increases intake of fat, protein and carbohydrate in non-deprived rats (Bhakthavatsalam and Leibowitz, 1986), which led to the idea that opioid, and particularly μ, agonists stimulate intake of the preferred macronutrient (Gosnell et al., 1990).

The μ receptor is involved in a wide variety of ingestive situations based on antagonist studies. Antagonism of μ receptors with β-FNA decreased spontaneous food intake and body weight in rats under acute and chronic conditions (Arjune et al., 1990; Cole et al., 1995), and reduces intake following food deprivation (Arjune et al., 1990), 2DG and insulin glucoprivation and mercaptoacetate-induced lipoprivation (Arjune et al., 1990; Beczkowska et al., 1992; Stein et al., 2000). β-FNA also reduced intake of fat, sucrose, and maltose dextrin (Beczkowska et al., 1992, 1993; Islam and Bodnar, 1990) as well as feeding elicited by tail-pinch or electrical stimulation of the lateral hypothalamus (Koch and Bodnar, 1993). Likewise, antisense probes directed against the MOR-1 clone reduce feeding under spontaneous, glucoprivic and lipoprivic

conditions (Leventhal et al., 1996; Burdick et al., 1997; Stein et al., 2000). Antagonism of μ_1 receptors with naloxonazine significantly reduced spontaneous intake and body weight under acute and chronic conditions (Cole et al., 1995; Mann et al., 1988), deprivation-induced feeding (Koch and Bodnar, 1993; Simone et al., 1985) and tail-pinch feeding (Koch and Bodnar, 1993). In contrast, naloxonazine failed to alter food intake under either glucoprivic or palatable intake (see review: Bodnar, 1996). The ability of μ and μ_1 antagonists to alter intake under spontaneous, deprivation or stress-related conditions implies a role for the μ_1 receptor in these ingestive responses. The ability of μ, but not μ_1 antagonists to alter palatable and glucoprivic intake implies a role for the μ_2 receptor in these ingestive responses.

Finally, feeding induced by selective μ agonists appears to be mediated by multiple opioid receptor subtypes in antagonist studies. DAMGO-induced feeding is diminished by pretreatment with either the selective μ-antagonist β-FNA or the κ_1-selective antagonist, Nor-BNI (Levine et al., 1990, 1991). These data either challenge the selectivity of the agents employed, or imply that multiple opioid receptors are modulating specific opioid receptor subtype agonist ingestive effects, probably through multiple, serially-linked synapses.

However, a more selective method of examining the effects of blocking DAMGO-induced feeding, using antisense oligonucleotides (AS), has shown that icv administered antisense probes, directed at exons 1 and 4 of the cloned MOR, significantly reduced DAMGO-induced feeding. In contrast, AS probes directed against exons 3 and 4 of the cloned MOR failed to alter DAMGO-induced feeding (Leventhal et al., 1997). Further, AS directed against the morphine metabolite, morphine-6β-glucuronide (M6G), itself a potent inducer of feeding showed the opposite effects to that of DAMGO-induced feeding. Whereas AS probes directed

against exons 2 and 3 of the MOR significantly reduced M6G-induced feeding, AS directed against exons 1 and 4 failed to significantly alter M6G-induced feeding (Leventhal et al., 1998).

2. δ receptor compounds and food intake. Central injections of δ-agonists increase spontaneous food intake (Gosnell et al., 1986), and selective icv administration of δ_1 and δ_2 receptor subtype agonists each increase spontaneous food intake in rats (Yu et al., 1997). δ receptor agonist also stimulate intake under palatable and challenge situations. The general δ-agonist, [D-Thr^2]-leucine enkephalin-Thr (DTLET), significantly increased saccharin and sodium chloride intake in rats (Gosnell and Majchrzak, 1989, 1990). While both DPDPE (δ_1) and deltorphin (δ_2) increased sucrose intake in rats (Ruegg et al., 1997), only deltorphin significantly enhanced 2DG-induced feeding (Yu et al., 1997). The ability of selective δ antagonists to block selective δ agonist-induced feeding is somewhat unclear since data obtained are somewhat conflicting. DSLET-induced feeding was blocked by central pretreatment with either μ or κ_1 antagonists (Levine et al., 1990). δ_1 (DPDPE) agonist-induced feeding was reduced by δ_2 (NTII) but not by δ_1 (DALCE) antagonist (Yu et al., 1997), yet deltorphin-induced feeding was blocked by DALCE and NTII (Yu et al., 1997). Further, AS probes directed against the exon 3 of the DOR-1 clone reduced deltorphin II-induced feeding, but failed to block M6G-induced feeding (Leventhal et al., 1998). Thus, whereas δ receptor subtype agonists reliably stimulate intake, a role for the δ receptor in modulating ingestion under a variety of situations appears to be limited in antagonists studies. Both chronic δ_1 (DALCE) and δ_2 (NTII) antagonism reduces spontaneous intake under deprivation, glucoprivic and palatable conditions (review: Bodnar, 1996) except for a reduction in saccharin intake (Beczkowska et al., 1993). The ability of δ agonists, but not δ antagonists, to alter intake is

consistent with a modulatory rather that a direct role for this receptor in ingestion in which agonists enhance the efficacy of a "final common pathway" for feeding which does not include δ receptors in its circuitry.

3. κ receptor compounds and food intake. Systemic administration of κ-selective drugs including, cyclazocine, ketocyclazocine, bremazocine, butorphanol and U50,488H each increase food intake (Gosnell et al., 1986; Levine and Morley, 1983a; Morley et al., 1982; Morley and Levine, 1983; Sanger and McCarthy, 1981). Whereas central injections of both selective κ_1 (U50,488H) and κ_3 (NalBzOH) agonists increase spontaneous food intake (Gosnell et al., 1986; Koch et al., 1992), both U50,488H and NalBzOH stimulate sucrose intake (Lynch and Burns, 1990; Ruegg et al., 1997). Feeding induced by U50,488H is significantly reduced by pretreatment with the κ_1 antagonist, Nor- BNI or with AS probes directed against exon 3 of the KOR-1 clone, but AS probes against exon 3 fail to block M6G-induced feeding (Koch et al., 1992; Leventhal et al., 1998).

The κ receptor has been implicated in ingestive behavior in antagonists studies with Nor-BNI producing potent reductions in nocturnal, sucrose and high-fat intake as well as intake induced by 2DG (Arjune and Bodnar, 1990; Beczkowska et al., 1992). In contrast, Nor-BNI produces only marginal reductions in deprivation-induced intake (Levine et al., 1990; Koch and Bodnar, 1994) and chronic spontaneous intake (Cole et al., 1995). Nor-BNI fails to alter intake induced by either insulin, tail-pinch, saccharin or maltose dextrin (Bodnar, 1996).

Summary: The foregoing section demonstrates that μ, δ and κ opioid receptor subtype agonists and antagonists differentially participate in either the elicitation and cessation of feeding under different ingestive situations following systemic and ventricular administration.

The following section provides details as to the anatomical sites of action at which these agonists and antagonists exert their ingestive effects.

V. Central sites of action of opioid agonists and antagonists upon feeding

Intracerebral sites of action of μ-agonist-induced feeding include the VMH (Tepperman and Hirst, 1982), PVN (McLean and Hoebel, 1983; Stanley et al., 1989; Woods and Leibowitz, 1985), amygdala (Gosnell, 1988; Stanley et al., 1989), VTA and NAcc (Mucha and Iverson, 1986; Bakshi and Kelley, 1993a, 1993b). δ feeding includes sites such as the VMH (Tepperman and Hirst, 1983), NAcc (Majeed et al., 1986), amygdala (Stanley et al., 1989) and PVN (McLean and Hoebel, 1983; Gosnell et al., 1986). Neither the VTA nor NAcc produce U50,488H-induced feeding (Bakshi and Kelley, 1993; Noel and Wise, 1993). Opioid agonists and/or antagonists also alter spontaneous feeding following administration into the perifornical area (e.g. Stanley et al., 1989), lateral parabrachial area (Carr et al., 1991) , ventral striatum (Bakshi and Kelley, 1993a, 1993b). Amygdala microinjections of DAMGO, morphine and D-Ala^2, Met^5-enkephalinamide (DALA) each elicit feeding, particularly from the central nucleus, suggesting mediation by at least μ and δ receptors (Gosnell, 1988; Stanley et al., 1989; Giraudo et al., 1998a, 1998b). In contrast, feeding is elicited by microinjections of μ (DAMGO), but not δ (DSLET) or κ (dynorphin) opioid agonists into the NTS (Kotz et al., 1997). Indeed, opioid receptors in the NTS appear to modulate feeding responses elicited by NPY since naloxone and naltrexone pretreatment in the NTS significantly reduced NPY-induced feeding elicited from the NTS (Kotz et al., 1995) and NPY pretreatment in the PVN is blocked by naltrexone into the NTS (Kotz et al., 2000). Levine and co-workers (Giraudo et al., 1998a, 1998b) have conducted several studies indicating interactions between brain sites

for opioid-induced feeding. An opioid-opioid interaction has been described between the PVN and the central nucleus of the amygdala (CNA) in that feeding elicited by DAMGO microinjected into the CNA is blocked by naltrexone microinjected into the PVN (Giraudo et al., 1998a). In contrast, feeding elicited by DAMGO microinjected into the PVN is unaffected by naltrexone microinjected into the CNA, suggesting the existence of an opioid-opioid signaling pathway from the CNA to the PVN. A bidirectional opioid-opioid signaling pathway between the rostral NTS and the CNA has been proposed based upon the ability of naltrexone microinjected into the rostral NTS to block feeding elicited by DAMGO microinjected into the CNA, and the ability of naltrexone microinjected into the CNA to block feeding elicited by DAMGO (Giraudo et al., 1998b).

Two main hypothalamic sites mediating opioid-induced feeding have been identified: the ventromedial (VMH) and paraventricular (PVN) nuclei. Feeding is elicited following VMH microinjections of β-endorphin, morphine, DADL, nociceptin and dynorphin (Grandison and Guidotti, 1977; Tepperman and Hirst, 1983; Gosnell et al., 1986b; Stratford et al., 1997; Tepperman et al., 1981; Tepperman and Hirst, 1982; Woods and Leibowitz, 1985), and following PVN microinjections of β-endorphin, morphine, dynorphin, DADL and DAMGO (Leibowitz and Hor, 1982; McLean and Hoebel, 1983; Gosnell et al., 1986b; Gosnell, 1988; Woods and Leibowitz, 1985; Stanley et al., 1989). Other hypothalamic nuclei, including the lateral, perifornical and dorsomedial areas produce either inconsistent effects, or have not been mapped thoroughly (Gosnell and Levine, 1996). Therefore, hypothalamic sites appear to support feeding elicited by agonists of each of the three major opioid receptor subtypes. The PVN is also an active site at which general and selective opioid antagonists act to inhibit food

intake. Deprivation-induced food intake is significantly reduced following microinjections of general, μ and κ, but not δ opioid antagonists into the PVN (Gosnell et al., 1986b; Woods and Leibowitz, 1985; Koch et al., 1995; Levine et al., 1989).

Summary: The previous section indicates that a number of brain areas traditionally related to motivational, homeostatic and gustatory sensory mechanisms appear to support feeding elicited by intracerebral microinjection of opioid agonists. A central premise of the dissertation is that the NAcc and the VTA are critical sites of action at which opioids induce feeding; the following section reviews the available literature on these relationships.

VI. Opioid-induced feeding along the VTA-NAcc Axis

Feeding is also elicited following opioid microinjections along the mesolimbic dopaminergic pathway, including the VTA and NAcc. Thus, VTA microinjections of either morphine, DPDPE, DAMGO or D-Ala^2-met-enkephalin elicit feeding (Stanley et al., 1989; Cador et al., 1986; Mucha and Iverson, 1986; Nencini and Stewart, 1990; Noel and Wise, 1993; Noel and Wise, 1995; Badiani et al., 1995a), implicating μ and δ opioid receptors in this response. A role for κ receptors in feeding elicited from the VTA is less clear. Although dynorphin stimulates spontaneous intake following VTA administration (Hamilton and Bozarth, 1988), and although U50, 488H in the VTA enhances feeding induced by electrical stimulation of the lateral hypothalamus (Jenck et al., 1987), κ-selective agonists in the VTA fail to stimulate deprivation-induced food intake (Noel and Wise, 1993). These results appear somewhat surprising given the presence of moderate to dense levels of μ and κ receptors in the VTA (Mansour et al., 1987, 1994; German et al., 1993; Speciale et al., 1993; Tempel and Zukin, 1987). Although μ agonists in the VTA potently increase dopamine release in the NAcc (Devine

et al., 1993a; DiChara and Imperato, 1988; Noel and Gratton, 1995; Spanagel et al., 1990), selective μ antagonists paradoxically produce the same effects (Devine et al., 1993b). Notably, locomotor activity is increased following VTA administration of both μ agonists and antagonists (Wise and Bozarth, 1987; Badiani et al., 1995b). It has been hypothesized that the parallel actions of μ-selective agonists and antagonists in the VTA upon mesolimbic dopamine release and locomotor activity may occur as a consequence of complex interactions between opioid actions on GABAergic afferents to the VTA, and GABAergic interactions within the VTA (Devine et al., 1993b). These data are consistent with the finding (Badiani et al., 1995a) calling the specificity of μ agonist actions into question since VTA microinjections of DAMGO elicit feeding, gnawing and drinking responses, and do not increase intake if rats must travel to the food source.

The δ agonist actions in the VTA are consistent with the presence of moderate densities of δ receptors (Mansour et al., 1987, 1994; German et al., 1993; Speciale et al., 1993; Tempel and Zukin, 1987), and are consonant with the contention that δ receptors serve a modulatory, rather than a direct role in ingestion. In this model, δ receptors in the VTA would not be in a hypothesized 'final common pathway' for the elicitation and maintenance of ingestion, but rather would be found in a hypothesized 'modulatory' pathway, that, if stimulated by δ receptor agonists, would increase the efficacy of the 'final common pathway' and thereby increase intake. The hypothesized pathway that δ receptors in the VTA would modulate is the dopaminergic projection from the VTA to the NAcc (see review: Moore and Bloom, 1978). In this regard, opiate microinjections into the VTA increase dopamine turnover and release in the NAcc (Devine et al., 1993a; Noel and Gratton, 1995; Swanson, 1982; Joyce and Iversen,

1979) as well as increase the firing rates of single dopamine neurons (Leone et al., 1991; Gysling and Wang, 1983).

According to the foregoing model, one proposed site involved in the 'final common pathway' for ingestive behavior is the NAcc which is a major site of action for opioid-induced feeding. The NAcc has immunoreactive cell bodies and terminals containing enkephalins and dynorphin (e.g., Fallon and Leslie, 1986; Jôngen-Relo et al., 1993; Khachaturian et al., 1982; Lewis et al., 1984, 1985; Van Bockstaele et al., 1994; Zamir et al., 1983, 1984) as well as visualized μ, δ and κ opioid receptors using autoradiography and mRNA gene expression (e.g., Herkenham et al., 1984; Lewis et al., 1984, 1985; Mansour et al., 1995, 1987; Pert et al., 1976). NAcc micro-injections of either morphine, DADL, DPDPE, β-endorphin, dynorphin, DAMGO, DPDPE and nociceptin stimulate feeding (Stratford et al., 1997; Mucha and Iverson, 1986; Yim and Mogenson, 1980; Majeed et al., 1986; Evans and Vaccarino, 1990; Bakshi and Kelley, 1993a). Kelley and co-workers have characterized both the sites of opioid action within the ventral striatum (Evans and Vaccarino, 1990) and the opioid agonist subtypes that elicit feeding within the NAcc (Bakshi and Kelley, 1993a). Thus, although morphine could elicit feeding following microinjection into such areas as the ventrolateral striatum, the anterior dorsal striatum and the posterior dorsal striatum, the magnitude of the effects were relatively small and occurred at high doses (Evans and Vaccarino, 1990). In contrast, morphine microinjections into either the NAcc or the ventromedial striatum elicited strong feeding responses at low doses which was dissociated from corresponding changes in locomotor behavior. The onset of morphine-induced feeding in the NAcc was initially delayed, and subject to sensitization following repeated morphine treatments.

Summary: The previous section indicates that the VTA and the NAcc are integral structures supporting the elicitation of feeding by opioid agonists, particularly those that stimulate μ and δ receptor subtypes. How these structures support opioid-induced feeding is a major goal of the present series of studies. The following section provides a detailed examination of the morphoiogy of the NAcc, as well as its afferent and efferent projections.

VII. Anatomy and Projections of the NAcc

A. Morphological distinctions of NAcc regions. The NAcc is a nuclear mass in the rostro-ventral part of the ventral striatum, bordered ventrally by the olfactory tubercle and medially by the septum. A distinction among the core, shell and rostral pole regions can be made within the NAcc on the basis of differing morphological characteristics with the core and shell being readily identified in caudal areas (Zaborszky et al., 1985; Zahm and Brog, 1992). The rostral pole appears to be contiguous with both the underlying olfactory tubercle and the overlying caudate-putamen (Zahm and Heimer, 1992). The core and shell appear to be comprised of two types of cellular compartments described as the patch and the matrix. Whereas the patch appears to be opiate-rich and responds strongly to naloxone, the matrix appears to be opiate-poor and responds strongly to calbindin. Patch cells receive inputs from deep cortical laminae and DA neurons in the ventral substantia nigra, pars compacta (SNC), and in turn send reciprocal projections to the SNC. Matrix cells receive inputs from superficial cortical laminae and DA neurons in the VTA, and project to the globus pallidus, entopeduncular nucleus and substantia nigra, pars reticulata (SNR) (see review: Zahm and Brog, 1992). Another substantial difference between the shell and the core is that while the core projects densely to the basal ganglia circuitry through the striato-fugal pathway to the ventrolateral

globus pallidus and the entopeduncular nucleus, the NAcc shell projects to the ventromedial globus pallidus, ventral bed nucleus of the stria terminalis, lateral pre-optic area, and the entire lateral hypothalamus (Heimer et al., 1991; Zahm and Heimer, 1990). The ventrolateral pallidum receives substantial input from the core area of the NAcc, making it appear as a rostro-ventral extension of the globus pallidus itself (Heimer et al., 1985). The shell and rostral pole, projections appear to fit well within the construct of the extended amygdala (Heimer et al., 1991) which has extensive outputs to brainstem autonomic and locomotor areas (Alheid and Heimer, 1988; Alheid et al., 1990; Brog et al., 1991; De Olmos, 1972, 1985; Heimer and Alheid, 1991; Heimer et al., 1991). Further, the core appears to have more in common with the overlying caudate-putamen, and may thus be more involved in voluntary motor movement (De Olmos and Heimer, 1999).

In contrast, the shell appears to be more closely linked with the "extended amygdala" and may be more involved with "motivational" systems (Alheid and Heimer, 1988). The afferents to the shell area of the NAcc, especially its caudal-medial component, share a great degree of similarity with the central nucleus of the amygdala with inputs from the basal amygdaloid complex, infra-limbic cortex, and paraventricular thalamus. Similarly, they share many of the same efferents, such as the ventral pallidum, pre-optic area, lateral hypothalamus and VTA. However, the shell region and amygdala also show some degree of divergence in their outputs to such caudal brainstem sites as the PAG and NTS in which practically no NAcc inputs are observed, but which receive robust projections from the central nucleus of the amygdala (see review: Zahm, 1998). Indeed, further evidence indicates that the core may be specifically involved in instrumental conditioning, while the shell may be involved in the control

of feeding (Kelley, 1999).

The pioneering work of Curt Richter and Eliot Stellar (see review: Kelley, 1999) indicated that motivated behaviors were used to maintain the organism in a state of homeostatic equilibrium for behaviors such as feeding and drinking and that excitatory and inhibitory control was exerted by specific neural substrates. The NAcc is particularly well-suited to play this role. The convergence of inputs originating from limbic structures, such as the hippocampus, amygdala, prefrontal cortex and brainstem autonomic areas, and outputs projecting to skeletal motor and visceral motor output centers, make this brain site particularly well-adapted to acting as a mediator between limbic inputs and motor outputs. Recent research has indicated that the shell region of the NAcc may be an important site for integrating feeding behavior, an effect which appears to be at least partly mediated through NAcc efferents to the lateral hypothalamus, and which appears to involve multiple neutotransmitter systems including GABA, excitatory amino acids and opioids (see review: Kelley, 1999). Opioid mediation of feeding within the NAcc (especially the shell region) is consistent with roles of this brain structure in a number of rewarding processes (see reviews: DiChiara et al., 1999; Everitt et al., 1999; Kelley, 1999; Koob, 1992, 1999; Robinson and Berridge, 1993; Salamone et al., 1994; Wise and Bozarth, 1987).

B. Immunohistological distinctions of NAcc regions. Immunohistological characterization of neurotransmitters and neuropeptides has indicated differences in the NAcc in terms of projection systems (Zaborszky et al., 1985; Zahm and Brog, 1992). Additionally, different neurotransmitters and their receptors exhibit differential distribution densities. Thus, whereas the shell region is dense in enkephalin, dopamine and D_1 receptors, it has few D_2

receptors. In contrast, the NAcc core exhibits a moderate density of enkephalin, dopamine, D_1 receptors and D_2 receptors (Voorn et al., 1989; Jôngen-Relo et al., 1995). The NAcc receives projections from the hippocampal region, basal amygdaloid complex, ventral pallidum, dopaminergic ventral tegmental (A10) and retro-rubral (A8) cell groups, the serotonergic medial raphé nucleus and the noradrenergic (A2) cell group of the NTS (see review: Groenewegen, 1999). These structures project preferentially to different parts of the NAcc, but, importantly, none goes exclusively to either the core or the shell. Thus, for example, although both core and shell receive inputs from the hippocampus, the dorsal subiculum projects to more to the core whereas the ventral subiculum projects exclusively to the shell (Brog et al., 1993). The core-shell difference continues when one looks at their efferents especially as it relates to the NAcc projections to the ventral pallidum and to the ventral mesencephalon. In a series of reciprocal connections, the medial shell projects to the ventromedial ventral pallidum, the lateral shell projects to the ventrolateral part of the ventral pallidum and the core projects to the dorsal ventral pallidum, sub-thalamic nucleus and substantia nigra (see review: Groenewegen, 1999). It should be noted that all three of these structures are classic basal ganglia output structures. Opioid peptides in the NAcc come from the medial basal hypothalamus (μ), interneurons (enkephalins) and the PVN (dynorphin). The connections with the ventral mesencephalon are more complicated. Generally, the shell connects to sub-cortical areas such as the lateral hypothalamus, VTA and ventromedial ventral pallidum. The VTA DA (A10) cell group projects primarily to the medial and ventral shell, but also has projections in the medial core (Voorn et al., 1986). The medial shell sends projections to the medial VTA, while the more lateral parts of the shell (ventromedial and

ventral parts) project to the lateral VTA (Groenewegen et al., 1996; Heimer et al., 1991; Zahm and Heimer 1990; Usada et al., 1998; Groenewegen et al., 1993; Voorn et al., 1986; Beckstead et al., 1979; Berendse et al., 1992). Core projections may be distinguished on the basis of the compartment of origination with patches projecting to substantia pars compacta and matrix projecting to dorsomedial pars reticulata (Heimer et al., 1991; Zahm and Heimer, 1990; Usada et al., 1998; Groenewegen et al., 1993; Voorn et al., 1986; Beckstead et al., 1979; Berendse et al., 1992; Gerfen et al., 1987; Deniau et al., 1994; Groenewegen et al., 1994).

Summary: Using morphology, differential anatomical projections and differential neurochemical distributions as markers for potential functional differentiation of the NAcc, it becomes clear that the shell and core regions of this nucleus subserve different aspects of motivated and motor behavior. Therefore, the aims of the dissertation will focus upon the shell region as the critical locus for opioid-induced feeding. The following section provides a rationale for the present studies.

VIII. Rationale

A. The Use of Multiple Feeding Models: This dissertation examines opioid effects on four types of feeding behaviors because although opioid agonists and antagonists respectively stimulate and reduce feeding, they do so in a differential manner (see reviews: Bodnar, 1996; Gosnell and Levine, 1996; Cooper, 1988; Levine et al., 1985). In most feeding behavior studies investigating opioid effects, the consequences of opioid agonist administration under spontaneous feeding conditions were studied. This approach will be used in the **Third and Fourth Specific Aims** to determine the underlying pharmacology of feeding elicited by μ and δ agonists administered into the shell region of the NAcc. In contrast, as detailed previously, and summarized again below, food intake paradigms using food deprivation, 2DG glucoprivation and exposure to such palatable ingesta as a sucrose solution display differential effects as a function of the opioid receptor subtype antagonist employed. These three situational conditions of feeding behavior constitute respectively a general challenge to homeostasis related to negative energy balance (e.g., food deprivation), a specific glucoprivic challenge to homeostasis (2DG), and exposure to palatable foods that result in increased spontaneous intake (sucrose). This approach will be used in the **First and Second Specific Aims**. Feeding induced by 24 h of food deprivation is significantly reduced by general opioid antagonists (e.g., Holtzman, 1974; see review: Morley et al., 1983) as well as ventricular microinjections of μ, μ_1 and κ opioid receptor subtype antagonists, (Arjune and Bodnar, 1990; Arjune et al., 1990; Koch and Bodnar, 1994; Levine et al., 1990, 1991; Simone et al., 1985). It should be noted that whereas μ opioid antagonists produced marked (~50%) decreases in deprivation-induced intake, κ antagonists produced marginal (~30%) reductions.

In contrast, ventricular administration of δ opioid receptor antagonists fail to alter deprivation-induced intake (Arjune et al., 1991; Koch and Bodnar, 1994). Thus, μ, μ_1, and to a lesser degree, κ opioid receptors participate in the regulatory homeostatic response to food deprivation.

Feeding induced by the anti-metabolic glucose analogue, 2-deoxy-D-glucose (Smith and Epstein, 1969) is significantly reduced by general opioid antagonists (Lowy et al., 1980). This effect is more pronounced than general opioid antagonism of insulin-induced feeding (Levine and Morley, 1981; Ostrowski et al., 1981). Ventricular antagonism of μ receptors with β-FNA reduces intake following 2DG and insulin glucoprivation (Arjune et al., 1990; Beczkowska and Bodnar, 1991). In contrast, μ_1 opioid receptor antagonism with naloxonazine failed to alter either 2DG or insulin-induced feeding (Simone et al., 1985; Beczkowska and Bodnar, 1991), suggesting μ_2 opioid receptor mediation of this response. The κ receptor has been implicated in feeding elicited by 2DG, but not insulin (Arjune and Bodnar, 1990; Beczkowska and Bodnar, 1991). Further, both κ_1 and κ_3 receptor agonists mildly enhance 2DG-induced feeding (Yu et al., 1997). Although δ receptor antagonists fail to alter 2DG-induced feeding (Arjune et al., 1991; Jackson and Sewell, 1985), the δ_2 opioid agonist, deltorphin significantly enhanced 2DG-induced feeding (Yu et al., 1997). Thus, μ_2 and κ opioid receptors participate in the specific glucoprivic regulatory challenge, and its compensatory feeding response.

Increased spontaneous intake has been observed for liquids containing simple sugars such as sucrose (Ackroff and Sclafani, 1988; Ramirez, 1990) which is reduced by pretreatment with general opioid antagonists with systemic (LeMagnen et al., 1980; Cooper,

1983; Siviy and Reid, 1983; Lynch, 1986) or ventricular (Beczkowska et al., 1992) administration. Whereas μ-opioid agonists increase intake of palatable solutions, including sucrose (Bertino et al., 1988; Cooper, 1983; Czirr and Reid, 1986; Gosnell and Majchrzak, 1989; Ruegg et al., 1997), selective μ, but not μ_1 antagonists potently reduce sucrose intake under both real-feeding (Beczkowska et al., 1992) and sham-feeding (Leventhal et al., 1995) conditions, indicating that these effects are mediated through the orosensory characteristics of sucrose intake. Similarly, κ antagonism potently reduces sucrose intake (Beczkowska et al., 1992). In contrast, δ receptor antagonism fails to alter sucrose intake, yet reduces the intake of saccharin (Beczkowska et al., 1992, 1993; Leventhal et al., 1995). Agonist studies reveal that selective μ, δ_1, δ_2, κ_1 and κ_3 agonists increase sucrose intake as a function of the sucrose concentration employed (Ruegg et al., 1997). Indeed, both general and selective opioid receptor subtype antagonists reduce sucrose intake by interfering with the maintenance rather than the initiation of intake (Kirkham and Blundell, 1984; Kirkham and Cooper, 1988; Leventhal et al., 1995). Thus, μ_2 and κ opioid receptors participate in the increased intake to simple carbohydrate solutions, and correspond to observed relationships between opioid peptide levels and sucrose intake (Levine et al., 1995).

B. The PVN as a model system for the study of differential opioid antagonist effects upon different forms of ingestive behavior: The hypothalamic PVN has been implicated in the mediation of ingestive behavior since food intake is increased following PVN microinjections of norepinephrine (Goldman et al., 1985; Leibowitz, 1978), neuropeptide Y (Stanley et al., 1985; Stanley and Leibowitz, 1984) and galanin (Kyrkouli et al., 1986; Tempel et al., 1988) as well as mineralcorticoids and corticosterone (Tempel and Leibowitz, 1989;

Tempel et al., 1992), and since food intake is decreased following PVN microinjections of serotonin (Shor-Posner et al., 1986; Weiss et al., 1986) and neurotensin (Stanley et al., 1983). Increases in spontaneous food intake also occur when either morphine (McLean and Hoebel, 1983; Woods and Leibowitz, 1985), β-endorphin (Grandison and Guidotti, 1977; Leibowitz and Hor, 1982), enkephalins and their analogues (Stanley et al., 1989; Tepperman and Hirst, 1982, 1983 or dynorphin (Gosnell et al., 1986a, 1986b) is microinjected into the PVN and surrounding medial hypothalamus. In like manner, PVN microinjections of μ-selective, but not κ-selective or δ-selective opioid agonists stimulate spontaneous water intake (Gosnell et al., 1986a, 1986b; Ukai and Holtzman, 1988). In contrast, administration of general opioid antagonists such as naloxone reduce feeding in deprived rats following PVN microinjection (Gosnell et al., 1986a, 1986b; Leibowitz and Hor, 1982; McLean and Hoebel, 1983; Woods and Leibowitz, 1985), as does β-endorphin antisera (Schulz et al., 1984). Deprivation-induced drinking is also reduced by PVN microinjections of naloxone (Ukai and Holtzman, 1987). Lesions placed in the PVN reduce, but do not eliminate the hyperphagic effects of morphine (Shor-Posner et al., 1986). Our laboratory (Koch et al., 1995) showed that general, μ and κ opioid antagonists microinjected into the PVN also reduce feeding elicited by deprivation, 2DG and sucrose intake. Deprivation intake was significantly reduced by nor-binaltorphamine (30-33%), β-funaltrexamine (26-29%) or naltrexone (26%) in the PVN. 2DG-induced feeding was significantly reduced only after 2 h by naltrexone (69%), nor-binaltorphamine (69%) or β-funaltrexamine (83%) in the PVN. Sucrose intake was significantly reduced by nor-binaltorphamine (27-36%), naltrexone (18-31%) and β-funaltrexamine (20%) in the PVN. These data indicate that general, μ and κ opioid antagonists administered into the

hypothalamic paraventricular nucleus produce similar patterns of effects upon different forms of food intake as ventricular administration, implicating this nucleus as part of the circuitry underlying opioid mediation of ingestion.

C. Specific Aim 1: Role of the Nucleus Accumbens in mediating selective opioid receptor subtype antagonist effects upon different forms of feeding:

Spontaneous feeding significantly increases following NAcc microinjections of either morphine, the μ-selective agonist, DAMGO or the δ_1-selective agonist, DPDPE, but not the κ_1-selective agonist, U50, 488H (Bakshi and Kelley, 1993a, 1993b, 1994; Evans and Vaccarino, 1990; Majeed et al., 1986; Mucha and Iverson, 1986). Further, food restriction increases levels of dynorphin A_{1-8} in the NAcc (Berman et al., 1994), but fails to alter selective μ opioid binding (Wolinsky et al., 1994). Since μ and κ receptors mediate feeding following either deprivation, 2DG glucoprivation or palatable sucrose intake following ventricular and PVN administration, the present study examined the effects of NAcc microinjections of general (naltrexone), μ (β-FNA) or κ (Nor-BNI) antagonists to inhibit these three different forms of food intake in rats. Sinceμ_1 antagonism with naloxonazine selectively decreases deprivation-induced intake, the present study evaluated its effectiveness in the NAcc. The following major forms and, when appropriate, alternate forms of hypotheses will be tested:

1. Since μ receptor agonists stimulate feeding in the NAcc, and since μ and μ_1 antagonists each reduce deprivation-induced intake, it is hypothesized that β-FNA and naloxonazine will each reduce deprivation-induced feeding following microinjection into the NAcc.

2. Since μ receptor agonists stimulate feeding in the NAcc, and since μ antagonists reduce 2DG and sucrose intake, it is hypothesized that β-FNA will reduce these responses following microinjection into the NAcc.

3a. Since κ receptor agonists fail to stimulate feeding in the NAcc, it is hypothesized

that Nor-BNI will not reduce these responses following microinjection into the NAcc.

3b. Since κ antagonists reduce deprivation, 2DG and sucrose intake, it is hypothesized that Nor-BNI will reduce these responses following microinjection into the NAcc.

This aim of the dissertation has been published in the journal, Brain Research (Bodnar et al, 1995).

D. Specific Aim 2: Role of the Ventral tegmental area in mediating selective opioid receptor subtype antagonist effects upon different forms of feeding:

The VTA has been implicated in the mediation of the reinforcing aspects of drugs, including opiates, particularly through its meso-limbic dopamine connections with the NAcc (e.g., see reviews: Self and Stein, 1992; Wise and Bozarth, 1987; Wise and Hoffman, 1992; Wise and Rompre, 1989). Administration of opiate drugs and opioid peptides and their analogues into the VTA differentially and selectively stimulate food intake (see review: Gosnell and Levine, 1996) with increased intake noted following microinjections of either the δ agonist, D-ala-met-enkephalin (Cador et al., 1986), the opioid peptide, dynorphin$_{1-13}$ (Hamilton and Bozarth, 1988), morphine (Mucha and Iverson, 1986; Nencini and Stewart, 1990; Noel and Wise, 1993), the δ_1 agonist, DPDPE (Noel and Wise, 1995) or the μ agonist, DAMGO (Noel and Wise, 1995). In contrast, VTA microinjections of the κ_1 agonist, U50,488H fail to stimulate deprivation-induced food intake (Noel and Wise, 1993), but enhance feeding elicited by electrical stimulation of the lateral hypothalamus (Jenck et al., 1986). The prophagic effects of μ-selective opioid agonists in the VTA occur under both spontaneous and deprived conditions, and act primarily upon the speed of intake rather than the latency to commence feeding (Noel and Wise, 1993, 1995). The specificity of μ agonist actions in the VTA to feeding responses has been called into question since VTA microinjections of DAMGO elicit feeding, gnawing and drinking, and do not increase intake if rats must travel to the food source (Badiani et al., 1995). VTA microinjections of the general opioid antagonist, naloxone reduces consumption of a sweet 50% apple juice solution with more marked decreases noted in normally-fed rats relative to rats maintained at 85% of

their normal body weight (Segall and Margules, 1989). Given the relationship between the VTA and the NAcc in opioid reward-mediated processes (Self and Stein, 1992; Wise and Bozarth, 1987; Wise and Hoffman, 1992; Wise and Rompre, 1989), the present study examined the effects of VTA microinjections of either general (naltrexone), μ (β-FNA), κ_1 (Nor-BNI), δ_1 (DALCE) or δ_2 (NTII) opioid antagonists to reduce either deprivation (24 h)-induced intake, 2DG (500 mg/kg)-induced intake or sucrose (10%) intake in rats. The following major forms and, when appropriate, alternate forms of hypotheses will be tested:

1. Since μ receptor agonists stimulate feeding in the VTA, and since μ antagonists reduce deprivation, 2DG and sucrose intake, it is hypothesized that β-FNA will reduce these responses following microinjection into the VTA.

2a. Since κ receptor agonists fail to stimulate feeding in the VTA, it is hypothesized that Nor-BNI will not reduce thee responses following microinjection into the VTA.

2b. Since κ antagonists reduce deprivation, 2DG and sucrose intake, it is hypothesized that Nor-BNI will reduce these responses following microinjection into the VTA. 3a. Since δ receptor agonists stimulate feeding in the VTA, it is hypothesized that DALCE and NTII will reduce these responses following microinjection into the VTA.

3b. Since δ antagonists fail to reduce deprivation, 2DG and sucrose intake, it is hypothesized that DALCE and NTII will fail to reduce these responses following microinjection into the VTA.

This aim of the dissertation has been published in the journal, Brain Research (Ragnauth et al, 1997).

E. Specific Aim 3: Define which opioid receptor subtypes mediate feeding induced by selective μ and δ opioid agonists in the NAcc:

The prototypical opioid agonist, morphine stimulates food intake following administration into the NAcc (Bakshi and Kelley, 1993, 1994; Evans and Vaccarino, 1990; Majeed et al., 1986; Mucha and Iverson, 1986). Receptor autoradiographic and mRNA gene expression studies have verified that μ, δ and κ opioid receptors can be found in the NAcc (Herkenham et al., 1984; Lewis et al., 1985; Mansour et al., 1995, 1987). In assessing the sensitivity of selective opioid receptor subtype agonists in the NAcc, Bakshi and Kelley (1993b) observed that spontaneous feeding was elicited following administration of the selective μ opioid agonist, DAMGO, or the selective δ_1 opioid agonist, DPDPE, but not the selective κ_1 opioid agonist, U50,488H in the shell region of the NAcc. Similarly, DAMGO and DPDPE, but not U50,488H or dynorphin, enhanced sucrose intake in the NAcc (Zhang and Kelley, 1997). Moreover, DAMGO microinjected into the NAcc selectively increased intake of a fat relative to a carbohydrate diet (Zhang et al., 1998).

While DAMGO has been generally accepted as a μ-selective opioid agonist (e.g., Simon and Hiller, 1994), pharmacological and biochemical data have suggested the existence of δ opioid receptor subtypes termed δ_1 and δ_2 (Jiang et al., 1990a, 1990b, 1991; Mattia et al., 1991, 1992; Negri et al., 1991; Sofuoglu et al., 1991). These distinctions were confirmed in analgesic assays such that the enkephalin analogue, DPDPE (Mosberg et al., 1993) acts as a δ_1-opioid receptor agonist based upon its sensitivity to selective δ_1 opioid antagonism by DALCE, but not to selective δ_2 opioid antagonism by naltrindole isothiocyanate (NTII) (Jiang et al., 1991; Sofuoglu et al., 1991). In contrast, analgesia elicited by the δ_2 opioid agonist,

Deltorphin (Kreil et al., 1989), is sensitive to δ_2, but not δ_1 opioid antagonism (Jiang et al., 1991; Mattia et al., 1992; Raffa et al., 1992; Sofuoglu et al., 1991). Further, analgesic cross-tolerance fails to occur between DPDPE and Deltorphin (Mattia et al., 1991). Moreover, supraspinal analgesia elicited from the ventrolateral periaqueductal gray and the rostral ventromedial medulla is observed following Deltorphin, but not DPDPE (Bodnar et al., 1988; Rossi et al., 1994). Indeed, distinctions between analgesic responses elicited by DAMGO and Deltorphin can be made in these sites such that the former response is blocked by μ, but not δ opioid antagonism, whereas the latter response is blocked by δ, but not μ opioid antagonism (Rossi et al., 1994). These data suggest receptor selectivity and specificity in producing analgesic responses elicited by opioid receptor subtype agonists.

Feeding responses induced by different opioid receptor subtype agonists do not produce this clean-cut specificity between opioid receptor subtype agonists and antagonists. Thus, ventricular administration of the κ_1 antagonist, Nor-BNI significantly reduced feeding induced by the κ_1 agonist, U50,488H, but not by the κ_3 agonist, naloxone benzoylhydrazone (Koch et al., 1992; Levine et al., 1990). However, Levine and co-workers subsequently demonstrated that effective doses of Nor-BNI also decreased feeding elicited by either DAMGO or the preferential δ agonist, [D-Ser^2, Leu^5, Thr^6]-enkephalin (DSLET). Although Nor-BNI was initially characterized as a selective κ antagonist (Portoghese et al., 1987), further studies using chronic Nor-BNI injections indicated μ and δ activity as well (Spanagel et al., 1994). Whereas β-FNA potently reduces feeding induced by ventricular administration of the μ agonist, DAMGO (Levine et al., 1990, 1991), this antagonist also reduces feeding induced by ventricular administration of the δ agonist, DSLET, but not the κ_1 agonist,

U50,488H. Similar separations between selective agonist and antagonist effects occurred for feeding responses induced by δ_1 and δ_2 mechanisms. Thus, feeding induced by ventricular administration of the δ_1 agonist, DPDPE, was blocked by general opioid antagonism with naltrexone and δ_2 antagonism with NTII, but not by δ_1 antagonism with DALCE (Yu et al., 1997). Alternatively, feeding induced by ventricular administration of the δ_2 agonist, Deltorphin was blocked by δ_1 (DALCE) and δ_2 (NTII) antagonist pretreatment, but not by the general opioid antagonist, naltrexone (Yu et al., 1997). Given that ventricular routes of administration were used in all of the above studies, it is possible that some of the multiple and anomalous results may be due to actions at multiple opioid receptor sites.

The different effects of opioid peptides on analgesia and feeding may be explained from an evolutionary perspective. Multiple receptors and receptor subtypes diversify the response characteristics of a neurotransmitter by providing different biophysical properties, thereby eliminating the need for the evolution of a completely different neurotransmitter system to serve a new function. They also help to ensure specificity for different systems. The existence of a common precursor for opioid peptides and opioid receptors may mean that while on the one hand there is specialization for peptide/receptor interactions, such as is seen in analgesia, on the other hand there may be some vestigial effect such that multiple peptides may act on multiple receptors, such as is seen in feeding.

Therefore, the present study had the following aims. First, our laboratory wanted to determine whether Deltorphin would produce feeding following microinjection into the shell region of the NAcc at similar dose levels and with similar magnitudes to that observed for DAMGO and DPDPE (Bakshi and Kelley, 1993; Zhang et al., 1998; Zhang and Kelley,

1997). Second, our laboratory examined whether feeding induced by either DAMGO, DPDPE or Deltorphin in the NAcc were mediated through single or multiple opioid receptor subtypes by pretreating rats in the NAcc with selective μ (β-FNA), μ_1 (NAZ), δ_1 (DALCE), δ_2 (NTII) or κ_1 (Nor-BNI) opioid receptor antagonists. The following major forms and, when appropriate, alternate forms of hypotheses will be tested:

1a. Since μ and δ receptor agonists stimulate feeding in the NAcc, and since μ and δ antagonists selectively reduce analgesia induced by their respective agonists, it is hypothesized that β-FNA will selectively reduce feeding elicited by DAMGO, but not DPDPE or deltorphin following microinjection into the NAcc. Further, it is hypothesized that DALCE will selectively reduce feeding elicited by DPDPE, but not DAMGO or deltorphin following microinjection into the NAcc. Finally, it is hypothesized that NTII will selectively reduce feeding elicited by deltorphin, but not DPDPE or DAMGO following microinjection into the NAcc.

1b. Since multiple opioid receptor antagonists reduce different forms of opioid agonist-induced feeding following ventricular or PVN injection, it is hypothesized that μ and δ antagonists will selectively reduce feeding elicited by DAMGO, DPDPE and deltorphin following micro-injections into the NAcc.

2a. Since κ receptor agonists fail to stimulate feeding in the NAcc, it is hypothesized that Nor-BNI will fail to reduce feeding elicited by DAMGO, DPDPE or deltorphin following micro-injections into the NAcc.

2b. Since multiple opioid receptor antagonists reduce different forms of opioid agonist-induced feeding following ventricular or PVN injection, it is hypothesized that κ

antagonists will selectively reduce feeding elicited by DAMGO, DPDPE and deltorphin following microinjections into the NAcc.

This aim of the dissertation has been published in the journal, Brain Research (Ragnauth et al, 2000a).

F. Specific Aim 4: Define whether dopamine receptor subtype antagonists mediate feeding induced by selective μ and δ opioid agonists in the NAcc:

A role of dopamine in the NAcc has been formulated within the General Anhedonia Model (Wise, 1982) in which dopamine innervation in the NAcc is conceived as a critical link in reward systems mediating natural reinforcers such as food. Although dopamine antagonism or depletion in the NAcc do not impair food-reinforced behaviors related to consumption and goal-seeking (see review: Salamone et al., 1997), dopamine agonists in the NAcc can either increase or decrease feeding, depending on dose and individual baseline intake (Carr and White, 1986; Evans and Vaccarino, 1986; Kelley et al., 1989; Sills and Vaccarino, 1996). Whereas injections of dopamine receptor antagonists into the NAcc suppress feeding elicited by a low dose of systemically-administered amphetamine (Sills et al., 1993) and by electrical stimulation of the medial forebrain bundle (Mogenson and Wu, 1982), lesions selectively depleting dopamine levels in the NAcc produce marginal decreases in spontaneous food intake (Koob et al., 1978; Salamone et al., 1993). Dopamine itself also stimulates feeding behavior within the shell region of the NAcc as compared to the core region (Swanson et al., 1997). Interactions between opioid and dopaminergic systems have been observed within the NAcc in anatomical studies (Van Bockstaele et al., 1994), and in neurochemical studies showing that fentanyl, the μ opioid receptor agonist, in the NAcc stimulates dopamine release which is blocked by general, μ and δ opioid receptor antagonists (Yoshida et al., 1999). Similarly, dopamine release is increased by μ (DAMGO), δ_1 (DPDPE) and δ_2 (deltorphin) infused into the NAcc (Yoshida et al., 1999). Dopamine release in the NAcc is also increased by systemic and VTA injections of opioids (e.g., DiChiara and Imperato, 1988; Kalivas and

Richardson-Carlson, 1986; Latimer et al., 1987; Spanagel and Shippenberg 1990), effects which appear necessary for the rewarding, locomotor and analgesic effects of opioids in this system (Altier and Stewart, 1998; Churchill and Kalivas, 1992; Cunningham et al., 1997; Cunningham and Kelley, 1992)

Both D_1 and D_2 receptors are localized on dendrites and presynaptic terminals in the shell and core regions of the NAcc (Koshikawa et al., 1996; Shetreat et al., 1996). Although proenkephalin and prodynorphin mRNA are co-localized with D_1, but not D_2 receptors (Curran and Watson, 1995), antisera raised against both the δ-opioid receptor (DOR) and the dopamine transporter (DAT) indicate that DOR appears on axon terminals apposed to DAT-immunoreactive terminals (Svingos et al., 1999). Both D_1 and D_2 have recently been implicated in reinforcement mechanisms (Braun and Chase, 1986; Nakajima et al., 1993; Walters et al., 1987; White et al., 1988). Therefore, to evaluate the relative roles of D_1 and D_2 receptor subtype involvement in feeding elicited by μ and δ opioid agonists in the NAcc, the present study examined the equimolar dose-dependent pretreatment effects of either the selective D_1 receptor antagonist, SCH23390 (Christensen et al., 1984; Iorio et al., 1983; Sidhu et al., 1986) or the selective D_2 receptor antagonist, raclopride (Kopp et al., 1992; Protais et al., 1994) upon feeding elicited by either the μ-selective opioid agonist, DAMGO or the δ_2-selective opioid agonist, deltorphin in the NAcc in rats. The shell region of the NAcc was chosen as the focal point for microinjections since dopamine concentrations are far higher in this segment of the nucleus than in the core region (Deutsch and Cameron, 1992). The following hypothesis will be tested:

1. Since μ and δ receptor agonists stimulate feeding in the NAcc, and since DA

antagonists selectively reduce analgesia induced by μ and δ receptor agonists, it is hypothesized that SCH 23390 and raclopride will selectively reduce feeding elicited by DAMGO and deltorphin following microinjection into the NAcc.

This aim of the dissertation has been published in the journal, Brain Research (Ragnauth et al, 2000b).

GENERAL METHODS:

Subjects, Surgery and Histology: Adult male albino Sprague-Dawley rats (Charles River Laboratories, Kingston, NY, 80-120 days of age) were housed individually in wire mesh cages and maintained on a 12 h light/12 h dark cycle with Purina rat chow and water available ad libitum. Each rat was pretreated with chlorpromazine (3 mg/kg, i.p.) and anesthetized with Ketamine HCl (120 mg/kg, i.m.). Bilateral stainless steel guide cannulae (26-gauge, Plastics One) were placed stereo-taxically (Kopf Instruments) in either the NAcc or the VTA using the following coordinates. NAcc coordinates were: incisor bar (+5), 3.5 mm anterior to the bregma suture, 1.7 mm lateral to either side of the sagittal suture and 6.4-6.6 mm from the top of the skull. VTA coordinates were: incisor bar (+5 mm), 5.6 mm posterior to the bregma suture, 2.5 mm lateral to and angled 10° towards either side of the sagittal suture, and 8.4 mm from the top of the skull. The cannulae were secured to the skull by 3 anchor screws with dental acrylic. To allow full drug clearance, all animals were allowed at least two weeks to recover from stereotaxic surgery before behavioral testing began. At the completion of testing, all rats were overdosed with an anesthetic (Nembutal) and received a transcardiac perfusion with 0.9% normal saline followed by 10% buffered formalin. Coronal 40-μm sections, stained with Cresyl violet, were examined by light microscopy by an observer unfamiliar with the behavioral data; only animals with confirmed cannula placements were included in the data analysis.

Statistical Analyses for Specific Aims 1 and 2: Cumulative intakes were evaluated since unequal levels of intake occur across the time course in deprivation, glucoprivic and palatable paradigms such that greater consumption occurs initially. Thus, separate analyses

of variance were performed at each cumulative intake point for each feeding paradigm. Tukey comparisons at the $p<.01$ level assessed specific antagonist effects relative to the corresponding control condition. The more conservative criterion was utilized to minimize the likelihood of false positives engendered by the cumulative repeated analyses.

Statistical Analyses for Specific Aims 3 and 4: Separate one-way repeated measures analyses of variance were performed at each cumulative intake point for each feeding paradigm. Tukey corrected comparisons ($P<0.05$) were used to assess significant alterations in agonist-induced feeding relative to corresponding control conditions as well as significant alterations induced by antagonist pretreatment upon agonist-induced feeding relative to corresponding agonist-induced feeding per se.

EXPERIMENT 1. Role of Opioid Receptor Subtype Antagonists in the Nucleus Accumbens in Mediating Feeding Elicited by Food Deprivation, 2DG Glucoprivation and Exposure to Sucrose Solutions.

Specific Methods, Experiment 1:

Deprivation Intake Protocol: Rats received the following bilateral microinjection conditions in counterbalanced order at weekly intervals: a) vehicle (n=17), naltrexone (Sigma Chemical Company) at total doses of b) 5 μg (n=6), c) 10 μg (n=6) and d) 20 μg (n=6), β-FNA (Research Biochemicals Intl.) at total doses of e) 1 μg (n=6) and f) 4 μg (n=6), g) naloxonazine (synthesized by Dr. GW Pasternak) at a total dose of 10 μg (n=6), and Nor-BNI (Research Biochemicals Intl.) at total doses of h) 1 μg (n=6) and i) 4 μg (n=6). In this and all subsequent experiments, each rat received a maximum of four bilateral microinjection conditions, and the subgroups of rats receiving different antagonist conditions were matched on the basis of the particular intake condition following vehicle treatment. At 5-7 h into the light cycle, food was removed for 24 h, and long-acting antagonists (β-FNA and naloxonazine) were administered. Short-acting antagonists (naltrexone and Nor-BNI) were administered 1 h prior to food re-introduction. Naltrexone, β-FNA and Nor-BNI were dissolved in normal saline, and naloxonazine was dissolved in distilled water and 0.2% glacial acetic acid. Food intake in deprived rats receiving either the saline vehicle (n=11) or the distilled water and 0.2% glacial acetic acid vehicle (n=6) failed to differ from each other across the time course. Thus, data from the two vehicle conditions were pooled. All microinjections were administered bilaterally in 1 μl volumes over 30 sec through a stainless steel internal cannula (33-gauge, Plastics One) connected to a Hamilton microsyringe by

polyethylene tubing. Cumulative intakes were assessed 0.5, 1, 2 and 4 h after food reintroduction by weighing food pellets before and after each condition, and adjusting for spillage which was collected by paper under the wire mesh cage.

Glucoprivic Intake Protocol: Rats received the following bilateral microinjection conditions in counterbalanced order at weekly intervals: a) vehicle (1 µl bilaterally, icv)/vehicle (1 ml normal saline/kg, i.p.) (n=11), b) vehicle/2DG (500 mg/kg, i.p., Sigma Chemical Company) (n=11), naltrexone at total doses of c) 10 µg (n=6) and d) 20 µg (n=7) paired with 2DG, β-FNA at total doses of e) 1 µg (n=5) and f) 4 µg (n=6) paired with 2DG, and Nor-BNI at total doses of g) 1 µg (n=6) and h) 4 µg (n=6) paired with 2DG. 2DG injections occurred 3-5 h into the light cycle, and cumulative intakes were assessed at 0.5, 1, 2 and 4 h thereafter.

Sucrose Intake Protocol: Rats were introduced to 10% sucrose solution (Sigma Chemical Company, 10%) in a sipper tube (Lab Products, 50 ml, 1 ml gradations) over 1 week and had to exceeded a criterion intake (10 ml in 60 min). Rats received the following bilateral microinjection conditions in counterbalanced order at weekly intervals: a) vehicle (n=11), naltrexone at total doses of b) 20 µg (n=8) and c) 50 µg (n=8), β-FNA at total doses of d) 1 µg (n=6) and e) 4 µg (n=7) and f) Nor-BNI at a total dose of 4 µg (n=5). At 3-5 h into the light cycle, cumulative sucrose intakes were assessed at 5, 10, 15, 30, 45 and 60 min after introduction of the sipper tube.

Results: Experiment 1:

Histological Placements: Figure 1 illustrates the bilateral cannula placements in the NAcc in the three ingestive paradigms. All of the placements were found in the core of the NAcc with a few placements bordering on the edge of the shell region. All NAcc placements were found in the rostral two-thirds of the nucleus, and the placements were equally distributed as a function of the ingestive paradigm.

NAcc Opioid Antagonists and Deprivation Intake: Significant differences in deprivation intake were observed among conditions after 0.5 ($F(8,128)= 24.22$, $p<.0001$), 1 ($F= 35.47$, $p<.0001$), 2 ($F= 37.26$, $p<.0001$) and 4 ($F= 45.07$, $p<.0001$) h of food reintroduction. Naltrexone in the NAcc significantly decreased deprivation-induced intake (Figure 2A): 5 μg (1-4 h, 15-24%, $p<.05$), 10 μg (0.5-2 h, 20-32%, $p<.01$), 20 μg (0.5-4 h, 26-44%, $p<.01$). β-FNA in the NAcc significantly decreased deprivation-induced intake (Figure 2B): 1 μg (4 h, 17%, $p<.05$), 4 μg (0.5-4 h, 36-55%, $p<.01$). In contrast, naloxonazine in the NAcc significantly increased ($p<.05$) deprivation-induced intake by 18-28% over 4 h (Figure 2B). Nor-BNI in the NAcc significantly decreased deprivation-induced intake (Figure 2C): 4 μg (1-4 h, 21-31%, $p<.01$).

NAcc Opioid Antagonists and Glucoprivic Intake: Significant differences in glucoprivic intake were observed among conditions after 0.5 ($F(7,70)= 16.45$, $p<.0001$), 1 ($F= 19.44$, $p<.0001$), 2 ($F= 21.35$, $p<.0001$) and 4 ($F= 14.24$, $p<.0001$) h. 2DG significantly increased food intake over 4 h relative to vehicle treatment. Naltrexone in the NAcc significantly decreased 2DG hyperphagia (Figure 3A): 10 μg (1 h, 34%, $p<.05$), 20 μg (0.5-2 h, 49-79%, $p<.01$). β-FNA in the NAcc significantly decreased 2DG hyperphagia (Figure

3B): 1 μg (0.5-4 h, 61-100%, p<.01), 4 μg (0.5-4 h, 47-79%, p<.01). Nor-BNI in the NAcc significantly decreased 2DG hyperphagia (Figure 3C): 1 μg (2 h, 31%, p<.05), 4 μg (0.5-4 h, 38-75%, p<.01).

NAcc Opioid Antagonists and Sucrose Intake: Significant differences in sucrose intake were observed among conditions after 5 ($F(5,50)= 13.22$, $p<.0001$), 10 ($F= 4.29$, $p<.0025$), 15 ($F= 2.70$, $p<.031$), 30 ($F= 2.96$, $p<.02$) and 60 ($F= 4.31$, $p<.0024$) min. Naltrexone in the NAcc significantly decreased sucrose intake (Figure 4A): 50 μg (15-30 min, 26-27%, p<.05). β-FNA in the NAcc significantly decreased sucrose intake (Figure 4B): 1 μg (5 min, 41%, p<.01), 4 μg (5-60 min, 25-37%, p<.01). In contrast, Nor-BNI in the NAcc failed to significantly alter sucrose intake (Figure 4B).

In contrast to these effects in the NAcc, microinjections delivered via cannulae misplaced 2mm medial-lateral and dorsal-ventral to the NAcc did not reduce ingestion induced by deprivation, glucoprivation or a palatable sucrose solution.

Figure 1. Histological verification of cannula placements (asterisks) in the NAcc. Note that virtually all placements are found in the core region, and occupy the rostral two-thirds of the nucleus. The number adjacent to the placement refers to the number of animals in the three paradigms with placements at that location.

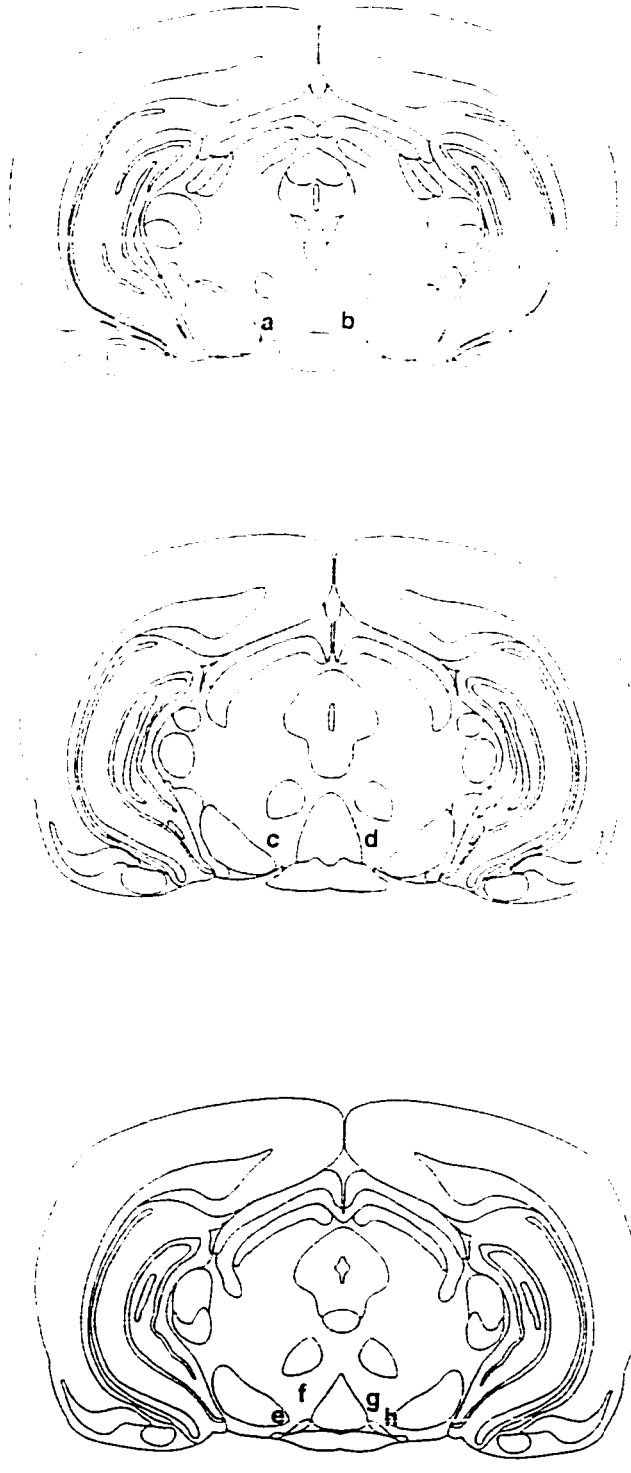


Figure 2. Alterations in cumulative intakes (g, ±S.E.M.) following micro-injections of either naltrexone (Panel A), β-funaltrexamine (B, Panel B), naloxonazine (N, Panel B) or nor-binaltorphamine (Panel C) into the NAcc in rats deprived of food for 24 h. The daggers in this and subsequent figures denote significantly reduced intake relative to vehicle treatment (Tukey comparisons).

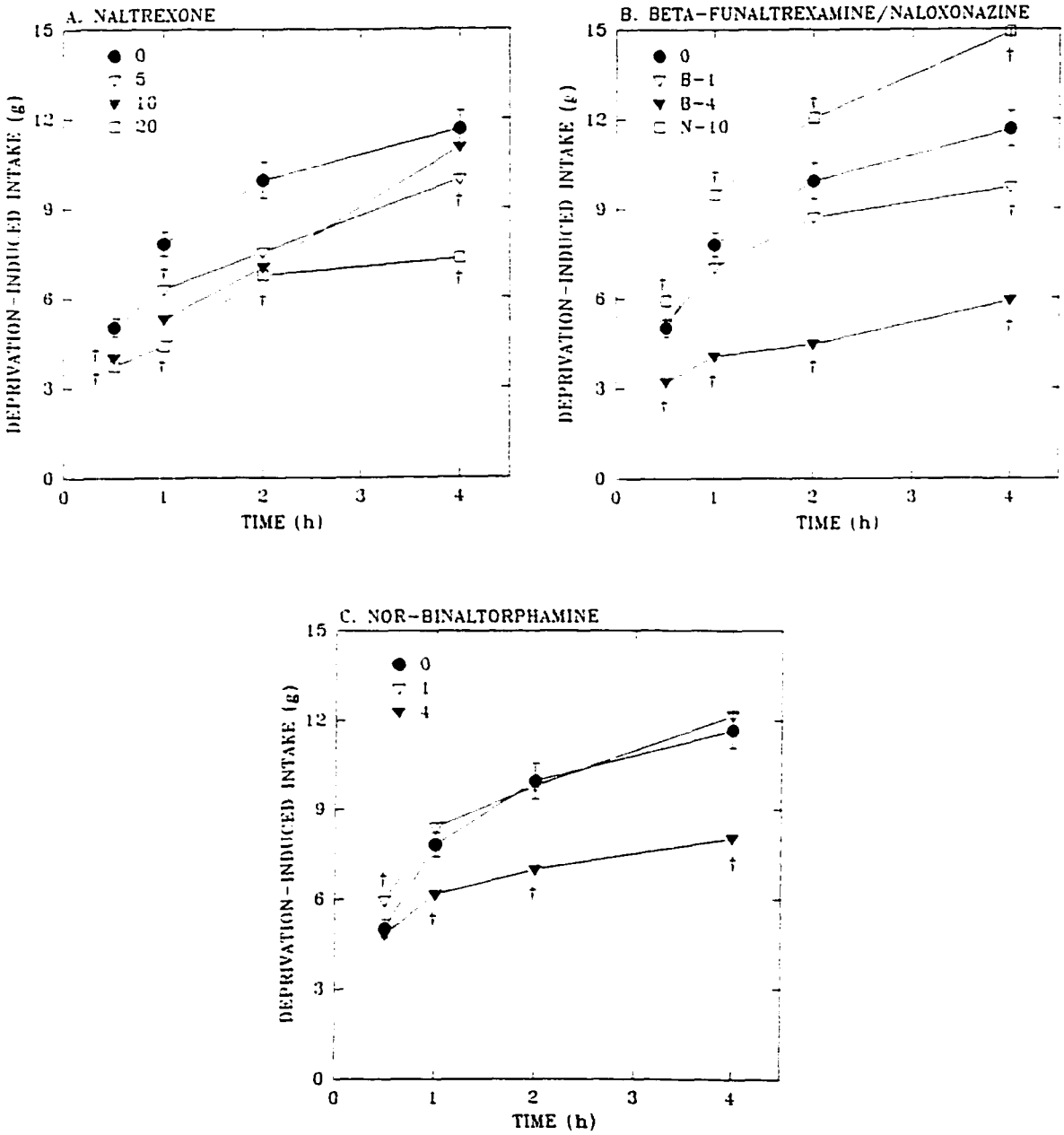


Figure 3. Alterations in cumulative intakes (g, ±S.E.M.) following micro-injections of either naltrexone (N, Panel A), β-funaltrexamine (B, Panel B) or nor-binaltorphamine (N, Panel C) into the NAcc in rats receiving 2-deoxy-D-glucose (2DG).

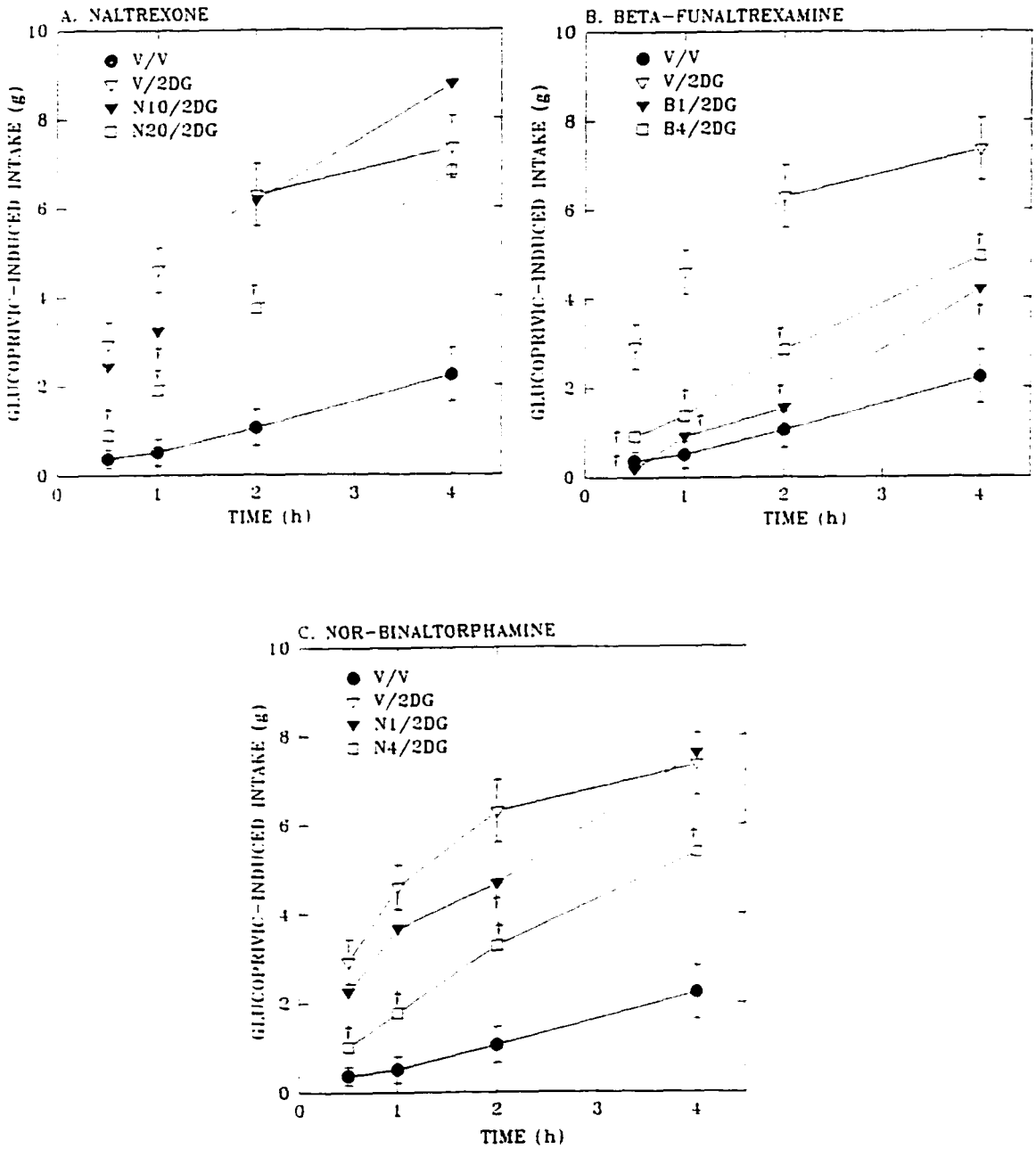
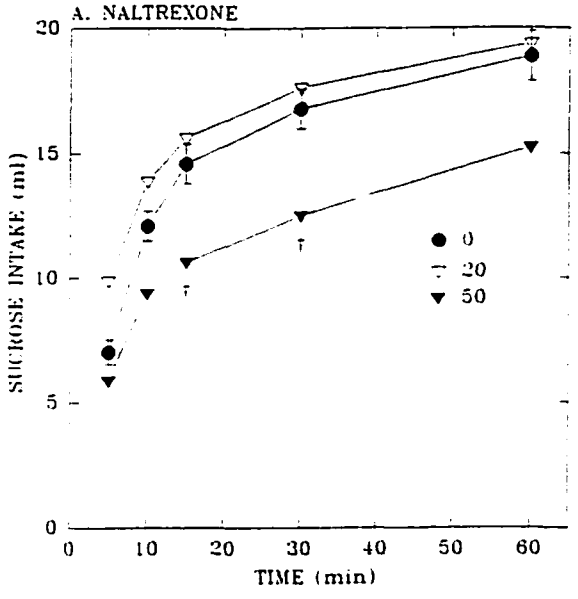
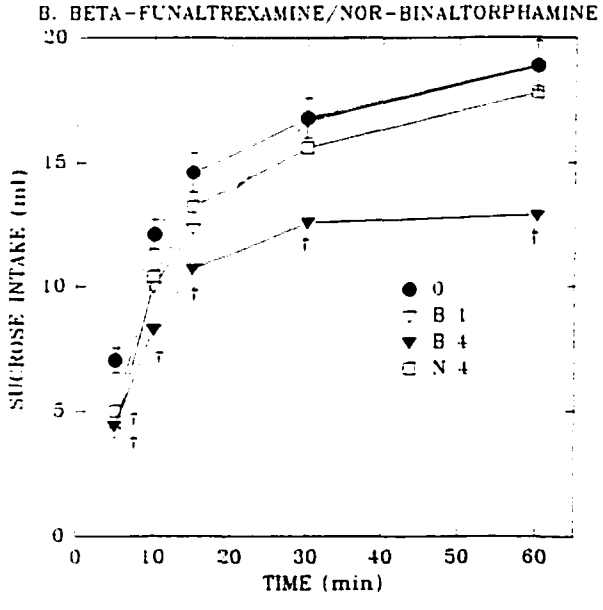


Figure 4. Alterations in cumulative sucrose (10%) intakes (g, ±S.E.M.) following microinjections of either naltrexone (Panel A), β-funaltrexamine (B, Panel B) or nor-binaltorphamine (N, Panel B) into the NAcc.





Discussion: Experiment 1:

Deprivation-Induced Intake: The present study found that whereas bilateral NAcc microinjections of either general (naltrexone), μ (β-FNA) or κ (Nor-BNI) opioid antagonists significantly reduced deprivation-induced intake, μ_1 antagonism with naloxonazine transiently stimulated this response. Naltrexone's reductions in deprivation-induced intake in the NAcc approached the magnitude (44%) and duration (4 h) of action observed for ventricular (Koch and Bodnar, 1994; Marks-Kaufman et al., 1985) or systemic (Brown and Holtzman, 1979; Frenk and Rogers, 1979; Simone et al., 1985) injections and appeared more potent than PVN administration (Koch et al., 1995). These data agree with previously-observed (Bless and Kelley, 1994) decreases in deprivation-induced intake and feeding duration after 0.5 h following naloxone (30 μg) or naltrexone (20 μg) in the NAcc. β-FNA's reduction in deprivation-induced intake in the NAcc also approached the magnitude (55%) and duration (4 h) observed for ventricular administration (Arjune et al., 1990; Koch and Bodnar, 1994; Levine et al., 1991), and appeared more potent than PVN administration (Koch et al., 1995). The present effects were far more pronounced than previous (Bless and Kelley, 1994) 10-15% reductions in deprivation-induced intake after 0.5 h. This difference can be explained by the increased magnitude of β-FNA's inhibition of deprivation-induced intake over time, and agrees with the premise that opioid antagonists interfere with the maintenance and not the initiation of intake (Kirkham and Blundell, 1984; Kirkham and Cooper, 1988a, 1988b). In contrast to reductions in deprivation-induced intake by following systemic (Simone et al., 1985) or ventricular (Koch and Bodnar, 1994) naloxonazine administration, NAcc naloxonazine significantly stimulated deprivation-induced intake over 4 h. Given the selective

antagonism by β-FNA ($\mu = \mu_1 + \mu_2$: e.g. Pasternak and Wood, 1986; Portogese et al., 1980) and naloxonazine (μ_1: e.g. Hahn et al., 1982; Pasternak and Wood, 1986), this suggests that the μ_2 opioid binding site is responsible for β-FNA's antagonism of deprivation-induced intake in the NAcc. The 31% reduction in deprivation-induced intake by Nor-BNI in the NAcc is in keeping with its limited though significant reductions following ventricular (Koch and Bodnar, 1994; Levine et al., 1990) and PVN (Koch et al., 1995) administration. These data differ from the failure (Bless and Kelley, 1994) to observe significant Nor-BNI effects upon deprivation intake in the NAcc 0.5 after food reintroduction. Time sampling procedures are important since Nor-BNI took 1 h to develop significant effects, and 2-4 h to produce its most pronounced effects. Since δ antagonists fail to alter deprivation-induced intake following ventricular administration (Arjune et al., 1991; Koch and Bodnar, 1994), they were not assessed in this intracerebral paradigm. Thus, μ_2, and to a lesser degree, κ receptors appear to mediate the opioid control of deprivation-induced intake in the NAcc. However, potential differences in the potency of μ and κ antagonists to reduce deprivation-induced intake may not only reflect the specific actions of the antagonists at their specific receptors. The contribution of antagonist differences in affinity and occupancy for their respective receptors should be considered as well as potential differences in antagonist diffusion within intracerebral sites. Finally, although the antagonist dose range (1-4 μg) was equated, less Nor-BNI (MW= 735, 5.4 nmol) was administered relative to β-FNA (MW= 491, 8.2 nmol) at the effective dose. In contrast to these effects in the NAcc, microinjections delivered via cannulae misplaced 2mm medial-lateral and dorsal-ventral to the NAcc did not reduce ingestion induced by deprivation.

Glucoprivic Intake: General, μ or κ antagonists in the NAcc significantly reduced 2DG hyperphagia. Naltrexone's reductions of 2DG hyperphagia in the NAcc were substantially shorter (2 h) than ventricular (Arjune and Bodnar, 1990; Koch and Bodnar, 1994) or systemic (Beczkowska et al., 1992; Lowy et al., 1980) administration, but more potent (49-79%) than PVN microinjections (Koch et al., 1995). The potent inhibition of 2DG-induced feeding by β-FNA or Nor-BNI in the NAcc was shorter than ventricular administration (Bordi et al., 1989; Koch and Bodnar, 1994), but similar to PVN administration (Koch et al., 1995). Since neither δ nor μ_1 antagonists reduce glucoprivic hyperphagia following ventricular (Arjune et al., 1991; Beczkowska and Bodnar1991; Koch and Bodnar, 1994) or systemic (Simone et al., 1985) administration, they were not assessed in this intracerebral paradigm. Again, the ability of β-FNA, but not naloxonazine to exert effects upon glucoprivic intake suggests a role of the μ_2 binding site. Thus, μ_2 and κ receptors appear to mediate opioid control of glucoprivic intake in the NAcc by affecting the initiation rather than the long-term maintenance of 2DG-induced feeding. In contrast to these effects in the NAcc, microinjections delivered via cannulae misplaced 2mm medial-lateral and dorsal-ventral to the NAcc did not reduce ingestion induced by glucoprivation.

Sucrose Intake: General and μ, but not κ antagonists in the NAcc significantly reduced sucrose intake. Although sucrose intake is quite sensitive to systemic (60-75%) and ventricular (ID_{40}= 6 nmol) naltrexone administration (Beczkowska et al., 1992, 1993; Kirkham and Cooper, 1988a; 1988b; Rockwood and Reid, 1982; Siviy and Reid, 1983), naltrexone (50 μg) in the NAcc only transiently (15-30 min) and marginally (27%) reduced sucrose intake, an effect similar to that observed in the PVN (Koch et al., 1995). The

significant reductions (25-41%) in sucrose intake across the time course by β-FNA in the NAcc were more pronounced than PVN administration (Koch et al., 1995) and comparable to ventricular administration (Beczkowska et al., 1992). In contrast to the potency (55-75%, ID_{40}= 4 nmol) of ventricular Nor-BNI to reduce sucrose intake (Beczkowska et al., 1992, 1993), Nor-BNI in the NAcc failed to alter sucrose intake. This pattern agrees with recent findings (Bless and Kelley, 1994) using a shorter (15 min) sampling interval. Since neither δ nor μ_1 antagonists reduce sucrose intake following ventricular administration (Beczkowska et al., 1992), they were not assessed in this intracerebral paradigm. Again, the ability of β-FNA, but not naloxonazine to exert effects upon sucrose intake suggests a role of the μ_2 binding site in the NAcc In contrast to these effects in the NAcc, microinjections delivered via cannulae misplaced 2mm medial-lateral and dorsal-ventral to the NAcc did not reduce ingestion induced by a palatable sucrose solution.

Caveats: Some concerns about diffusion from the injection site to other brain areas have been raised in other studies involving opioids (Schroeder et al., 1991). However, because micro-injections via misplaced cannulae did not affect feeding, this may not be a valid concern.

Conclusions: Whereas general and μ antagonists in the NAcc significantly reduce intake under deprivation, glucoprivic and palatable conditions, κ antagonists reduce intake in the first two conditions. This opioid antagonist pattern is similar to the greater efficacy of general and μ agonists to stimulate spontaneous intake in the NAcc relative to κ agonists (Bakshi and Kelley, 1993a, 1993b, 1994; Evans and Vaccarino, 1990; Majeed et al., 1986; Mucha and Iversen, 1986). Since the NAcc is involved in reinforcement and reward

mechanisms in addiction, drug self-administration and palatability studies (e.g. Bozarth, 1994; Evans and Vaccarino, 1990; Koob and Bloom, 1988; Salamone, 1994; Wise and Bozarth, 1987), it was expected that opioid antagonists in the NAcc would potently affect palatable intake, and that the κ receptor subtype most effective in modulating sucrose intake in ventricular studies (Beczkowska et al., 1992) would also be most effective in the NAcc. Both our and other recent (Bless and Kelley, 1994) data are at variance with these expectations. Interestingly, the NAcc has also been considered an interface for, or a chimera of, limbic-striatal interactions, gating motivational and motor processes (Churchill and Kalivas, 1992; Kalivas et al., 1983; Mogenson, 1987, 1980), including stereotyped oral behavior (Bordi et al., 1989; Kelley et al., 1988; Koene et al., 1993; Prinssenet al., 1994). Our laboratory (Beczkowska et al., 1993; Koch and Bodnar, 1994) has hypothesized that the μ receptor mediates the amount of intake per se, and not its hedonic consequences, and based this upon the consistent, steady inhibition of different forms of intake by β-FNA. Since β-FNA's respective inhibitions of intake for the three ingestive situations were similar following either NAcc or ventricular administration, it would appear that the NAcc is a site of action in exerting this form of ingestive control. Since β-FNA ($\mu = \mu_1 + \mu_2$), but not naloxonazine (μ_1) antagonism was effective, this suggests that the μ_2 opioid binding site may be the receptor within the NAcc mediating ingestive consequences. The second experiment in this series examined whether these selective opioid antagonists exerted a similar pattern of effects upon these forms of ingestive behavior following microinjection into the VTA.

EXPERIMENT 2. Role of Opioid Receptor Subtype Antagonists in the Ventral Tegmental Area in Mediating Feeding Elicited by Food Deprivation, 2DG Glucoprivation and Exposure to Sucrose Solutions.

Specific Methods, Experiment 2:

Deprivation Intake Protocol: Rats received the following bilateral microinjection conditions at weekly intervals: vehicle, naltrexone at total doses of 10, 20 and 40 μg, β-FNA at a total dose of 4 μg, Nor-BNI at a total dose of 4 μg, DALCE (synthesized by Dr. W.D. Bowen) at a total dose of 8 μg and NTII (Research Biochemicals Intl.) at a total dose of 4 μg. At 5-7 h into the light cycle, food was removed for 24 h, and μ (β-FNA) and δ (DALCE and NTII) antagonists were administered. General (naltrexone) and κ_1 (Nor-BNI) antagonists were administered 1 h prior to food re-introduction. All antagonists were dissolved in normal saline, except for DALCE which was dissolved initially in 0.2 M HCl in distilled water with the pH adjusted to 7.5-8.0 with 0.2 M NaOH. In order to administer the proper amount of antagonist doses into the VTA, a 1 μl volume was needed to address solubility limitations. For those antagonists in the following sections that failed to produce significant effects, the dose used was the maximum due to solubility limitations. Cumulative intakes were assessed 0.5, 1, 2 and 4 h after food reintroduction by weighing food pellets before and after each condition, and adjusting for spillage which was collected by paper under the wire mesh cage.

Glucoprivic Intake Protocol: Rats received the following bilateral microinjection conditions at weekly intervals: vehicle/vehicle, vehicle/2DG (500 mg/kg, i.p.), naltrexone at total doses of 20 and 50 μg paired with 2DG, β-FNA at a total dose of 4 μg paired with 2DG, Nor-BNI at total doses of 1 and 4 μg paired with 2DG, DALCE at a total dose of 4 μg

paired with 2DG, and NTII at total doses of 1 and 4 μg paired with 2DG. 2DG injections occurred 3-5 h into the light cycle, and cumulative intakes, adjusted for spillage, were assessed at 0.5, 1, 2 and 4 h thereafter.

Sucrose Intake Protocol: Rats were introduced to the 10% sucrose solution over 1 week and intake had to exceed a criterion (10 ml in 60 min). Rats received the following bilateral microinjection conditions at weekly intervals: vehicle, naltrexone at total doses of 20 and 50 μg, β-FNA at a total dose of 4 μg, Nor-BNI at a total dose of 4 μg, DALCE at a total dose of 4 μg and NTII at doses of 1 and 4 μg. At 3-5 h into the light cycle, cumulative sucrose intakes were assessed at 5, 10, 15, 30, 45 and 60 min after introduction of the sipper tube.

Results: Experiment 2:

Histological Placements: Figure 5 illustrates the bilateral cannula placements within and immediately adjacent to the VTA for animals in the three ingestive paradigms (Paxinos and Watson, 1997). Of the 38 rats that were tested in one of the three ingestive protocols, 31 rats had proper bilateral cannula placements which were included in the data analysis. Twenty-four of these rats had bilateral cannulae localized within the VTA. The remaining seven rats had one cannula in the VTA, and the second cannula placement in a zone between the VTA and medial aspect of the substantia nigra, pars compacta. These two groups of animals failed to differ from each other in their antagonist-induced effects, and their data for each protocol were therefore pooled. The remaining seven rats had one or both cannula placements located either dorsal or dorsolateral to the VTA; none of these animals displayed any significant antagonist effects upon their specific intake paradigm.

VTA Opioid Antagonists and Deprivation-Induced Intake: Significant differences in deprivation-induced intake were observed among conditions after 1 ($F(7,70)=2.11$, $p<.05$), 2 ($F= 4.64$, $p<.0003$) and 4 ($F= 3.52$, $p<.003$), but not 0.5 ($F= 1.02$) h of food reintroduction. Figure 6 illustrates the cumulative effects over 4 h of opioid antagonists in the VTA upon deprivation-induced intake. Only the high (40 μg) total dose of naltrexone in the VTA significantly decreased (21%) deprivation-induced intake after 1, 2 and 4 h. Neither the 10 nor 20 μg naltrexone doses in the VTA significantly altered deprivation-induced intake. Neither the μ (β-FNA), κ_1 (Nor-BNI) nor δ_1 (DALCE) opioid antagonists in the VTA were effective in significantly altering deprivation-induced intake at any point across the 4 h time course. The δ_2 opioid antagonist, NTII in the VTA significantly decreased (19%) deprivation-

induced intake after 2 and 4 h.

VTA Opioid Antagonists and Glucoprivic-Induced Intake: Significant differences in glucoprivic-induced intake were observed among conditions after 0.5 ($F(9,81)= 10.76$, $p<.0001$), 1 ($F= 12.94$, $p<.0001$), 2 ($F= 16.14$, $p<.0001$) and 4 ($F= 27.43$, $p<.0001$) h. 2DG significantly increased food intake at each time point over 4 h relative to vehicle treatment. Figure 7 illustrates the cumulative effects over 4 h of opioid antagonists in the VTA upon 2DG-induced intake. Only the high (50 μg) total dose of naltrexone in the VTA significantly decreased (64%) 2DG-induced intake across the 4 h time course. The lower 20 μg naltrexone doses in the VTA failed to significantly alter 2DG-induced intake. The μ opioid antagonist, β-FNA in the VTA only transiently decreased 2DG-induced intake after 1 and 2 h (data not shown), but these effects failed to persist after 4 h. 2DG-induced intake was also transiently decreased by the 1 (1 h) and 4 (0.5-2 h) μg doses of the κ_1 opioid antagonist, Nor-BNI. The δ_1 opioid antagonist, DALCE in the VTA transiently decreased 2DG-induced intake after 1 h, but produced subsequent significant increases (26%) in 2DG-induced intake after 4 h. The higher 4 μg dose of the δ_2 opioid antagonist, NTII in the VTA significantly decreased (27%) 2DG-induced intake across the time course while the lower 1 μg dose was ineffective.

VTA Opioid Antagonists and Sucrose Intake: Significant differences in sucrose intake were observed among conditions after 5 ($F(7,63)= 7.87$, $p<.0001$), 10 ($F= 4.49$, $p<.0004$), 15 ($F= 5.06$, $p<.0001$), 30 ($F= 7.98$, $p<.0001$), 45 ($F= 6.47$, $p<.0001$) and 60 ($F= 5.14$, $p<.0001$) min. Figure 8 illustrates the cumulative effects over 1 h of opioid antagonists in the VTA upon sucrose intake. Sucrose intake was significantly decreased by

VTA microinjections of both the 20 (22%: 45-60 min) and 50 (39%: 10-60 min) μg doses of naltrexone. The μ opioid antagonist, β-FNA in the VTA failed to significantly alter sucrose intake at any time point. Sucrose intake was significantly though only transiently decreased by VTA microinjections of either the κ_1 opioid antagonist, Nor-BNI (10-15 min) or the δ_1 opioid antagonist, DALCE (5-10 min). The higher 4 μg dose of the δ_2 opioid antagonist, NTII in the VTA significantly decreased sucrose intake by 25% across the 1 h time course; the lower 1 μg dose produced significant, though transient (5-45 min) reductions.

Figure 5. Histological verification of cannula placements in the VTA. Note that 24 of the animals had bilateral (n=48) cannula placements in the VTA. Seven animals had a unilateral (n=7) cannula placement in the VTA and the second (n=7) cannula placement in the medial aspect of the substantia nigra (SN), pars compacta. The following numbers of cannula placements were in the depicted areas: a (n=3), b (n=2), c (n=11), d (n=14), e (n=3), f (n=14), g (n=11) and h (n=4). Rats with bilateral VTA placements or VTA/SN combination placements did not differ in their antagonist-induced effects, and their data were pooled. The remaining seven animals had misplaced cannulae dorsal and/or lateral to the VTA, and were not included in the data analysis. They failed to display antagonist-induced effects.

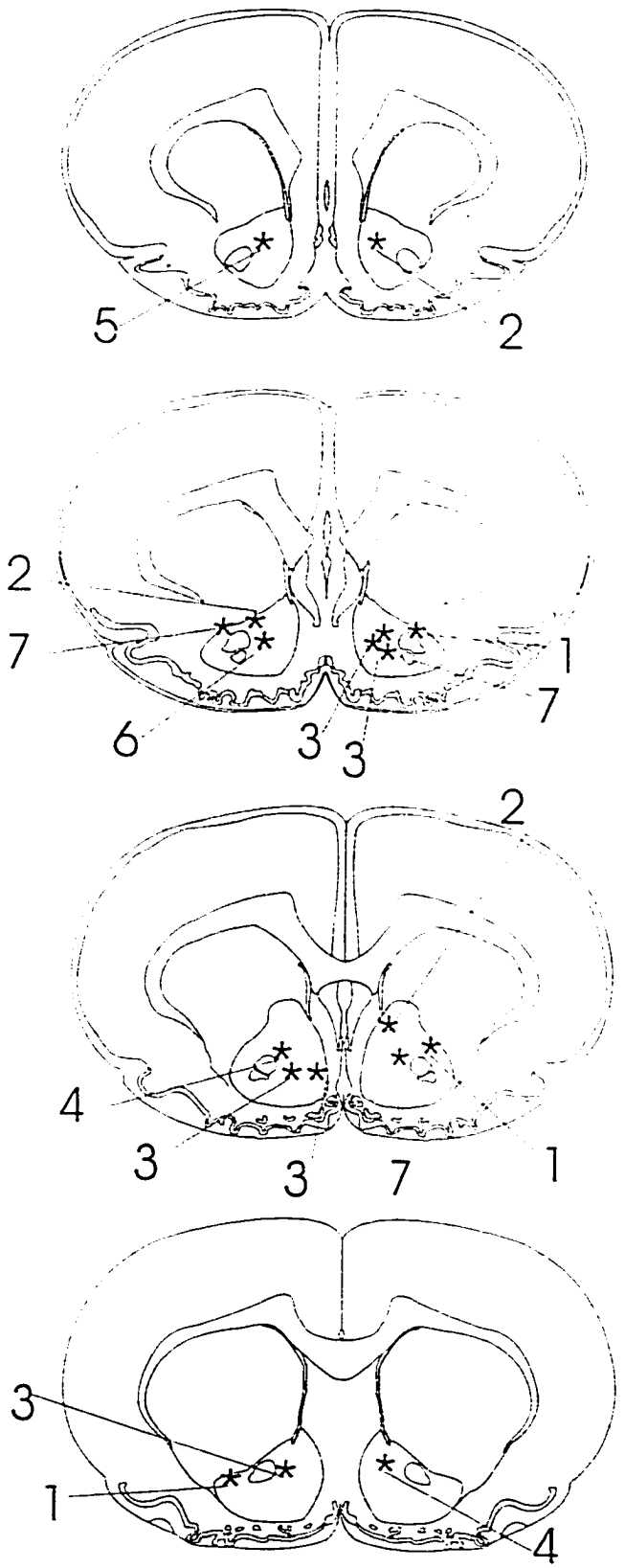


Figure 6. Alterations (Mean, ±SEM) in food intake (4 h) following microinjections (μg) of either vehicle (n=11), the general opioid antagonist, naltrexone (NTX) at doses of 10 (n=8), 20 (n=7) and 40 (n=7) μg, the μ opioid antagonist, β-funaltrexamine (β-FNA, n=8), the κ_1 opioid antagonist, nor-binaltorphamine (Nor-BNI, n=8), the δ_1 opioid antagonist, DALCE (n=5) or the δ_2 opioid antagonist, naltrindole isothiocyanate (NTII, n=5) into the VTA in rats deprived of food for 24 h. The daggers denote significantly reduced intake relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$).

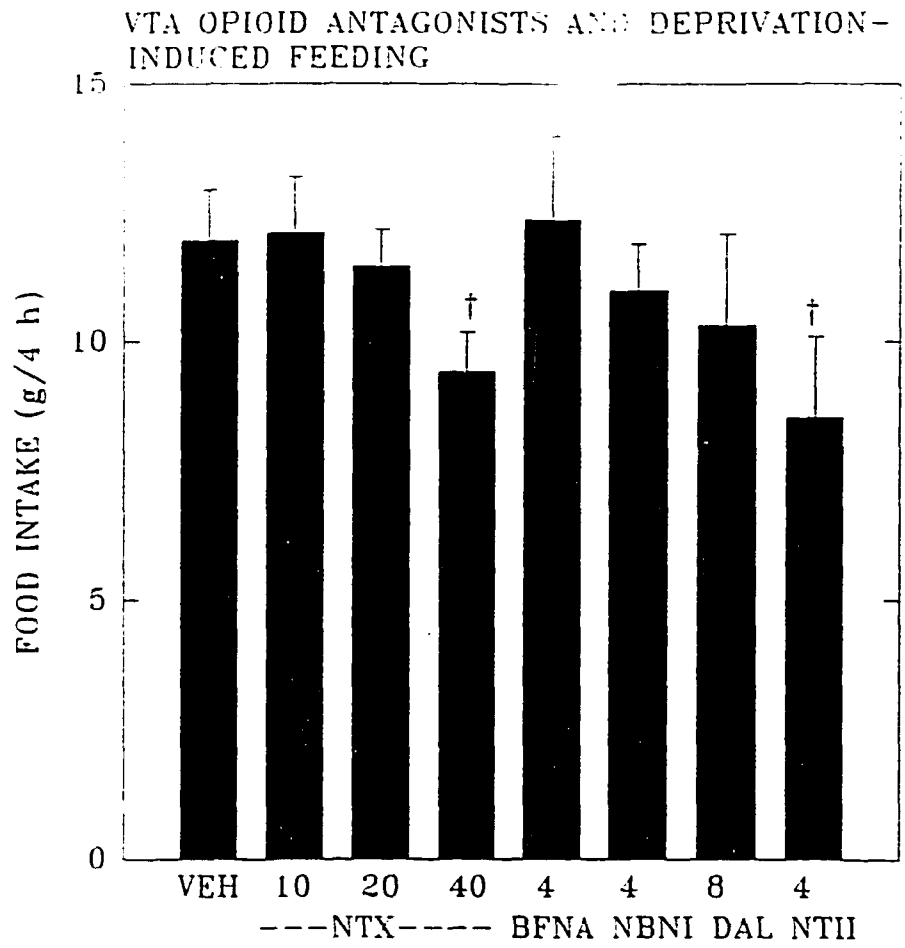
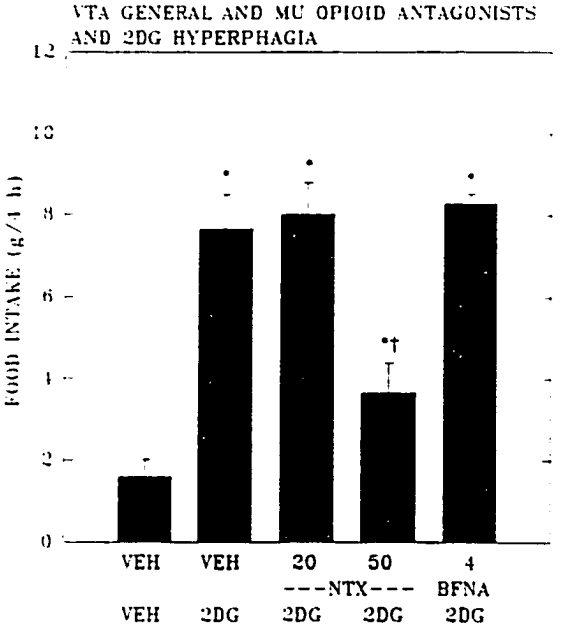


Figure 7. Alterations (Mean, ±SEM) in food intake (4 h) following microinjections (μg) of either vehicle (n=10), naltrexone (NTX, upper panel) at doses of 20 (n=4) and 50 (n=5) μg, β-funaltrexamine (β-FNA, n=5, upper panel), nor-binaltorphamine (Nor-BNI, lower panel) at doses of 1 (n=4) and 4 (n=4) μg, DALCE (n=6, lower panel) or naltrindole isothiocyanate (NTII, lower panel) at doses of 1 (n=4) and 4 (n=5) μg into the VTA in rats receiving 2-deoxy-D-glucose (2DG: 500 mg/kg, ip). The stars denote significant increases in food intake in groups receiving 2DG relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$). The daggers denote significantly reduced 2DG-induced hyperphagia relative to VEH-2DG treatment (Tukey comparisons, $p<.01$).



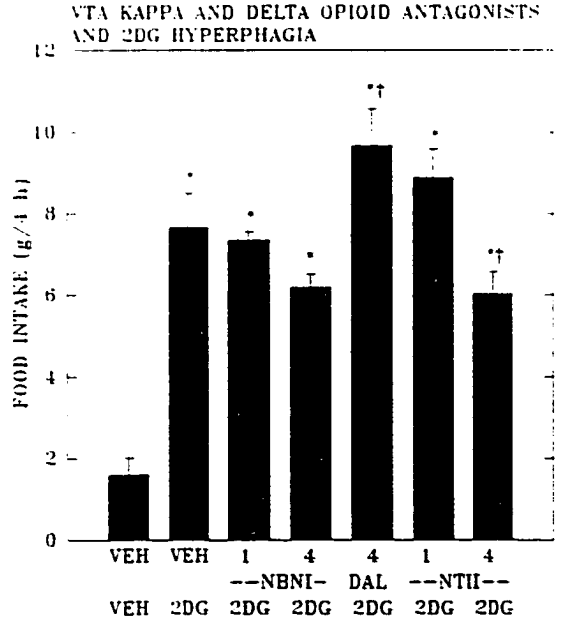
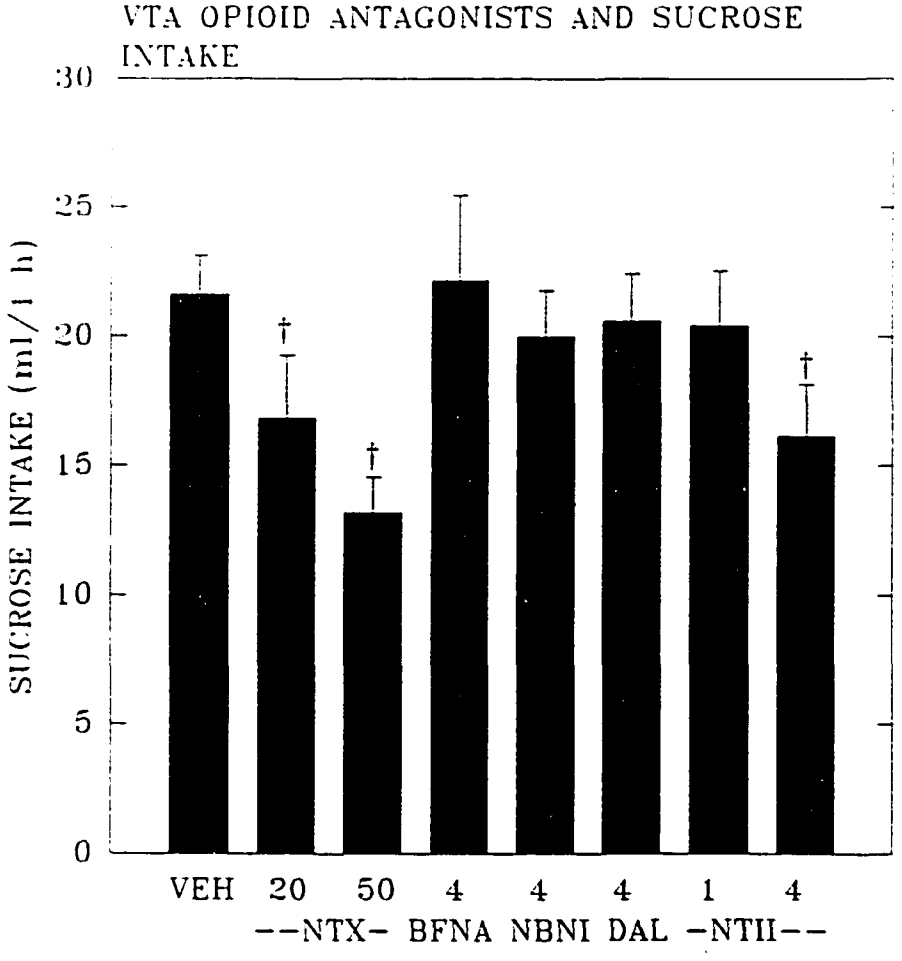


Figure 8. Alterations (Mean, ±SEM) in sucrose (10%) intake (1 h) following microinjections (μg) of either vehicle (n=10), naltrexone (NTX) at doses of 20 (n=6) and 50 (n=6) μg, β-funaltrexamine (β-FNA, n=6), nor-binaltorphamine (Nor-BNI, n=7), DALCE (n=5) or naltrindole isothiocyanate (NTII) at doses of 1 (n=7) or 4 (n=7) μg into the VTA. The daggers denote significantly reduced intake relative to vehicle (VEH) treatment (Tukey comparisons, $p<.01$).



Discussion: Experiment 2

General Opioid Antagonism in the VTA: The present study found that bilateral VTA microinjections of general (naltrexone) opioid antagonists significantly reduced deprivation-induced intake, 2DG-induced intake and sucrose intake. Naltrexone's reductions of deprivation-induced intake in the VTA only occurred at a high (40 μg), but not lower (10-20 μg) doses, persisted from 1-4 h after food reintroduction, and only produced an overall 21% decrease in intake over the 4 h paradigm. While the duration of naltrexone's effects in the VTA was comparable to that observed following either systemic (Brown and Holtzman, 1979; Frenk and Rogers 1979) or ventricular (Koch and Bodnar, 1994; Marks-Kaufman, 1985) administration for deprivation-induced intake, the magnitude of naltrexone's effects in the VTA was considerably smaller than the approximate 50% reduction in intake observed following systemic and ventricular administration. One might question whether the relative inability of naltrexone to exert effects in the VTA was due to the modest role that the opioid system plays in this site, or alternatively, whether the lipophilic characteristics of naltrexone allowed it to disperse from the injection site and thereby raise the need for more compound for receptor antagonism. The present study has no data indicating the dispersion rate of naltrexone, but comparison of naltrexone's effects in other sites may provide insight. Far lower doses (5-20 μg) of naltrexone produced larger (44%) reductions in deprivation-induced intake over the same time course following microinjection into the NAcc (Experiment 1), while it failed to produce significant reductions in shorter (0.5 h) feeding tests (Kelley et al., 1996). A hypothesis citing rapid dispersion of naltrexone would predict the opposite pattern with maximal effects immediately after injection and dissipating effects thereafter. Indeed, if

naltrexone is administered into the PVN, a comparably-sized structure to the VTA, it reduced deprivation-induced intake by 29% at far lower (10 μg) doses (Koch et al., 1995). A note of caution is appropriate however in that some of the protocols or statistical tests used in other studies were not always the same as those used in the current study. Therefore, these data suggest, but do not prove that naltrexone's relative inaction in the VTA in this and the following procedures is indicative of the limited role that the VTA plays in the mediation of these behaviors, and not due to non-selective effects of rapid dispersion.

Naltrexone's reductions in the VTA of 2DG-induced intake again only occurred at a high (50 μg), but not lower (20 μg) dose, persisted across the 4 h time course, and produced an overall 64% decrease in intake. Both the magnitude and duration of naltrexone's effects in the VTA were comparable to that observed following either systemic (Beczkowska et al., 1992; Lowy et al., 1980) or ventricular (Arjune and Bodnar, 1990; Koch and Bodnar, 1994) administration for 2DG-induced intake. However, the VTA dose (50 μg) was considerably higher than those doses used in ventricular studies (5-20 μg). Although the magnitude of naltrexone's effects in the VTA upon 2DG-induced intake was comparable to that observed following naltrexone administration into either the NAcc (10-20 μg: 79%; 10) or the hypothalamic PVN (10 μg: 69%; 32), a 2.5-fold higher dose was needed to produce this effect in the VTA. However, naltrexone's effects in the VTA were more prolonged (4 h) than naltrexone's effects (2 h) in either the NAcc or PVN. Therefore, it appears that VTA naltrexone effects upon 2DG-induced intake appeared comparable to other intracerebral loci in terms of magnitude, was more prolonged, but was less potent.

Naltrexone's reductions in the VTA of sucrose intake were dose-dependent (20-50

μg), and produced 22-39% reductions in intake. The magnitude of the dose-dependent reductions in sucrose intake by naltrexone in the VTA was quite comparable to this antagonist's dose-dependent reductions in apple juice intake (Segall and Margules, 1989). The magnitude and potency of naltrexone in the VTA to reduce sucrose intake were also quite comparable to that observed for naltrexone administered into either the PVN (5-10 μg, 18-31%; 36) or the NAcc (30-50 μg, 27-35%; (Experiment 1; Kelley et al., 1996). The limited ability of either the VTA, NAcc or PVN to support naltrexone-induced reductions in sucrose intake is in marked contrast to the 60-75% reductions observed following either systemic or ventricular administration of naltrexone (Beczkowska et al., 1992; Kirkham and Cooper, 1988; Rockwood and Reid, 1982; Siviy and Reid, 1983).

μ Opioid Antagonism in the VTA: The inability of μ opioid antagonism to reduce either deprivation-induced intake, 2DG-induced intake or sucrose intake following VTA microinjections was quite surprising given its purported roles in these forms of intake following ventricular and other intracerebral microinjection studies. While naltrexone and β-FNA block the μ receptor, the former, but not the latter produced effects in the VTA upon these ingestive responses. Whereas β-FNA is a highly selective antagonist for the μ receptor particularly after its reversible κ agonist actions (0-6 h) have dissipated (Portoghese et al., 1980; Takemori et al., 1981; Ward et al., 1982), naltrexone also has activity at δ and, to a lesser degree, κ receptors (see reviews: Sawynok et al., 1979; Zukin and Zukin, 1981). Thus, the limited naltrexone effects appear to be due to δ, and not μ antagonism. μ opioid antagonism with β-FNA significantly reduced either deprivation-induced intake, 2DG-induced intake or sucrose intake following ventricular microinjections (Arjune et al., 1990;

Beczkowska et al., 1992; Koch and Bodnar, 1994; Levine et al., 1991). Administration of μ opioid antagonists into either the PVN or NAcc significantly reduced all three forms of intake (Experiment 1; Kelley et al., 1996; Koch et al., 1995).

On the basis of autoradiographic, receptor binding and in situ hybridization mRNA expression studies (German et al., 1993; Mansour et al., 1995, 1987; Tempel and Zukin, 1987), one would expect μ-mediated opioid actions given the presence of moderate to dense levels of opioid receptors in the VTA. Further, μ agonists administered into the VTA increase dopamine release in the NAcc in a manner more potent than δ and κ opioid agonists (Devine et al., 1993; DiChiara et al., 1988; Noel and Gratton, 1995; Spanagel et al., 1990). However, the μ antagonists, CTOP and β-FNA also increase dopamine release in the NAcc (Devine et al., 1993), indicating that both μ agonists and μ antagonists are producing similar and seemingly paradoxical actions. It should be further noted that increased locomotor activity is observed following VTA microinjections of both μ agonists (see review: Wise and Bozarth, 1987) and μ antagonists (Badiani et al., 1985).

The parallel actions of μ-selective agonists and antagonists in the VTA upon mesolimbic dopamine release and locomotor activity may occur as a consequence of complex interactions between opioid actions on GABAergic afferents to the VTA and GABAergic interactions within the VTA (Devine et al., 1993). μ-selective agonists in the VTA stimulate intake, but produce more potent effects in deprived animals (Mucha and Iversen, 1986; Nencini and Stewart, 1990; Noel and Wise, 1993, 1995). However, the μ agonist effects in the VTA may not be selective for ingestion since VTA DAMGO also increases gnawing and drinking when these objects were available, and since VTA DAMGO did not stimulate intake

of palatable food when it was only accessible by traversing a tunnel to another cage (Badiani et al., 1995a). The present failure to observe VTA antagonist effects upon intake under deprivation, glucoprivic and palatable conditions extends the observation that CTOP in the VTA failed to alter palatable intake during the onset of the dark cycle (Badiani et al., 1995b), and argues strongly for the proposition that the VTA is not an integral site for μ-mediated actions upon ingestion.

κ Opioid Antagonism in the VTA: The inability of κ_1 opioid antagonism to reduce deprivation-induced intake, 2DG-induced intake and sucrose intake following VTA microinjections was not surprising given the questionable actions of κ agonists upon ingestion at this site. VTA administration of the selective κ_1 agonist, U50,488H fails to alter food intake (Noel and Wise, 1993) and fails to alter dopamine release in the NAcc (Devine et al., 1993), yet it enhances feeding elicited by electrical stimulation of the lateral hypothalamus (Jenck et al., 1986). While dynorphin stimulates feeding in the VTA (Hamilton and Bozarth, 1988), this study did not demonstrate that it was mediated specifically by κ receptors. Therefore, while κ receptors are present in the VTA (Mansour et al., 1995, 1987; Speciale et al., 1993, Tempel and Zukin, 1987), they do not appear to be important in mediating ingestive effects.

δ Opioid Antagonism in the VTA: The ability of δ_2 (NTII), but not δ_1 (DALCE) opioid antagonists to reduce either deprivation-induced intake, 2DG-induced intake or sucrose intake following VTA microinjections was also quite surprising given previous failures to observe consistent δ antagonist effects in ingestion. Neither δ (naltrindole, ICI174864) nor δ_1 (DALCE) opioid antagonists reduce deprivation-induced intake, 2DG-induced intake or sucrose intake following ventricular or systemic administration (Arjune et

al., 1991; Beczkowska et al., 1992; Jackson and Sewell, 1985; Koch and Bodnar, 1994). Further, naltrindole administered into the NAcc actually enhanced the duration of chow feeding in deprived rats (Kelley and Bless, 1996). The δ_2 opioid antagonist, NTII has only been tested in limited ingestive paradigms, and ventricular administration reduced spontaneous food intake and deprivation-induced water intake, but failed to reduce maltose dextrin intake (Cole et al., 1995; Leventhal and Bodnar, 1996).

The present finding that δ_2, but not δ_1 antagonists in the VTA reduced all three ingestive paradigms suggests a specific δ subtype action, but several factors need to be considered. The specificity of DALCE and NTII for respective δ_1 and δ_2 opioid actions has been confirmed largely in analgesic assays (Jiang et al., 1991; Mattia et al., 1992, 1991; Sofuoglu et al., 1991). However, DALCE significantly reduced food intake elicited by the δ_2 opioid agonist, (D-Ala^2,Glu^4)-deltorphin (Delt II), but not the δ_1 opioid agonist, DPDPE. In contrast, NTII significantly reduced food intake elicited by either δ subtype agonist (Yu et al., 1997). That δ_1 and δ_2 receptor subtypes are differentially involved in 2DG-induced intake and sucrose intake is supported by agonist studies. Ventricular administration of Delt II, but not DPDPE produces 3-fold leftward shifts in 2DG's hyperphagic dose-response curve (Yu et al., 1997). Whereas DPDPE stimulates sucrose intake at moderate (2.5%) and high (10%) concentrations, Delt II stimulates sucrose intake at low (0.5%) and moderate (2.5%) concentrations (Ruegg et al., 1997). Further, both general δ (D-ala-met-enkephalin: Cador et al., 1986) and selective δ_1 (DPDPE: Noel and Wise, 1995) agonists stimulate food intake following microinjection in the VTA. The δ agonist and antagonist actions in the VTA are consistent with the presence of moderate densities of δ receptors (German et al., 1993,

Mansour et al., 1995, 1987; Speciale et al., 1993; Temple and Zukin, 1987).

The effects of δ opioid agonists and antagonists in the VTA upon ingestive behavior appear to support the contention that δ opioid receptors serve a modulatory, rather than direct role in ingestion (see review: Bodnar, 1996). In this model, δ receptors in the VTA would not be in a hypothesized "final common pathway" for the elicitation and maintenance of ingestion, but rather would be found in a hypothesized "modulatory" pathway, that, if stimulated by δ receptor agonists, would increase the efficacy of the "final common pathway" and thereby increase intake. The hypothesized pathway that δ receptors in the VTA would modulate is the dopaminergic projection from the VTA to the NAcc. In this regard, systemic administration of naltrexone and selective dopaminergic antagonists interact with each other to significantly reduce both deprivation-induced intake and 2DG-induced intake (Hobbs et al., 1994; Schaefer et al., 1994). Further, injections of opiates into the VTA increases dopamine turnover and release in the NAcc (Devine et al., 1993; Joyce and Iversen, 1979; Leone et al., 1991; Noel and Gratton, 1995), and increases the firing rates of single dopamine neurons (Gysling and Wang, 1983; Yim and Mogenson, 1980). Further studies should examine whether opioid agonist prophagic actions in the VTA can be altered by receptor antagonists in the NAcc.

Conclusions: The VTA was chosen for the study of intracerebral opioid antagonist effects because of its well-established role in the mediation of the reinforcing and "rewarding" aspects of drugs, including opioids (see reviews: Self and Stein, 1992; Wise and Bozarth, 1987; Wise and Hoffman, 1992; Wise and Rompre, 1989). Since opioid receptor agonists appear to act in part through the "rewarding" aspects of feeding (see reviews: Cooper et al.,

1988, Gosnell and Levine 1996; Levine et al., 1985), the VTA would appear to be an ideal candidate at which these mechanisms work. However, the present set of studies largely fails to support this hypothesis that opioid antagonists in the VTA would interfere with the rewarding aspects of feeding. Although naltrexone in the VTA reduced intake in the three feeding paradigms, it did so at higher doses than was needed for ventricular effects. Further, neither μ nor κ_1 antagonists, highly effective in blocking intake under both challenging and rewarding conditions following ventricular administration, were active following VTA administration. Finally, δ_2 antagonists produced small, but consistently significant reductions in each of the three forms of intake. Thus, these data indicate that the VTA plays a relatively minor role in the elicitation of these forms of food intake, and that δ_2 receptors appear responsible for this limited level of mediation. The results from the first and second experiments of this dissertation indicate that selective opioid receptor subtype mediation of feeding under deprivation, glucoprivic and palatable conditions occurs in the NAcc rather than in the VTA. Therefore, the third and fourth experiments in this dissertation focus upon the pharmacological mechanisms underlying opioid-mediated ingestive effects in the NAcc.

EXPERIMENT 3. Role of Opioid Receptor Subtype Antagonists in the Nucleus Accumbens Shell in Mediating Feeding Elicited by Opioid Receptor Subtype Agonists in the Nucleus Accumbens Shell.

Specific Methods, Experiment 3:

μ Agonist Intake Protocol: In all experiments, each rat received bilateral microinjection conditions in counterbalanced order at weekly intervals. Subgroups of rats receiving different antagonist conditions were matched on the basis of the particular intake conditions following vehicle treatment. All drugs were dissolved in a 0.9% normal saline solution except for DALCE (0.2 M HCl in distilled water with the pH raised to 7.5 to 8.0 by adding 0.2 M NaOH) and NAZ (distilled water and 0.2% glacial acetic acid). In assessing opioid receptor subtype antagonism upon μ-opioid agonist-induced feeding, subgroups of rats received the following bilateral microinjection conditions: a) vehicle (n=22), b) DAMGO (n=22, Peninsula Laboratories, Belmont, CA, 2.5 μg: 1.25 μg each side), β-FNA (Research Biochemicals Intl., Natick, MA) at doses of either c) 0.1 (n=7), d) 1 (n=10) or e) 4 (n=9) μg paired with DAMGO, f) NAZ (synthesized by Dr. G.W. Pasternak) at a 4 μg dose paired with DAMGO (n=9), DALCE (synthesized by Dr. W.D. Bowen) at doses of either g) 1 (n=7) or h) 4 (n=10) μg paired with DAMGO, NTII (Research Biochemicals Intl.) at doses of either i) 1 (n=7) or j) 4 (n=8) μg paired with DAMGO, and Nor-BNI (Research Biochemicals Intl.) at doses of either k) 1 (n=12) or l) 4 (n=8) μg paired with DAMGO.

δ_1 Agonist Intake Protocol: In assessing opioid receptor subtype antagonism upon δ_1-opioid agonist-induced feeding, subgroups of rats received the following bilateral

microinjection conditions: a) vehicle (n=16), b) DPDPE (n=16, Peninsula Laboratories, 5 μg: 2.5 μg each side), β-FNA at doses of either c) 1 (n=8) or d) 4 (n=7) μg paired with DPDPE, e) NAZ at a 4 μg dose paired with DPDPE (n=7), DALCE at doses of either f) 1 (n=7) or g) 4 (n=8) μg paired with DPDPE, NTII at doses of either h) 1 (n=7) or i) 4 (n=7) μg paired with DPDPE, and Nor-BNI at doses of either j) 1 (n=9) or k) 4 (n=7) μg paired with DPDPE.

δ_2 Agonist Intake Protocol: In assessing opioid receptor subtype antagonism upon δ_2-opioid agonist-induced feeding, subgroups of rats received the following bilateral microinjection conditions: a) vehicle (n=17), b) Deltorphin (n=17, Peninsula Laboratories, 5 μg: 2.5 μg each side), β-FNA at doses of either c) 1 (n=7) or d) 4 (n=7) μg paired with Deltorphin, NTII at doses of either e) 1 (n=9), f) 4 (n=7) or g) 8 (n=8) μg paired with Deltorphin, and h) Nor-BNI at a 4 μg dose paired with Deltorphin (n=8). It should be noted that neither NAZ nor DALCE was available for the last part of this protocol, and therefore these antagonists were not tested against Deltorphin-induced feeding. Antagonist treatments, reflecting peak dose and time intervals, preceded agonist treatments by 1 h for Nor-BNI, and by 24 h for β-FNA, NAZ, DALCE and NTII. On the test day at 3-5 h into the light cycle, food was removed from the cages and antagonist-treated animals received the particular opioid agonist. All microinjections were administered bilaterally in 1 μl volumes over 30 s through a stainless steel internal cannula (33-gauge, Plastics One) connected to a Hamilton microsyringe by polyethylene tubing. This relatively high injection volume was necessary because of limited solubility of some of the antagonists. Cumulative intakes were assessed at

1, 2 and 4 h after the last injection by measuring preweighed food which was adjusted for spillage collected by paper towels placed under the wire mesh cages.

Results: Experiment 3:

Histological Verification: Figure 9 is a schematic of representative bilateral guide cannulae placements into the NAcc shell. Cannulae placements were all localized to the NAcc shell as far rostral as Figure 9, and as far caudal as Figure 14 of the Paxinos and Watson atlas (Paxinos and Watson, 1986). The blackened area represents the multiple locations of cannula tips in the NAcc shell.

NAcc Opioid Receptor Subtype Antagonists and NAcc DAMGO-induced Feeding: Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(11,231)= 23.12$, $p<0.0001$), 2 ($F= 21.30$, $p<0.0001$) and 4 ($F= 19.82$, $p<0.0001$) h. DAMGO significantly increased food intake relative to control treatment after 1, 2 and 4 h (Figures 10 and 11). Pretreatment in the NAcc with the selective μ opioid receptor subtype antagonist, β-FNA, dose-dependently reduced DAMGO-induced feeding across the 4h time course (Figure 10A). Whereas rats pretreated with the 0.1 μg dose of β-FNA displayed normal DAMGO-induced feeding in the NAcc, rats pretreated with either 1 or 4 μg doses of β-FNA failed to display significant feeding responses to DAMGO in the NAcc over the 4h time course.

In contrast, pretreatment with the selective μ_1 opioid receptor subtype antagonist, NAZ in the NAcc failed to alter NAcc DAMGO-induced feeding at any time point (Figure 10B). Similarly, pretreatment with the selective δ_1 opioid receptor subtype antagonist, DALCE, in the NAcc failed to alter NAcc DAMGO-induced feeding at any time point (Figure 11A). NAcc DAMGO- induced feeding was dose-dependently reduced by pretreatment with the selective δ_2 opioid receptor antagonist, NTII in the NAcc with the 4, but not the 1 μg

antagonist dose, thereby preventing the expression of NAcc DAMGO- induced feeding after 2 and 4 h (Figure 11B). Similarly, pretreatment of the selective κ_1 opioid receptor subtype antagonist, Nor-BNI, in the NAcc significantly reduced NAcc DAMGO- induced feeding following the 4 but not the 1 μg dose across the 4 h time course (Figure 11C). Therefore, it appears that the μ, δ_2 and κ_1 opioid receptors antagonists, but not μ_1 or δ_1 opioid receptor antagonists administered into the NAcc block feeding induced by the μ opioid agonist, DAMGO in the NAcc.

NAcc Opioid Receptor Subtype Antagonists and NAcc DPDPE-induced Feeding: Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(10,150)= 12.15$, $p<0.0001$), 2 ($F= 9.69$, $p<0.0001$) and 4 ($F= 10.89$, $p<0.0001$) h. DPDPE significantly increased food intake relative to control treatment after 1, 2 and 4 h (Figures 12 and 13). Pretreatment in the NAcc with the selective μ opioid receptor subtype antagonist, β-FNA, significantly reduced DPDPE-induced feeding following both antagonist doses over the 4 h time course (Figure 12A). In contrast, pretreatment with the selective μ_1 opioid receptor subtype antagonist, NAZ in the NAcc failed to alter NAcc DPDPE-induced feeding at any time point (Figure 12B). Pretreatment with the selective δ_1 opioid receptor subtype antagonist, DALCE, in the NAcc prevented NAcc DPDPE-induced feeding after 4 h following the 1 μg antagonist dose, and after 2 and 4 h following the 4 μg antagonist dose (Figure 13A). Pretreatment with the selective δ_2 opioid receptor antagonist, NTII in the NAcc unexpectedly prevented NAcc DPDPE-induced feeding across the time course following the 1, but not the 4 μg antagonist dose (Figure 13B). Pretreatment with the selective κ_1 opioid receptor antagonist, Nor-BNI in the NAcc prevented DPDPE-induced

feeding across the time course following the 4, but not the 1 μg antagonist dose (Figure 13C). Thus it appears that μ, δ_1, δ_2 and κ_1, but not μ_1 opioid receptor antagonists injected into the NAcc block feeding induced by the δ_1 opioid agonist, DPDPE in the NAcc.

NAcc Opioid Receptor Subtype Antagonists and Deltorphin-induced Feeding:

Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(7,119)= 18.74$, $p<0.0001$), 2 ($F= 16.39$, $p<0.0001$) and 4 ($F= 10.84$, $p<0.0001$) h. Deltorphin significantly increased food intake relative to control treatment after 1, 2 and 4 h (Figure 14). Pretreatment with the selective δ_2 opioid receptor subtype antagonist, NTII, in the NAcc failed to alter NAcc Deltorphin-induced feeding at any time point and at any of the antagonist doses ranging from 1 to 8 μg (Figure 14A). Pretreatment with the selective μ opioid receptor subtype antagonist, β-FNA, in the NAcc, failed to alter NAcc Deltorphin-induced feeding except for a transient (1 h) though significant increase in intake following the 4 μg antagonist dose (Figure 14B). Indeed, pretreatment with the selective κ_1 opioid receptor subtype antagonist, Nor-BNI, in the NAcc, significantly increased NAcc Deltorphin-induced feeding across the entire 4 h time scale (Figure 14C). Thus, it appears that none of the selective opioid receptor subtype antagonists tested decreased Deltorphin-induced feeding in the NAcc, and that μ and κ_1 opioid antagonist pretreatment augmented this ingestive response.

Figure 9. Schematic depiction of directions of bilateral guide cannulae placements positioned towards the shell region of the NAcc (AcbSh). The majority of the tips of the confirmed cannula placements were found in the shaded area. All placements were found medial to the core region of the NAcc (AcbC) and lateral to the ventral diagonal band (VNB) area. aca: anterior commissure. This schematic was based upon the Paxinos and Watson Brain Atlas (42).

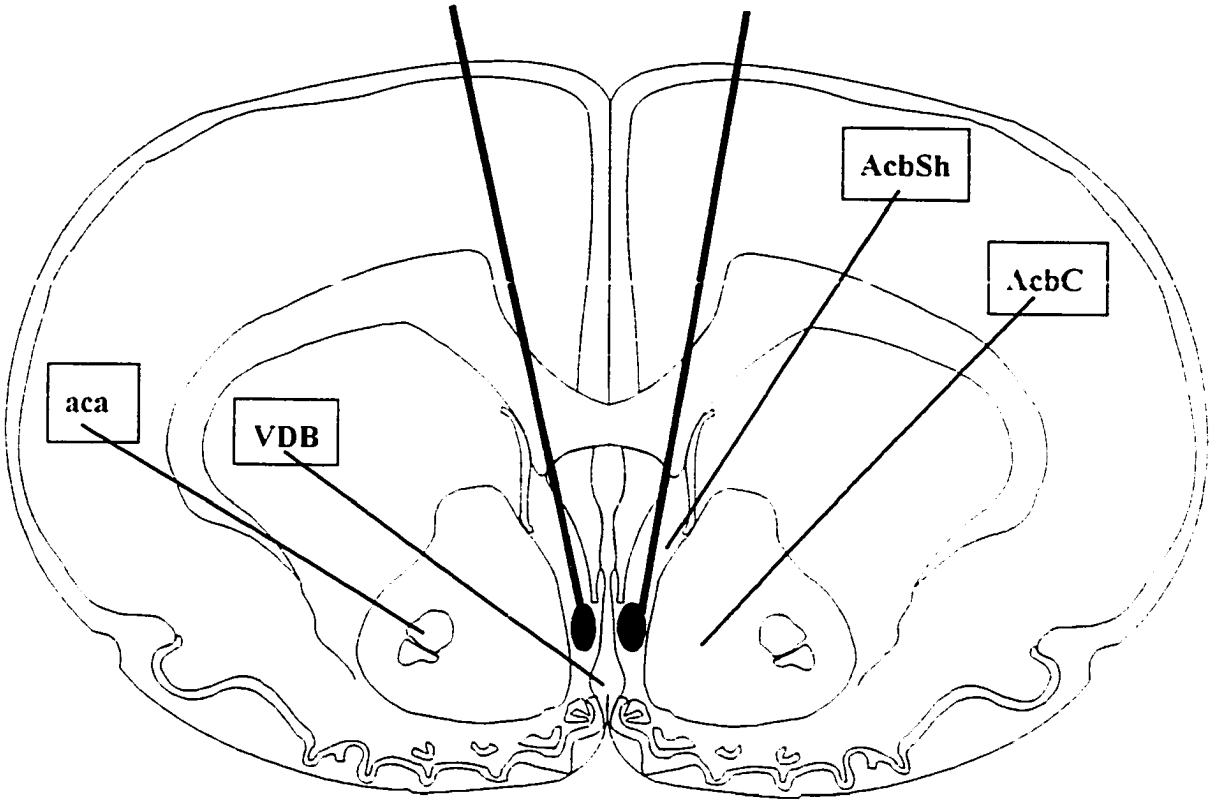
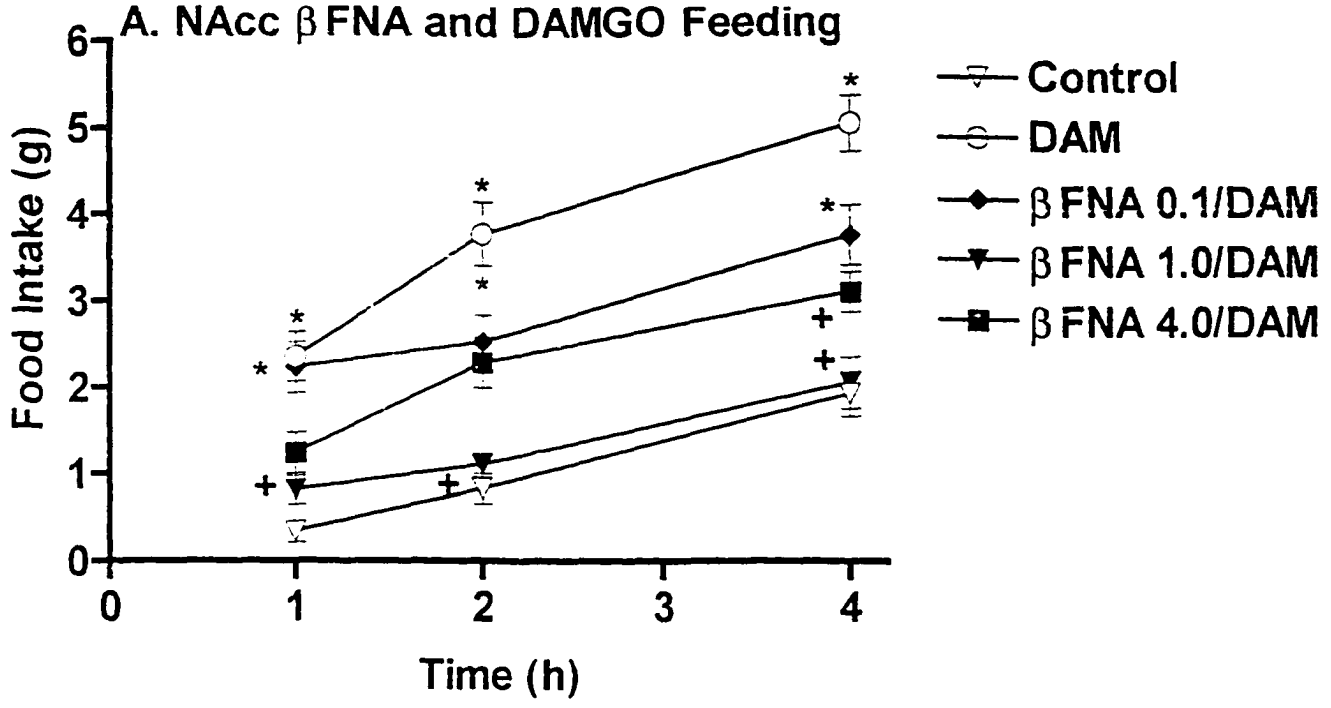


Figure 10. Alterations in spontaneous food intake (g, ± SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the NAcc shell relative to Control treatment. The upper and lower panels depict effects upon DAMGO-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective μ-opioid antagonist, β-funaltrexamine (β-FNA) or the selective μ_1-opioid antagonist, naloxonazine (NAZ) respectively. The asterisks (*) in this and subsequent figures indicate significant increases in food intake following opioid agonist treatment relative to Control treatment (Tukey comparisons, $p<0.05$). The crosses (+) in this and subsequent figures indicate significant alterations in food intake following opioid antagonist pretreatment relative to opioid agonist treatment alone (Tukey comparisons, $p<0.05$).



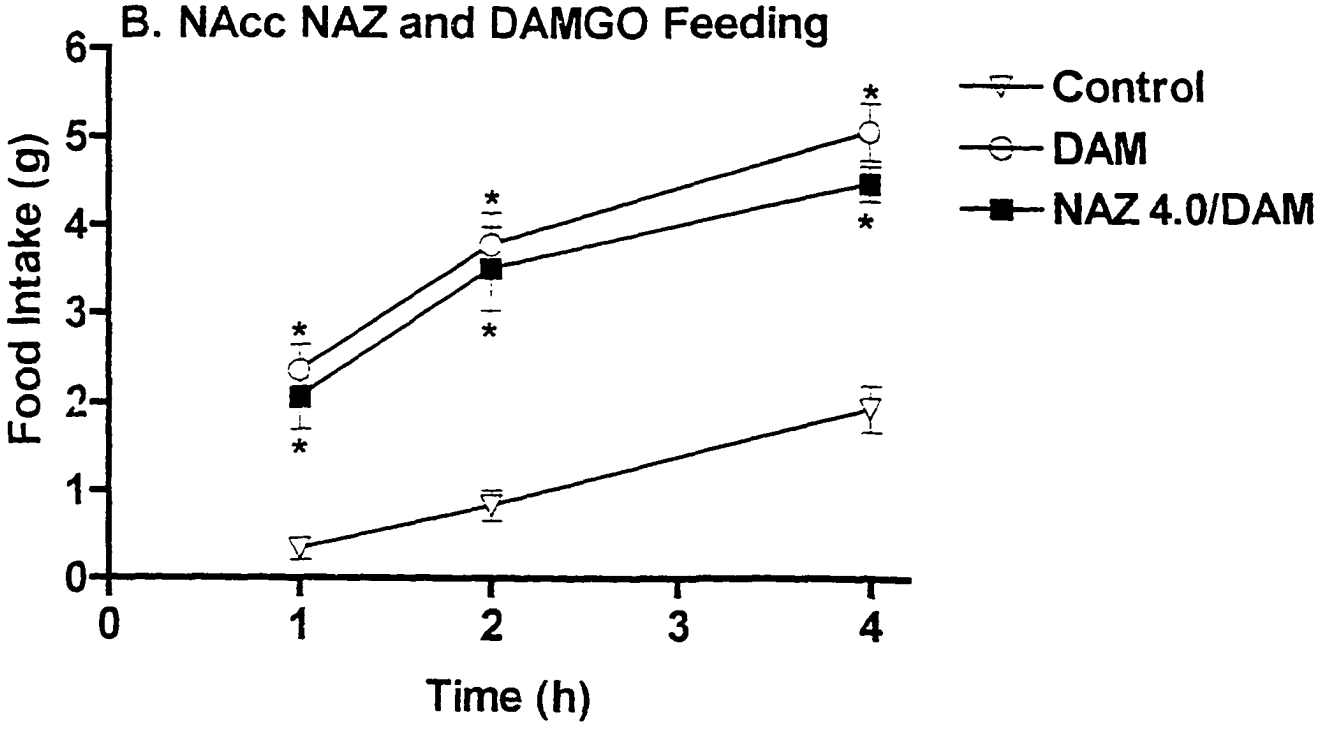
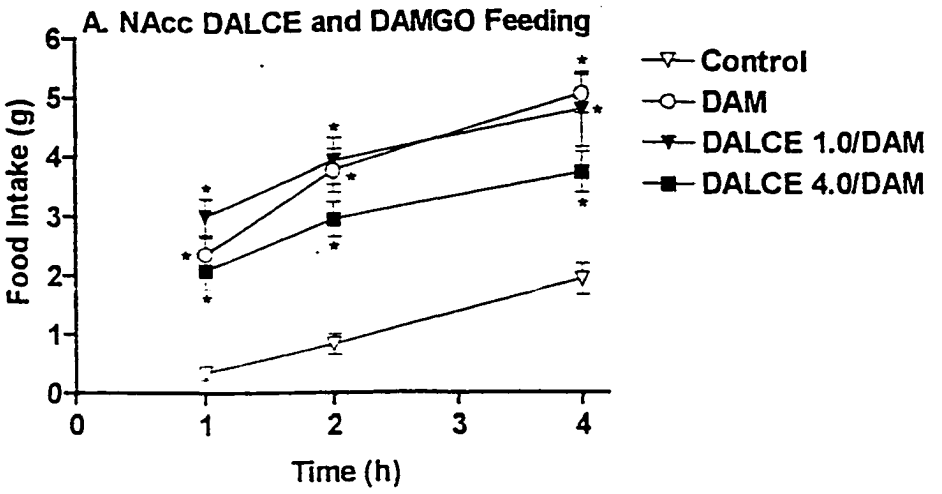
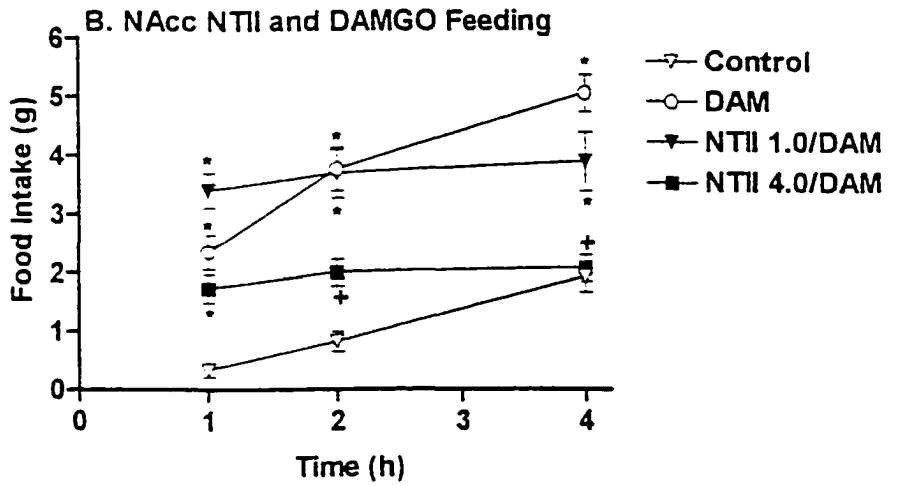


Figure 11. Alterations in spontaneous food intake (g, ± SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon DAMGO-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective δ_1-opioid antagonist, DALCE or the selective δ_2-opioid antagonist, naltrindole isothiocyanate (NTII) respectively. The lower panel depicts effects upon DAMGO-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).





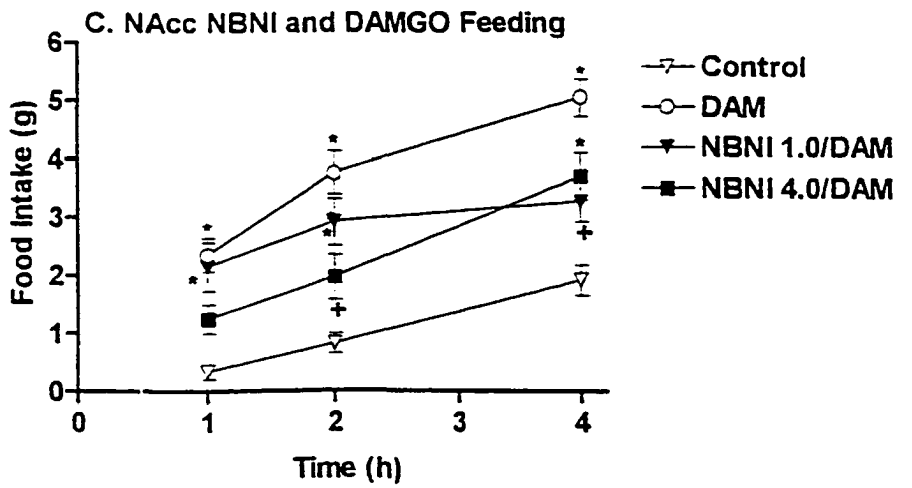
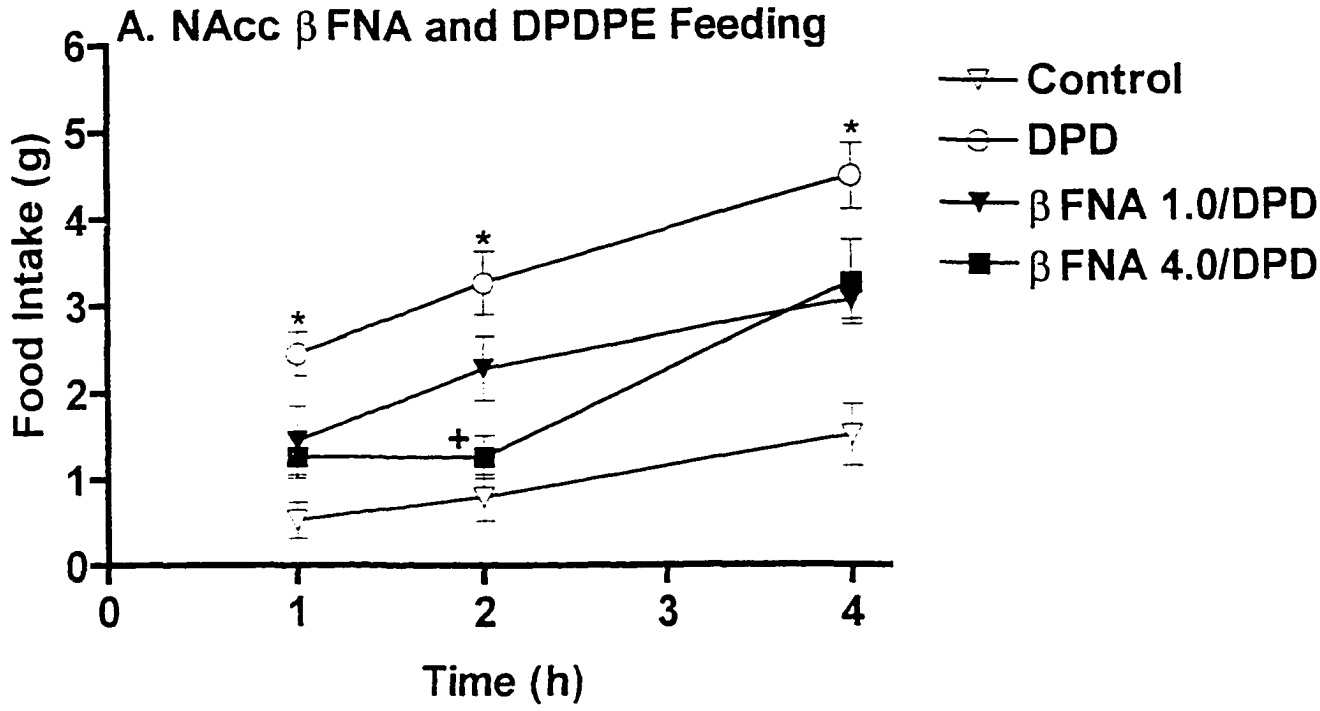


Figure 12. Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_1-selective opioid agonist, DPDPE (DPD) bilaterally in the NAcc shell relative to Control treatment. The upper and lower panels depict effects upon DPDPE-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective μ-opioid antagonist, β-funaltrexamine (β-FNA) or the selective μ_1-opioid antagonist, naloxonazine (NAZ) respectively.



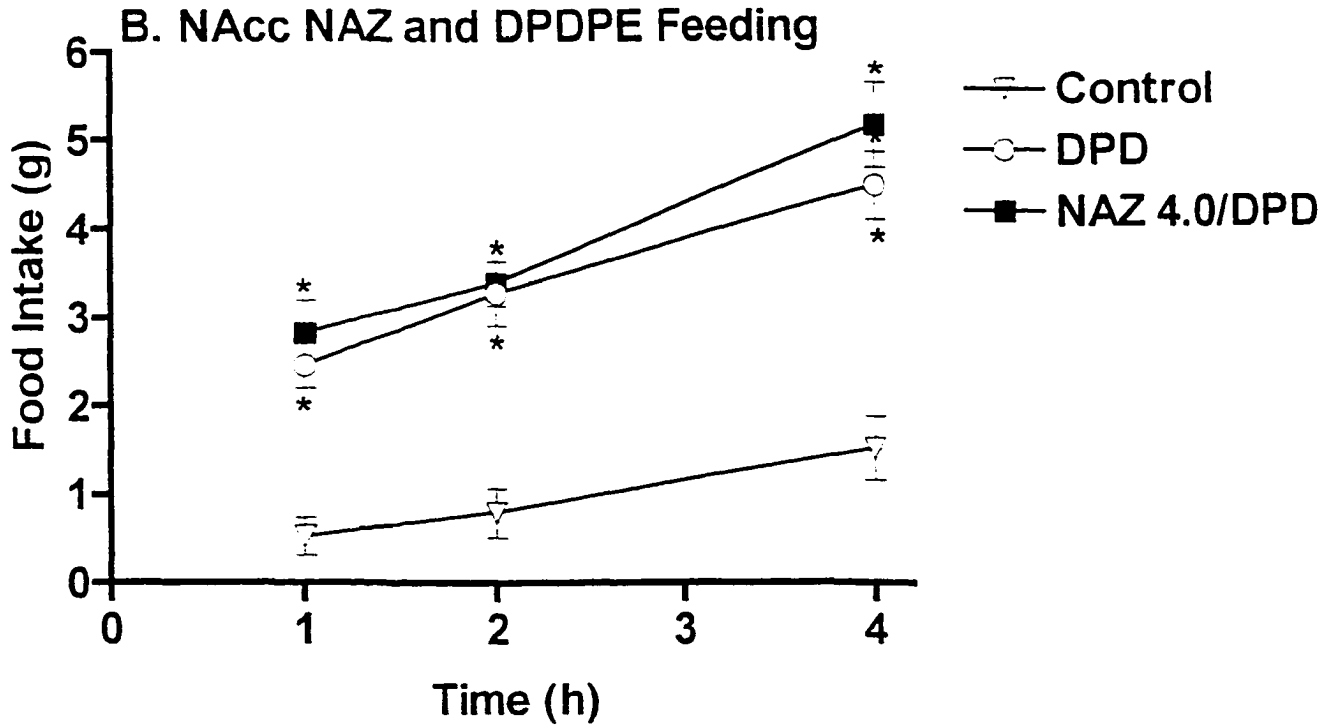
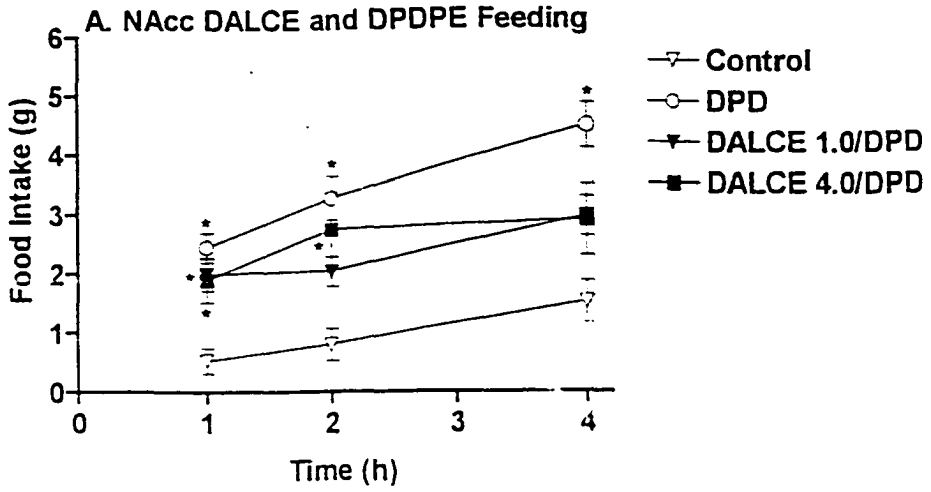
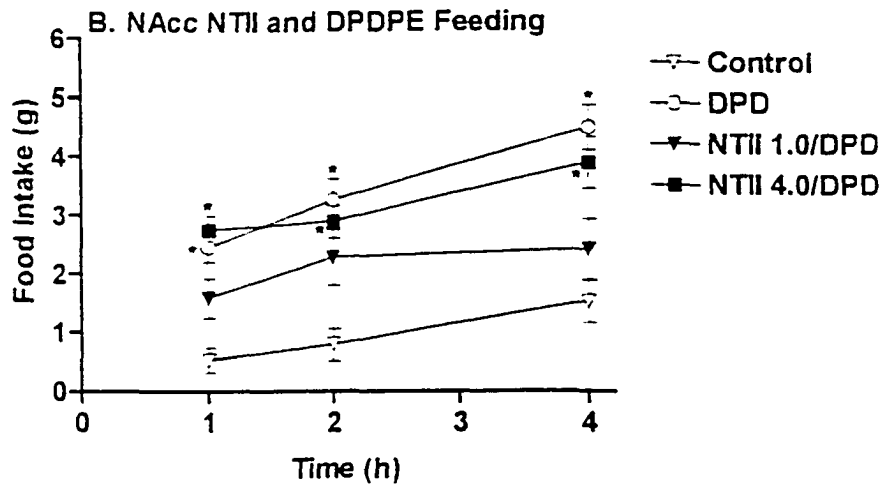


Figure 13. Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_1-selective opioid agonist, DPDPE (DPD) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon DPDPE-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective δ_1-opioid antagonist, DALCE or the selective δ_2-opioid antagonist, naltrindole isothiocyanate (NTII) respectively. The lower panel depicts effects upon DPDPE-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).





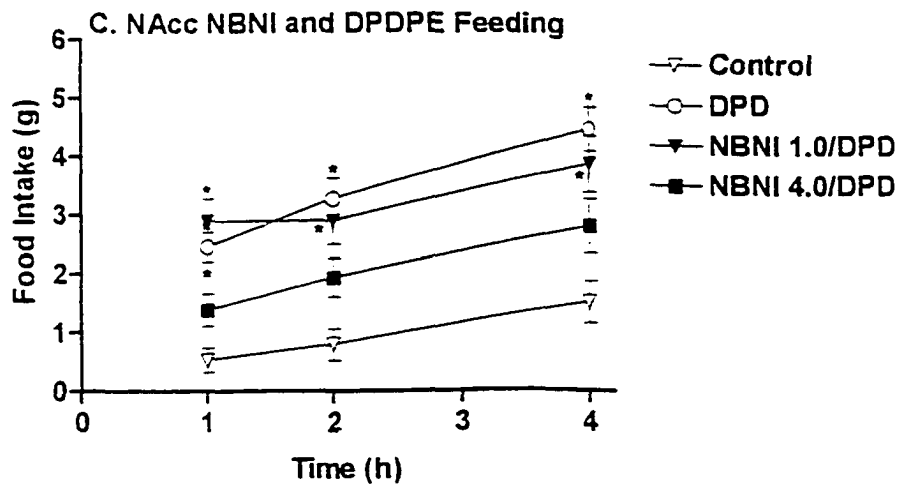
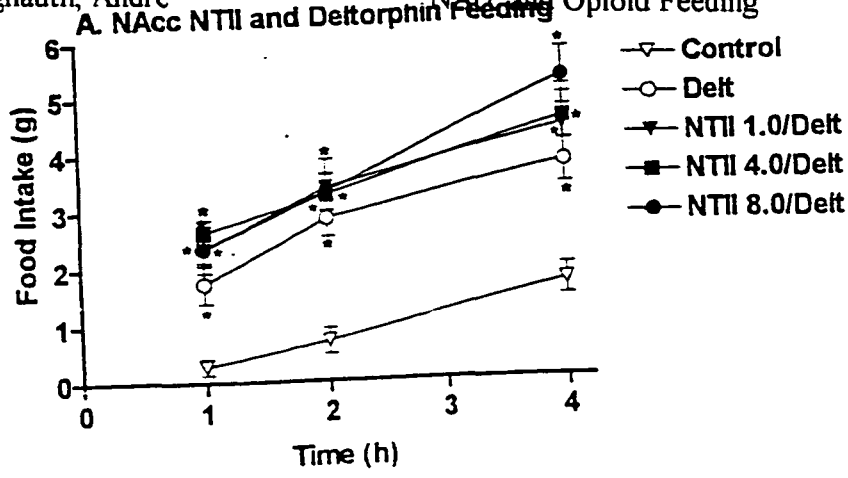
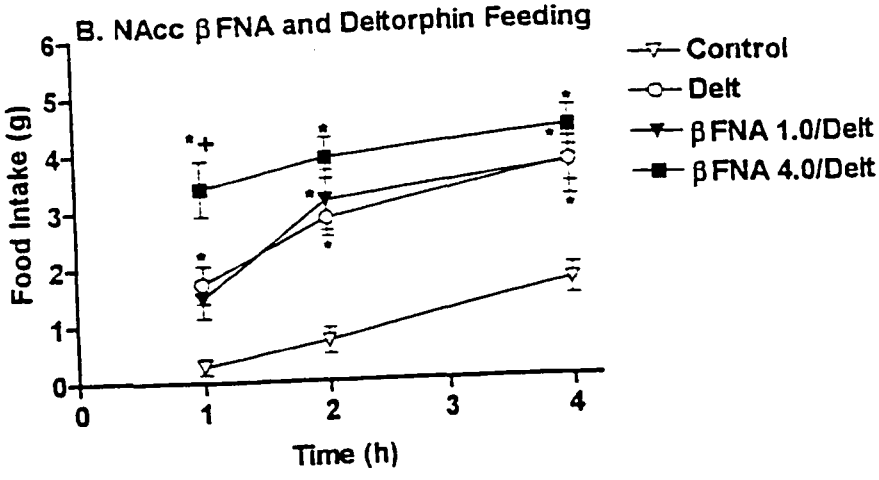
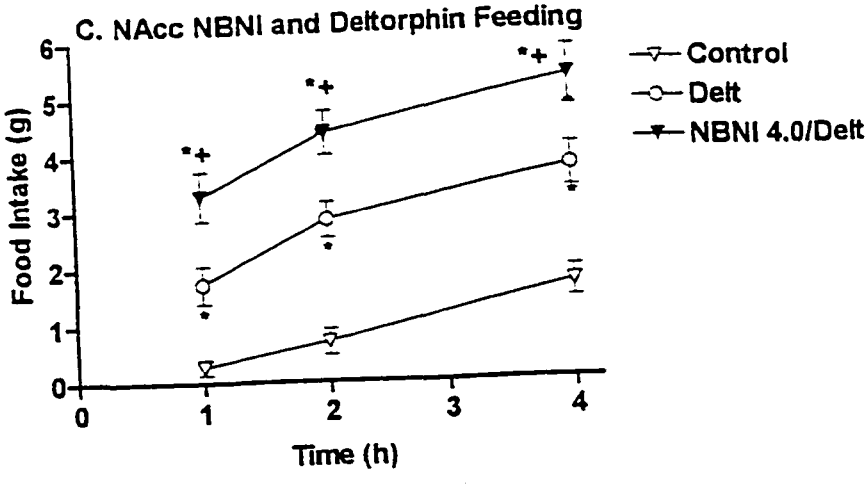


Figure 14. Alterations in spontaneous food intake (g, ± SEM) following a 5 μg dose of the δ_2-selective opioid agonist, Deltorphin (Delt) bilaterally in the NAcc shell relative to Control treatment. The upper and middle panels depict effects upon Delt-induced feeding following pretreatment (24 h) with different doses (μg) of either the selective δ_2-opioid antagonist, NTII or the selective μ-opioid antagonist, β-FNA respectively. The lower panel depicts effects upon DAMGO-induced feeding following pretreatment (1 h) with different doses (μg) of the selective κ_1-opioid antagonist, nor-binaltorphamine (Nor-BNI).







Discussion: Experiment 3:

Similar magnitudes and time courses of feeding responses were elicited from the NAcc following microinjections of either the μ-selective opioid agonist, DAMGO, the δ_1-selective opioid agonist, DPDPE, or the δ_2-selective opioid agonist, Deltorphin. In contrast to intracerebral and supraspinal analgesic studies in which antagonist-specific effects produced selective blockade of analgesic responses following either DAMGO, DPDPE or Deltorphin (Jiang et al., 1990a, 1990b, 1991; Mattia et al., 1992, 1991; Rossi et al., 1991; Sofuoglu et al., 1991), selective antagonists for different opioid receptor subtypes exerted effects upon multiple opioid agonist-induced feeding responses within the NAcc.

First, the fact that DAMGO, like morphine, elicited feeding following microinjection into the NAcc (Bakshi and Kelley, 1993a , 1993b, 1994; Evans and Vaccarino, 1990; Majeed et al., 1986; Mucha and Iversen, 1986; Zhang et al., 1998; Zhang and Kelley, 1997) suggested that the μ opioid receptor was implicated in the mediation of opioid-induced feeding within the NAcc. This hypothesis was supported by the observation that β-FNA, a μ-selective opioid receptor antagonist that alkylates this receptor (Portoghese et al., 1980) significantly and dose-dependently reduced DAMGO-induced feeding in the NAcc. The μ opioid receptor has been subdivided into high-affinity μ_1 and lower-affinity μ_2 opioid binding sites (see review: Pasternak and Wood, 1986) which can be pharmacologically differentiated using the selective μ_1 opioid antagonist, naloxonazine (Hahn et al., 1982). The respective ability of β-FNA (μ_1 + μ_2) and the inability of naloxonazine (μ_1) to alter DAMGO-induced feeding in the NAcc strongly suggest that the μ_2 opioid receptor subtype mediates DAMGO-induced feeding in the NAcc, a pattern that was also observed for selective μ antagonist effects in the NAcc for

feeding under deprivation, glucoprivic and palatable conditions (Experiment 1; Kelley et al., 1996).

Differentiations between μ_1-mediated and μ_2-mediated effects upon other forms of opioid-induced feeding following ventricular administration of opioid antagonists have been described as well (e.g., see review: Bodnar, 1996). Although DAMGO-induced feeding in the NAcc was largely unaffected by NAcc pretreatment with the selective δ_1-opioid antagonist, DALCE (Bowen et al., 1987), this response was significantly reduced by the higher, but not lower dose of the selective δ_2-opioid antagonist, NTII (Portoghese et al., 1990) in the NAcc. Further, NAcc pretreatment with the higher, but not lower dose of the κ_1 opioid antagonist, Nor-BNI (Portoghese et al., 1987) significantly reduced DAMGO-induced feeding in the NAcc as well. This latter effect is quite similar to the abilities of ventricular administration of the same μ and κ_1 opioid antagonists to reduce feeding induced by ventricular administration of DAMGO (Levine et al., 1990, 1991).

Three explanations can account for these results: a) DAMGO is not a selective μ opioid agonist in feeding studies; b) the antagonists are not entirely selective for their respective opioid receptor subtypes; and/or c) feeding elicited by μ opioid agonists in the NAcc is mediated through multiple opioid receptors. The first of these explanations is quite unlikely given the convergence of evidence using antisense oligodeoxynucleotide (AS ODN) probes directed against either the MOR-1, KOR-1 or DOR-1 opioid receptor clones (see reviews: Pasternak and Standifer, 1995; Rossi and Pasternak, 1997; Uhl et al., 1994). DAMGO-induced feeding is selectively reduced following ventricular pretreatment with AS ODN probes directed against either exons 1 or 4 of the MOR-1 clone, but unaffected by

probes directed against either exons 2 or 3 of the MOR-1 clone or a missense probe (Leventhal et al., 1997). These AS ODN effects upon DAMGO-induced feeding were comparable in magnitude to those reductions observed following β-FNA administration (Leventhal et al., 1997).

In contrast, AS ODN probes directed against the MOR-1 clone that significantly reduced DAMGO-induced feeding failed to affect feeding induced by ventricular administration of either the δ_2 opioid agonist, Deltorphin or the κ_1 opioid agonist, U50,488H (Leventhal et al., 1998). Although Nor-BNI was initially proposed as a selective and reversible κ_1 opioid antagonist (Portoghese et al., 1987), both characteristics have been called into question such that the antagonist has shown some longer-acting behavioral and biochemical effects (Horan et al., 1992; Jones and Holtzman, 1992;, Paronis et al., 1993). Further, chronic, but not acute administration of Nor-BNI appears to act at multiple opioid receptor subtypes rather than selectively at the κ_1 site (Spanagel et al., 1994). In contrast, the selectivity of NTII as a δ_2 opioid antagonist (Portoghese et al., 1990) has not been open to as many questions. The lack of further opioid subtype receptor antagonists, particularly for the κ_1 receptor, precludes further and definitive rectification of this issue. Even if the third hypothesis indicating that DAMGO-induced feeding in the NAcc is mediated through multiple opioid receptors in this structure is true, the potency and magnitude of these effects strongly suggest that the μ receptor, and particularly its low-affinity μ_2 opioid binding site play more

critical roles in this response than either the δ_2 or κ_1 opioid receptor subtypes.

Both DPDPE (δ_1) and Deltorphin (δ_2) opioid agonists in the NAcc produced comparable magnitudes and durations of feeding, confirming previous suggestions that the δ receptor is involved in agonist-induced feeding in this structure (Bakshi and Kelley, 1993; Zhang and Kelley, 1997). Feeding elicited by DPDPE in the NAcc was reduced by NAcc pretreatment with either μ, δ_1, δ_2 or κ_1 opioid receptor subtype antagonists, but not by μ_1 opioid antagonism. Indeed, it appeared that μ receptor antagonism with β-FNA produced more pronounced effects upon DPDPE-induced feeding than either DALCE, NTII or Nor-BNI. Therefore, unlike ventricular analgesic studies in which DPDPE-induced analgesia is selectively reduced by pretreatment with DALCE, but not NTII (Jiang et al., 1990, 1991; Sofuoglu et al., 1991), it appears that multiple δ receptor subtypes mediate the ingestive response elicited by DPDPE within the NAcc. Indeed, ventricular administration of DPDPE elicits feeding which was significantly reduced by the general opioid antagonist, naltrexone and the δ_2 opioid antagonist, NTII, but not by the dose range (20-40 μg) of the δ_1 opioid antagonist, DALCE employed in this study (Yu et al., 1997). In the present study, doses of 1-4 μg of DALCE prevented the full expression of DPDPE-induced feeding in the NAcc; both solubility and availability issues precluded us from testing higher doses of this antagonist in the NAcc.

The putative δ_2 opioid receptor subtype agonist, Deltorphin displayed an entirely different pattern of sensitivity to multiple opioid receptor subtype antagonists. Deltorphin-induced feeding was unaffected by NAcc pretreatment of the δ_2 opioid antagonist, NTII, even at doses as high as 8 μg, the limit of solubility. The inability of NTII to affect Deltorphin-

induced feeding in the NAcc could not be attributed to any perturbation in antagonist efficacy since NAcc NTII pretreatment concomitantly reduced feeding elicited by either DAMGO or DPDPE in the NAcc. Previous work (Yu et al., 1997) indicated that ventricular administration of Deltorphin produced feeding that was sensitive to both δ_1 and δ_2 antagonism; unanticipated unavailability of the antagonist, DALCE precluded testing upon Deltorphin-induced feeding within the NAcc. Further support for the ability of δ receptor antagonists to reduce Deltorphin-induced feeding following ventricular administration (Yu et al., 1997) has been provided by the ability of AS ODN probes directed against the DOR-1 clone to significantly reduce Deltorphin-induced feeding (Leventhal et al., 1998). Indeed, it has been proposed in analgesic AS ODN studies that the DOR-1 clone possesses more similarities with the pharmacologically-described δ_2 opioid receptor subtype (Rossi et al., 1997). Therefore, it appears that the inability of NTII to alter Deltorphin-induced feeding in the NAcc as expected is probably due to the ineffectiveness of delivering high enough antagonist doses into the structure rather than to the failure of Deltorphin to act at a δ_2 receptor in this site and paradigm. In marked contrast to the respective abilities of β-FNA and Nor-BNI to reduce feeding elicited by either DAMGO or DPDPE in the NAcc, pretreatment with each of these antagonists significantly and dose-dependently enhanced Deltorphin-induced feeding in the Nacc. While this may be explained by minuscule, non-significant, differences between the squads of rats used for the DAMGO study and the rats used for the deltorphin study (data not shown), the same cannot be used as an explanation for the differences between the data obtained from the DPDPE and the deltorphin studies. Further, while deltorphin may be biochemically different from the other opioid peptides, and while this

may be a possible explanation for the differences seen in these studies, the biochemical differences may not be a valid explanation since these types of contradictory data are not obtained in analgesia studies. One possible area of differences between the peptides, at the receptor level, has been noted but it is not known how this may affect behavior (Befort et al., 1996). It may also be possible that there were technical differences, but it is unlikely that this would have been selective only for the deltorphin test animals. The most likely explanation is pharmacological differences, either. Thus, while DPDPE and DAMGO share some common opioid receptor substrates in mediating their ingestive responses in the NAcc, the feeding response induced by Deltorphin appears to activate different pathways in the NAcc that are modulated differentially by opioid receptor subtypes.

As indicated previously, μ, δ and κ receptors have been localized in the NAcc using receptor autoradiographic and mRNA gene expression techniques (Herkenham et al., 1984; Lewis et al., 1985; Mansour et al., 1995, 1987). While these techniques verify the presence of these receptors within the nucleus, they cannot specify the synaptic arrangement of these receptors relative to each other and to other transmitter and modulator systems within the nucleus. Ultrastructural studies have suggested that dynorphin activation of κ opioid receptors, Met^5-enkephalin activation of δ opioid receptors, and Leu^5-enkephalin activation of μ opioid receptors in the NAcc may act on spiny neurons modulating responses to excitatory and inhibitory amino acids (Svingos et al., 1998, 1999; 1996). Further, co-localization in the shell region of the NAcc have been observed for both μ and NMDA receptors (Gracy et al., 1997) as well as for μ opioid receptors and GABA-containing neurons (Svingos et al., 1997). It is also known that proenkephalin and prodynorphin mRNA are co-

localized with dopamine and substance P neurons (Van Bockstaele et al., 1995; 1994) and with D_1, but not D_2 dopamine receptors (Curran and Watson, 1995). Further, the DOR-1 opioid receptor clone appears to be on axon terminals apposed to terminals immunoreactive to the dopamine transporter (Svingos et al., 1999). Moreover, agonists at μ and δ_2 receptors stimulate dopamine release in the NAcc (Longoni et al., 1991; Pentney and Gratton, 1991; Yoshida et al., 1999). Taken together, regulation of the NAcc by these multiple transmitter systems, and particularly dopamine, appear to play an integral role in the elucidation of ingestive and other (e.g., see reviews: Ikemoto and Panksepp, 1999; Salamone, 1994) motivated behaviors, and ingestion mediated by μ and δ opioid receptor agonists appear to employ multiple opioid receptors within the NAcc in the differential expression of this ingestive response. The fourth experiment of this dissertaion evaluated the role of dopamine receptor subtypes in the mediation of opioid receptor subtype agonist-induced feeding in the NAcc.

EXPERIMENT 4. Role of Dopamine Receptor Subtype Antagonists in the Nucleus Accumbens Shell in Mediating Feeding Elicited by Opioid Receptor Subtype Agonists in the Nucleus Accumbens Shell.

Specific Methods, Experiment 4:

μ Agonist Intake Protocol: In all experiments, each rat received bilateral microinjection conditions in counterbalanced order at weekly intervals. Subgroups of rats receiving different antagonist conditions were matched on the basis of the particular intake conditions following vehicle treatment. All drugs were dissolved in a 0.9% normal saline solution. In assessing dopamine receptor antagonism upon μ-opioid agonist-induced feeding, subgroups of rats received the following bilateral microinjection conditions: a) vehicle (n=13), b) DAMGO, (Peninsula Laboratories, Belmont, CA, 2.5 μg: 1.25 μg each side), SCH23390 (Sigma Chemical Company, St. Louis, MO) at total doses of either c) 5 (n=6), d) 10 (n=6), e) 16.5 (n=6), or f) 33 (n=7) μg paired with DAMGO, and raclopride (Sigma Chemical Company) at total doses of either g) 6 (n=6), h) 10 (n=6), i) 25 (n=6), or j) 50 (n=6) μg paired with DAMGO. At 3-5h into the light cycle, food was removed from the cages, and animals received the dopamine receptor subtype antagonist followed 20 min thereafter with the opioid agonist. All microinjections were administered bilaterally in 1 μl volumes over 30 sec through a stainless internal cannula (33-gauge, Plastics One) connected to a Hamilton microsyringe by polyethylene tubing. This relatively high injection volume was necessary because of limited solubility problems of some of the pharmacological agents. Cumulative intake was assessed at 1, 2 and 4 h after the last injection by measuring preweighed food

which was adjusted for spillage collected by paper towels placed under the wire mesh cages.

δ_2 Agonist Intake Protocol: In assessing dopamine receptor antagonism upon δ_2-opioid agonist-induced feeding, subgroups of rats received the following bilateral microinjection conditions: a) vehicle (n=16), b) Deltorphin, (Peninsula Laboratories, 5 µg: 2.5 µg each side), SCH23390 at total doses of either c) 5 (n=10), d) 10 (n=10), e) 16.5 (n=6), or f) 33 (n=7) µg paired with deltorphin, and raclopride at total doses of either g) 6 (n=5), h) 10 (n=8), i) 25 (n=11), or j) 50 (n=11) µg paired with deltorphin. These SCH23390 and raclopride doses were equimolar with respect to each other.

Results: Experiment 4:

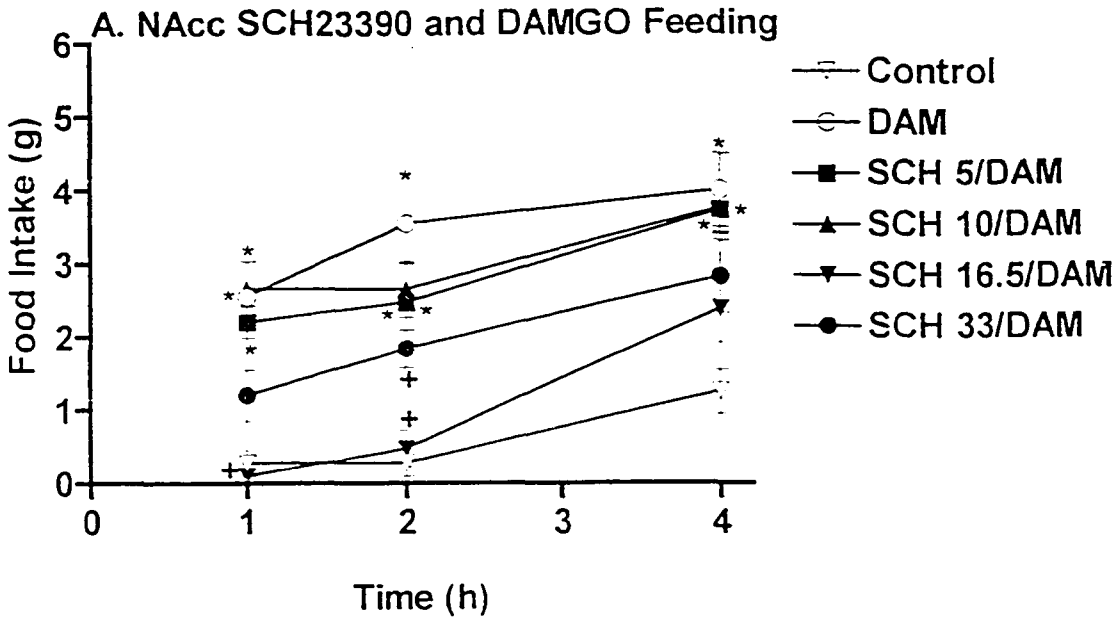
Histological Verification: Bilateral cannulae placements were all localized to the shell region of the NAcc which were medial to the core region and lateral to the ventral diagonal band area. The shell region placements extended in the rostral-caudal dimension from Figures 9 through 14 of the stereotaxic atlas of Paxinos and Watson (Paxinos and Watson, 1986).

NAcc DA Antagonists and NAcc DAMGO-induced Feeding: In examining D_1 receptor antagonist actions, significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(5,60)= 19.58$, $p<0.0001$), 2 ($F= 23.28$, $p<0.0001$) and 4 ($F= 10.63$, $p<0.0001$) h. DAMGO significantly increased food intake relative to control treatment across the time course (Figure 15). Pre-treatment with the selective D_1 antagonist SCH 23390 dose-dependently and significantly reduced NAcc DAMGO-induced feeding (Figure 15A). Whereas the two lower (5 and 10 μg) doses of SCH 23390 were without effect, NAcc DAMGO-induced feeding was significantly reduced after 1 and 2 h by a 16.5 μg dose and after 2 h by the 33 μg dose. Both of these effective doses also prevented the significant expression of DAMGO-induced feeding. In examining D_2 receptor antagonist actions, significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(5,60)= 8.82$, $p<0.0001$), 2 ($F=10.63$, $p<0.0001$) and 4 ($F= 16.99$, $p<0.0001$) h. Pretreatment with the selective D_2 antagonist, raclopride, produced less consistent effects upon NAcc DAMGO-induced feeding (Figure 15B) despite the use of doses equimolar to those of SCH23390. Thus, a 25 μg dose of raclopride prevented DAMGO-induced feeding after 1 h, and significantly reduced this ingestive response after 2 and 4 h. A

higher (50 μg) raclopride dose prevented DAMGO-induced feeding only after 1 h, while the lowest (6 μg) raclopride dose prevented DAMGO-induced feeding only after 4 h.

NAcc DA Antagonists and NAcc Deltorphin-induced Feeding: In examining D_1 receptor antagonist actions, significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(5,75)= 18.26$, $p<0.0001$), 2 ($F= 19.38$, $p<0.0001$) and 4 ($F= 20.52$, $p<0.0001$) h. Deltorphin significantly increased food intake relative to control treatment across the time course (Figure 16). In contrast to its more consistent dose-response actions upon DAMGO-induced feeding in the NAcc, pretreatment with SCH23390 at doses of 5 and 16.5 μg significantly reduced deltorphin-induced feeding after 1 and 2 h, and prevented this ingestive response after 4h (Figure 16A). In contrast, the other SCH23390 doses of 10 and 33 μg failed to alter deltorphin-induced feeding across the time course. In examining D_2 receptor antagonist actions, significant differences in spontaneous food intake were observed among injection conditions after 1 ($F(5,75)= 23.58$, $p<0.0001$), 2 ($F= 19.71$, $p<0.0001$) and 4 ($F= 21.76$, $p<0.0001$) h. Pretreatment with raclopride had very limited actions upon deltorphin-induced feeding (Figure 16B) with this ingestive response prevented by the lowest (6 μg) antagonist dose only after 1 h, and by the highest (50 μg) antagonist dose only after 4h.

Figure 15. Alterations in spontaneous food intake (g, +SEM) following a 2.5 μg dose of the μ-selective opioid agonist, DAMGO (DAM) bilaterally in the shell region of the NAcc relative to Control treatment. The upper and lower panels depict effects upon DAMGO-induced feeding following pretreatment (20 min) with different doses (μg) of either a D_1-selective (SCH23390) or D_2-selective (raclopride) antagonist respectively. The asterisks (*) in this and the subsequent figure indicate significant increases in food intake following opioid agonist treatment relative to Control treatment (Tukey comparisons, $p<0.05$). The crosses (+) in this and the subsequent figure indicate significant decreases in food intake following dopaminergic antagonist pretreatment relative to opioid agonist treatment alone (Tukey comparisons, $p<0.05$).



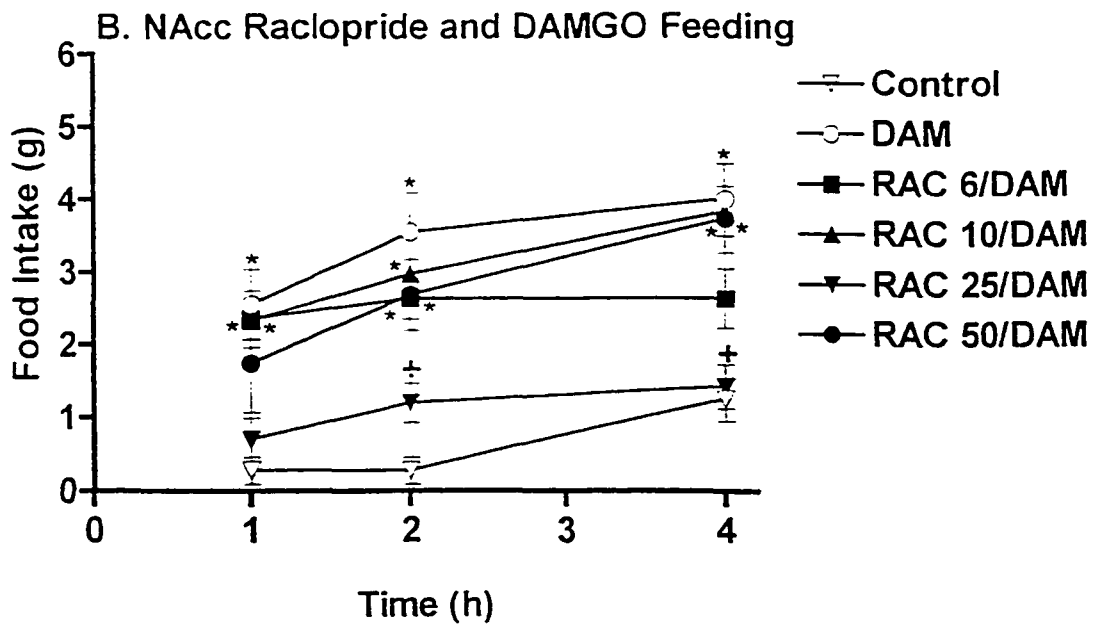
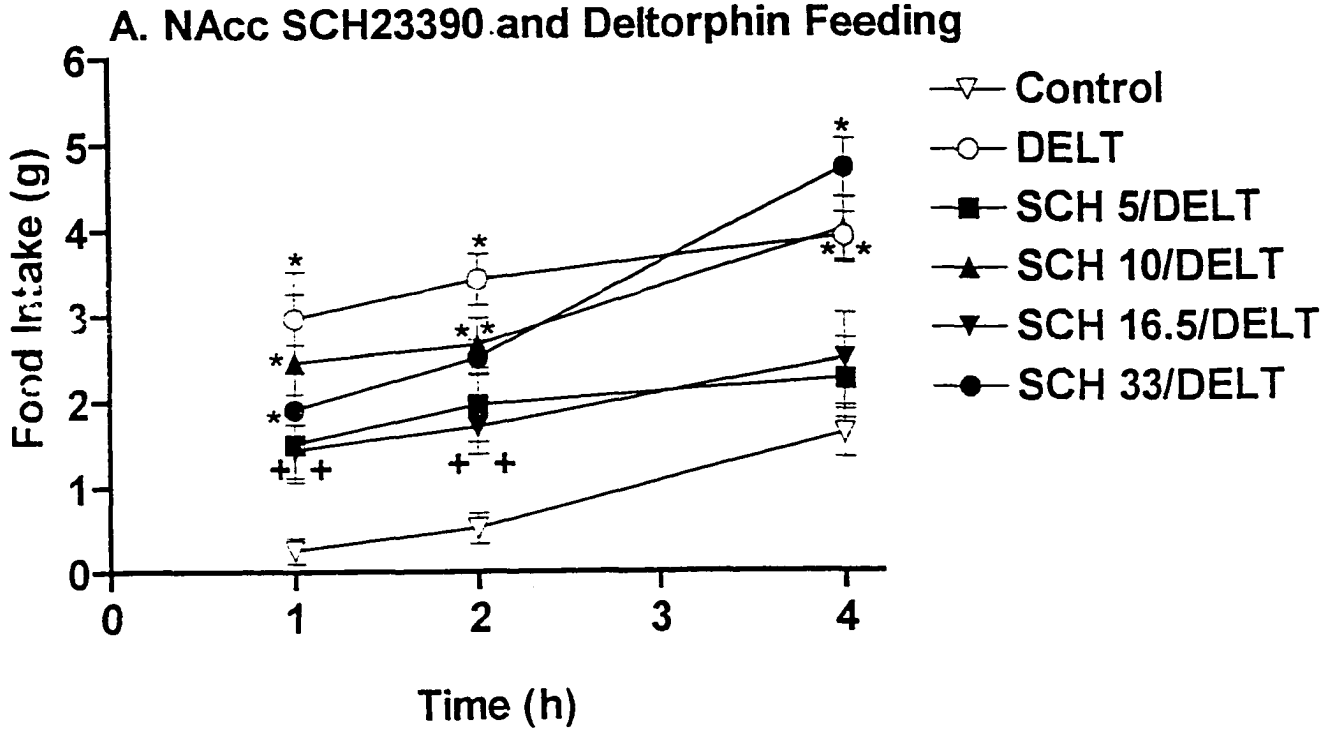
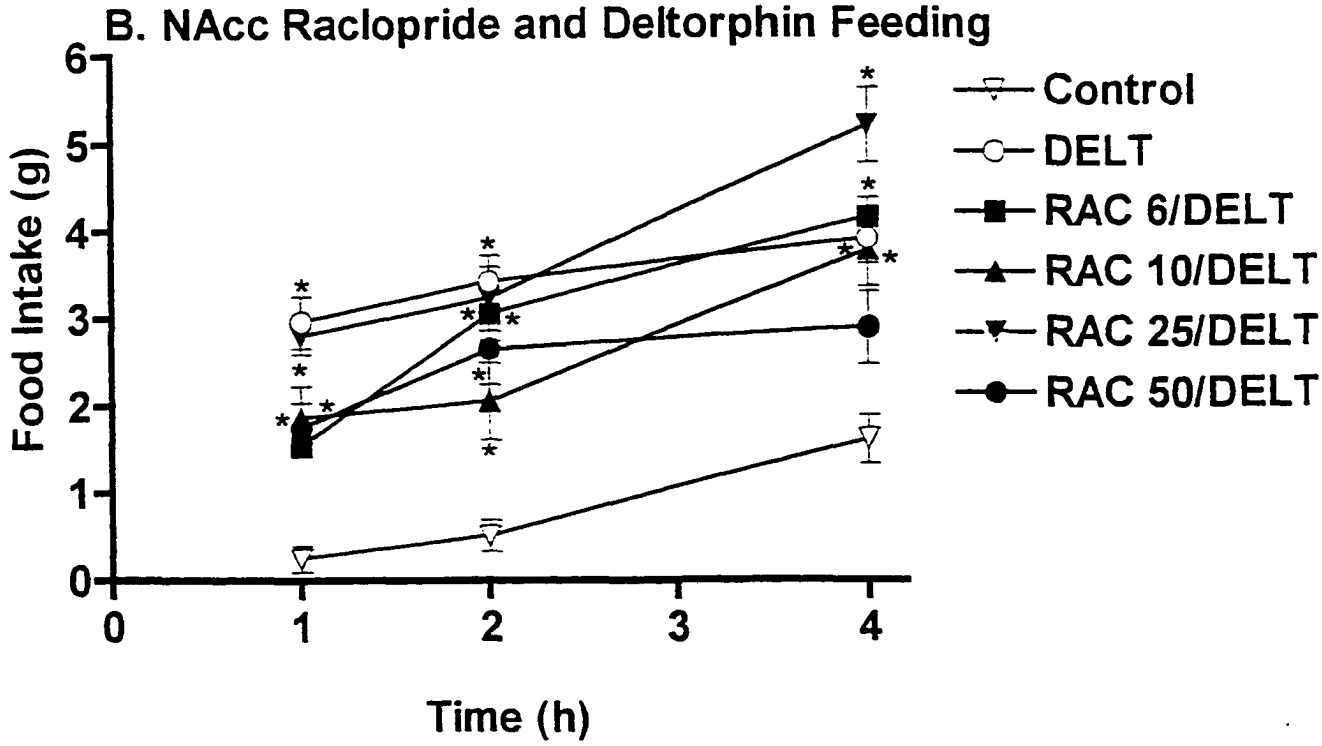


Figure 16. Alterations in spontaneous food intake (g, +SEM) following a 5 μg dose of the δ_2-selective opioid agonist, Deltorphin (DELT) bilaterally in the shell region of the NAcc relative to Control treatment. The upper and lower panels depict effects upon Deltorphin-induced feeding following pretreatment (20 min) with different doses (μg) of either a D_1-selective (SCH23390) or D_2-selective (raclopride) antagonist respectively.





Discussion: Experiment 4:

The role of dopamine receptors in mediating opioid-induced feeding within the shell region of the NAcc was both dependent upon the dopamine receptor subtype that was blocked (D_1 vs. D_2) as well as the opioid receptor subtype that was being stimulated (μ vs. δ_2). Hence, NAcc DAMGO-induced feeding was significantly reduced by NAcc pretreatment with the D_1 antagonist, SCH23390 across a 4 h time course, indicating that feeding induced by μ-selective opioid agonists in the NAcc is partially dependent upon the full integrity of D_1 receptors in the NAcc. In contrast, equimolar doses of the D_2 antagonist, raclopride in the NAcc produced far less consistent effects upon DAMGO-induced feeding in the NAcc, suggesting that dopaminergic mediation of feeding elicited by selective μ agonists is selective to D_1, but not D_2 receptors. It should be noted that in behavioral activity studies, D_1 agonists produce more potent locomotor effects than D_2 agonists in the NAcc (Breese et al., 1987; Dreher et al., 1989; Gong et al., 1999; Phillips et al., 1995; Swanson et al., 1997). Moreover, the magnitude and potency of these effects appear less than the ability of both D_1 and D_2 antagonists administered into the NAcc to produce equipotent reductions at lower effective doses in the analgesic responses elicited by either morphine or substance P administered into the VTA (Altier and Stewart, 1998). Although the present study showed that the δ_2-selective opioid agonist, deltorphin produced very comparable patterns and magnitudes of feeding responses in the NAcc to that of μ-selective agonist actions, the profiles of D_1 and D_2 antagonist pretreatment effects were markedly different from that observed with μ-selective opioid agonists. Therefore, pretreatment with either the D_1 antagonist, SCH23390 or the D_2 antagonist, raclopride in the NAcc produced mild and inconsistent effects upon deltorphin-

induced feeding in the NAcc across the time course, suggesting minimal participation of dopamine receptor systems in mediating feeding induced by δ_2 opioid agonists in the NAcc. The differential effects of dopamine receptor subtype antagonism upon feeding induced by μ and δ_2 agonists in the NAcc complement differences observed in the opioid mediation of these respective responses in this nucleus. Thus, DAMGO-induced feeding in the NAcc was significantly reduced by NAcc pretreatment with μ, δ_2 and κ_1, but not μ_1 or δ_1 opioid receptor subtype antagonists. In contrast, deltorphin-induced feeding in the NAcc was largely unaffected by NAcc δ_2 antagonist pretreatment, and was actually enhanced by NAcc pretreatment with μ or κ_1 antagonists (Experiment 3).

Evidence exists linking potential mechanisms of action for dopaminergic mediation of opioid-induced feeding in the NAcc. Dopamine stimulates feeding behavior within the shell, but not the core region of the NAcc (Swanson et al., 1997). Anatomical interactions between opioid and dopaminergic systems have been observed within the NAcc (Van Bockstaele et al., 1994). The ability of D_1 and D_2 antagonists in the NAcc to reduce analgesia elicited by morphine in the VTA (Altier and Stewart, 1998) correlates with the inhibitory actions on NAcc neurons by VTA morphine reversed by dopamine antagonists in the NAcc (Hakan and Henriksen, 1989). In neurochemical studies, the μ opioid receptor agonists, fentanyl and DAMGO in the NAcc stimulate dopamine release which is blocked by general, μ and δ opioid receptor antagonist pretreatment (Yoshida et al., 1999). These data are complementary to the present findings that D_1, and to a far lesser extent D_2, receptor antagonists reduce DAMGO-induced feeding in the NAcc. However, dopamine release has also been observed following infusions of δ_1 (DPDPE) and δ_2 (deltorphin) agonists in the NAcc (Yoshida et al., 1999). Yet,

deltorphin-induced feeding in the NAcc appears quite impervious to either D_1 or D_2 antagonism, suggesting that such release is not a necessary and sufficient condition to elicit this agonist-induced feeding response. It should also be noted that dopamine release in the NAcc is also increased by systemic and ventral tegmental injections of opioids (e.g., DiChiara and Imperato, 1988; Kalivas and Richardson-Carlson, 1986; Latimer et al;., 1987; Spanagel et al., 1990), effects which appear necessary for the rewarding, locomotor and analgesic effects of opioids in this system (Altier and Stewart, 1998; Churchill and Kalivas, 1992; Cunningham and Kelley, 1997, 1992; Harris and Aston-Jones, 1994; Kalivas et al., 1983; Kiyatkin et al., 1993).

Both D_1 and D_2 receptors are localized on dendrites and presynaptic terminals in the shell and core regions of the NAcc (Koshikawa et al., 1996; Shetreat et al., 1996). There is only limited anatomical and cytoarchitectural information concerning dopamine-opioid interactions in the NAcc. Proenkephalin and prodynorphin mRNA are co-localized with D_1, but not D_2 receptors (Curran and Watson, 1995), suggesting a possible mechanism of action for the greater ability of D_1 relative to D_2 receptor antagonists to reduce DAMGO-induced feeding in the NAcc. This suggestion must however be tempered by further evidence. Although D_1 and D_2 receptors appear to be segregated in the neighboring dorsal neostriatum, there is a substantial sub-population (20-25%) of medium spiny neurons that co-express these receptors as well as enkephalins and substance P (Surmeier et al., 1996). However, these levels of co-expression are not consistent with the present behavioral findings in that the more effective D_1 receptor displays expression with substance P, while the less effective D_2 receptor displays expression with enkephalin (LeMoine and Bloch, 1996; Lu et al., 1998; Schwartz et

al., 1998). Finally, antisera raised against both the δ-opioid receptor (DOR) and the dopamine transporter (DAT) indicate that DOR appears on axon terminals apposed to DAT-immunoreactive terminals (Svingos et al., 1999). Although pre-synaptic and/or post-synaptic effects have been proposed for D_1 and D_2 receptors, the technique of intracerebral administration of these selective dopamine receptor subtype antagonists in the NAcc does not allow us to definitively indicate the pre-synaptic or post-synaptic location of the receptors selectively blocked by these antagonists.

A simple explanation does not appear to be forthcoming for the differential actions of dopamine receptor subtype antagonists (D_1 vs. D_2) upon feeding induced by μ and δ_2 opioid agonists in the NAcc in which DAMGO-induced feeding is reduced by D_1, but not D_2 antagonism, while Deltorphin-induced feeding appears to act independently of dopamine receptor manipulations. Part of this has to do with the difficulty in elucidating the precise mechanisms by which dopamine modulates neural function within the NAcc (see review: Nicola et al., 2000). Nevertheless, emerging evidence strongly suggests that feeding elicited by the μ-selective opioid agonist, DAMGO and the δ_2 opioid agonist, Deltorphin, employ separable mechanisms of action within the NAcc which may have implications for other opioid-mediated responses subserved by this nucleus, including locomotor activity, reward and drug self-administration.

GENERAL DISCUSSION

Based upon ventricular actions of these antagonists (see review: Bodnar, 1996), and based upon the predominant role for μ receptor agonists in stimulating feeding in the NAcc (Bakshi and Kelley, 1993a, 1994), it was predicted that naltrexone, naloxonazine and β-FNA microinjected into the NAcc would each reduce deprivation-induced feeding. Our data, however, indicated that naloxonazine potentiated deprivation-induced feeding, while the other antagonists had the expected effect of reducing deprivation-induced feeding. Thus, deprivation-induced feeding is mediated primarily through low-affinity μ_2 opioid receptors. It was further predicted that since ventricular administration of the κ antagonist, Nor-BNI marginally affected deprivation-induced feeding (Levine et al., 1990), and based upon the lack of feeding elicited by κ receptor subtype agonists in the NAcc (Bakshi and Kelley, 1993a), Nor-BNI should have limited effects upon deprivation-induced feeding in the NAcc. Like ventricular administration, Nor-BNI in the NAcc produced significant, though marginal reductions in deprivation-induced feeding, suggesting that μ_2 and, to a lesser extent κ, opioid receptors in the NAcc are involved in mediating deprivation-induced feeding. As predicted (see review: Bodnar, 1996), general, μ and κ opioid antagonists each significantly and potently blocked 2DG-induced feeding, indicating that the NAcc is involved in the opioid mediation of this regulatory challenge.

Based upon ventricular studies, it was expected that general, μ and κ antagonists would each reduce sucrose intake following microinjection into the NAcc (Beczkowska et al., 1992). Whereas naltrexone and β-FNA each potently reduced this palatable response, Nor-BNI failed to affect sucrose intake in the NAcc. Therefore, whereas μ receptors, and

particularly the μ_2 receptor subtype mediated the three disparate types of feeding responses in the NAcc, κ receptors were maximally involved in mediating glucoprivic intake, marginally involved in deprivation-induced intake, and failed to affect palatable intake. The mediation of palatable intake by the μ receptor in the NAcc is quite compatible with recent findings demonstrating that the μ opioid agonist, DAMGO in the NAcc, stimulated intake of both palatable fat and sucrose diets (Zhang and Kelley, 1997; Zhang et al., 1998). Thus, the NAcc, like the PVN (Koch et al., 1995) appears to be a critical locus for the opioid mediation of different ingestive behaviors.

Given the intimate and reciprocal connections between the VTA and the NAcc (see review: Heimer et al., 1991), it was expected that the pattern of opioid mediation of ingestive behaviors in the VTA would be similar to that observed in the NAcc. Further, agonist studies indicate that μ and δ, but not κ receptors were responsible for eliciting feeding in the VTA. Thus, in the VTA, it was predicted that VTA general, μ and δ opioid antagonists would block deprivation, 2DG and sucrose-induced feeding, while κ antagonists would not. Surprisingly, while naltrexone, the general opioid antagonist reduced deprivation-induced feeding in the VTA, it did so to a much lesser degree and at higher doses than was observed in systemic, ventricular and intracerebral studies. Moreover, neither selective μ, δ_1 nor κ antagonists in the VTA reduced feeding elicited by either deprivation, 2DG or sucrose. In contrast, δ_2 receptor antagonism with NTII in the VTA reduced each of these forms of feeding. These data indicate that the VTA does not appear to be a significant site of action for most opioid receptor subtype antagonists despite its ability to support stimulation of spontaneous intake by opioid receptor subtype agonists. The ability of δ_2 receptor antagonism in the VTA to

affect deprivation, 2DG and sucrose-induced feeding, together with the ability of δ agonists in the VTA to stimulate spontaneous intake suggests a modulatory action for this receptor subtype in this nucleus. It is apparent that the endogenous opioid system in the VTA and NAcc provide differential types of action upon these different types of feeding behavior with the latter structure far more intimately involved in the direct opioid action upon the ingestive behaviors per se.

The third experiment was based upon two competing hypotheses about selective serial actions of opioid agonists upon their receptors as compared to parallel system actions. As summarized in detail previously, analgesic studies demonstrate specificity such that selective μ (β-FNA), δ_1 (DALCE) and δ_2 (NTII) opioid antagonists would respectively and only block analgesia elicited by their selective opioid agonists like DAMGO, DPDPE and deltorphin; such data are examplars of selective serial actions. In this model, NBNI, a κ antagonist should fail to exert actions upon these agonist effects. Alternatively, a parallel system action of opioids has been observed in opioid-induced feeding studies such that multiple opioid receptor subtype antagonists are capable of reducing feeding elicited by specific opioid agonists. Thus, this experiment evaluated different opioid antagonist effects in the NAcc upon feeding elicited by DAMGO, DPDPE or deltorphin. It was found that DAMGO-induced feeding in the NAcc was potently reduced by the μ opioid antagonist β-FNA across the time course, while δ_2 and κ_1 opioid antagonists reduced feeding only at high antagonist doses. Neither μ_1 (NAZ) nor δ_1 (DALCE) opioid antagonists in the NAcc affected NAcc DAMGO-induced feeding, implying that the μ_2 opioid receptor primarily mediates DAMGO-induced feeding in this nucleus. NAcc DPDPE-induced feeding was blocked by antagonists specific

for μ, δ_1, δ_2 and κ_1, but not μ_1 receptors. That both δ receptor subtype antagonists reduced intake elicited by a putative δ_1 agonist in the NAcc is similar to feeding studies using ventricular routes of administration (Yu et al., 1997), but differs markedly from analgesic studies used to delineate δ_1 and δ_2 receptors (Jiang et al., 1990; Mattia et al., 1992; Sofouoglu et al., 1991). Interestingly, feeding elicited by the putative δ_2 agonist, deltorphin in the NAcc was not blocked by the selective δ_2 antagonist, NTII, an effect reminiscent of ventricular feeding studies (Yu et al., 1997). In fact, no selective opioid antagonist in the NAcc could reduce deltorphin-induced feeding since both κ and μ antagonists enhanced deltorphin-induced feeding. Two major patterns of effects are observed for opioid agonist effects upon feeding behavior in the NAcc. It appears that μ agonist-induced feeding follows quite predictable pharmacological effects with μ opioid antagonism capable of eliminating this response. A role for parallel system mediation is supported by the significant, though less potent effects of κ and δ_2 antagonists.

In contrast, the δ_1/δ_2 pharmacological dichotomy used so well in analgesic assays, seems less relevant in feeding studies using ventricular (Yu et al., 1997) and now intracerebral NAcc injections. Indeed, the δ_1 agonist, DPDPE, exerts more traditional opioid sensitivity, though through parallel mechanisms of action, whereas deltorphin, the δ_2 agonist, has far less predictable sensitivity to opioid antagonism in the NAcc. Since DAMGO and deltorphin had such great differential responses to opioid receptor subtype antagonists in the third experiment, these agonists were chosen for study to assess dopaminergic mediation of opioid-induced feeding in the NAcc.

The final series of experiments of this dissertation examined the effects of D_1 and D_2

dopamine antagonists upon opioid agonist-induced feeding in the NAcc. Since we knew that both DAMGO and deltorphin induced feeding in the NAcc, and that SCH23390 (D_1) and raclopride (D_2) both reduced DAMGO and deltorphin analgesia (Altier and Stewart, 1998), it was hypothesized that these antagonists would reduce opioid-induced feeding in the NAcc. Our results indicate that SCH23390, but not raclopride, dose-dependently reduced DAMGO-induced feeding particularly at higher doses. In contrast, deltorphin-induced feeding was inconsistently blocked by different SCH23390 and raclopride doses. These data indicate that whereas the D_2 receptor may play a minor role in mediating opioid agonist-induced feeding behaviors in the NAcc, the consistent effects of SCH23390 upon opioid-agonist feeding imply a more active role for the D_1 receptor. In any case, it is quite clear that there is not an absolute dependence upon dopamine receptors for the full expression of either μ or δ_2 opioid agonist-induced feeding responses in the NAcc. These data appear to be consistent with the recent findings of Unterwald and Cuntapay (2000) who failed to find clear-cut interactions between opioid and dopaminergic systems within the rat striatum in their examination of dopamine receptor subtype modulation of opioid receptor-mediated signal transduction of adenylyl cyclase or G-protein activity.

These data appear to indicate that the NAcc is more directly involved than the VTA in mediating opioid effects upon a wide variety of ingestive situations. Further, whereas δ opioid receptors, and particularly δ_2 receptor antagonism, appear to be more robust in the VTA, it would appear that this receptor subtype is primarily responsible for opioid modulation of feeding in this nucleus. These data are consistent with the hypothesis that δ receptors play an indirect, modulatory role upon feeding, whereas μ and κ opioid receptors

have been shown to exert more direct effects upon different feeding paradigms in ingestive studies (see review: Bodnar, 1996). These data are also consistent with the finding that μ agonists in the VTA may be eliciting feeding responses through a primary effect upon stereotypic motor behaviors related to chewing and gnawing (Badiani et al., 1995). Taken together, these data indicate that the VTA acts to modulate feeding activity following stimulation with opioid agonists, rather than act as part of some "final common pathway" for feeding. Further, these data suggest that the dopaminergic input from the VTA to the NAcc may also be participating in an indirect, modulatory way upon opioid-induced feeding in the NAcc based upon the relatively-limited actions dopamine receptor antagonists have upon opioid agonist-induced feeding in the NAcc.

Thus, these studies also suggest that the NAcc is the critical site in the VTA-NAcc pathway for the direct mediation of opioid-induced feeding. The first study showed that μ and to a far lesser degree, κ opioid receptor antagonists were highly effective in reducing feeding under deprivation, glucoprivic and palatable conditions following injection into the NAcc. Mapping studies using selective opioid agonists indicate a crucial role for μ and δ, but not κ receptor agonists in stimulating spontaneous intake in the NAcc (Bakshi and Kelley, 1993). Finally, the third study of this dissertation indicated that selective μ, δ_1 and δ_2 opioid receptor agonists each stimulate spontaneous intake in the NAcc, but each utilize multiple opioid sysnapses within the NAcc to elicit effects. While both δ_1 and μ opioid receptor agonists appear to mediate feeding through fairly common multiple (μ_2, δ_1, δ_2, κ) opioid receptor subtypes, feeding elicited by δ_2 opioid agonists is actually potentiated by κ and secondarily μ opioid receptor antagonists. The greater ability of D_1 antagonists to reduce DAMGO-

induced feeding, relative to deltorphin-induced feeding in the NAcc is a further illustration of differential processes mediating different types of opioid-induced feeding. If dopamine is not a major mechanism of action through which opioids elicit feeding in the NAcc, what are some other potential candidates?

Two other transmitter systems have been identified in the NAcc that may also subserve feeding responses, and may thereby interact with the opioid system in mediating ingestive responses. The first candidate is the excitatory amino acid transmitter system, particularly through its interaction with AMPA/kainate receptors. Kelley and colleagues recently found that bilateral microinfusion into the NAcc of 6,7-dinitroquinoxaline-2,3-dione (DNQX), 6-cyano-7-nitroquinoxaline (CNQX), and 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(F) quinoxaline (NBQX) markedly and dose-dependently stimulated food intake immediately following infusion (Maldonado-Irizarry and Kelley, 1995; Maldonado-Irizarry et al., 1995; Kelley and Swanson, 1997; Stratford et al., 1998; Stratford and Kelley, 1999). Moreover, 2-amino-5-phosphonopentanoic acid (AP5), an NMDA antagonist, infused into the NAcc core blocks spatial learning on a food gathering task (Kelley, 1999), indicating a possible role for both the core and shell in mediating feeding responses, instrumental learning and direct control of feeding respectively. In contrast, infusion of AMPA into the NAcc suppressed both sucrose intake and deprivation-induced intake (Stratford et al., 1998). Synaptic connections between the NAcc and the lateral hypothalamus appear to mediate this ingestive response through $GABA_A$ receptors in the latter structure (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1999). Dopamine receptors also appear to mediate feeding elicited by AMPA antagonists in the NAcc, although systemic antagonist administration precluded identification

of the site(s) at which these antagonists acted (Maldonado-Irizarry et al., 1995). Further, pretreatment with naltrexone failed to affect DNQX-induced feeding. These findings demonstrate a selective role for non-NMDA receptors in the nucleus accumbens shell in feeding behavior, and indicates the importance of functional links between two major brain regions involved in reward, the NAcc and the lateral hypothalamus (Maldonado-Irizarry et al., 1995). Our laboratory (Echo et al., 2000) has begun to examine the relationship, if any, between excitatory amino acid systems and opioid systems in mediating feeding behavior in the NAcc, and has found some surprising results. If excitatory amino acid antagonists and opioid agonists each stimulate feeding, it would be expected that excitatory amino agonists might antagonize opioid-mediated responses. However, the opposite effect occurred such that simultaneous treatment of AMPA and DAMGO produced feeding that was greater than either agonist alone. Indeed, AMPA itself induced feeding, although with a longer-latency time course than its antagonist, DNQX. AMPA-induced feeding was reduced by pretreatment with naltrexone and β-FNA, suggesting μ receptor mediation, and the additive ingestive effects of AMPA and DAMGO were also blocked by general and μ opioid antagonism. Thus, multiple ingestive systems within the shell region of the NAcc interact with each other.

The second candidate system in the NAcc that may interact with opioid-induced feeding is GABA. GABA interneurons and receptors are localized within the shell compartment of the NAcc (Meredith et al., 1993). A GABA-opioid interaction within the NAcc exists, such that there are both GABA and enkephalin projections from the NAcc to the ventral pallidum, and from both the NAcc and ventral pallidum to the VTA (Zahm et al., 1985; Kalivas et al., 1993). Based on their observation that AMPA antagonists stimulated

feeding behavior by reducing neural activity in the NAcc, Stratford and Kelley (1997) hypothesized that GABA receptor subtype agonists should stimulate feeding in the shell region of the NAcc by similarly reducing neural activity. In this vein, both the $GABA_A$ receptor agonist, muscimol and the $GABA_B$ receptor agonist, baclofen elicited feeding following microinjection into the shell region of the NAcc. These effects were receptor-selective since the $GABA_A$ receptor antagonist, bicuculline reduced feeding induced by muscimol, but not baclofen, and the $GABA_B$ receptor antagonist, saclofen reduced feeding induced by baclofen, but not muscimol (Stratford and Kelley, 1997). Further, muscimol in the shell region of the NAcc also stimulated both high-fat and high-carbohydrate diets as well as palatable sucrose intake (Basso and Kelley, 1999). Our laboratory (Znamensky et al., 2000) showed differential GABA receptor interactions with opioid-induced feeding in the shell region of the NAcc. Whereas simultaneous treatment of the $GABA_A$ antagonist, bicuculline and DAMGO resulted in a potentiated ingestive response in the NAcc relative to DAMGO alone, pretreatment with the $GABA_B$ antagonist, saclofen dose-dependently reduced DAMGO-induced feeding in the shell region of the NAcc. Ongoing studies are exploring further relationships between opioid, excitatory amino acid and GABA systems in the NAcc.

In conclusion, the metamorphosis in our understanding of how opioids induce feeding behavior has moved markedly in the past two decades from initial observation that opiate drugs stimulate food intake. The development of selective opioid receptor subtype agonists and antagonists allowed us to delineate which neural opioid receptors mediated specific ingestive effects. The use of ventricular routes of microinjections firmly established the role of central opioid receptors in this response. The use of intracerebral microinjection techniques

in studying agonist effects upon spontaneous intake identified possible sites of action. This dissertation firmly established the NAcc, and secondarily the VTA, as putative sites of action at which opioid peptides and their receptors act to modulate feeding under a wide variety of conditions. The next step for ingestive behavior is to follow in the footsteps of its sister function, analgesia, in elucidating specific synaptic connections between brain sites that mediate specific pharmacological responses including those elicited by opioids. This approach will hopefully identify the specific substrates by which feeding is modulated by opioid peptides and receptors under natural conditions, and establish relationships, if any, with other behaviors related to reinforcement, reward and other primary motivational functions modulated by this important system.

References

Ackroff, K., & Sclafani, A. (1988) Sucrose-induced hyperphagia and obesity in rats fed a macronutrient self-selection diet. Physiology and Behavior, 44, 181-187.

Akil, H., Watson, S.J., Young, E., Lewis, M.E., Khachaturian, H., & Walker, J.M. (1984) Endogenous opioids: biology and function. Annual Review of Neuroscience, 7, 223-255.

Alheid, G.F., & Heimer, L. (1988) New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid, and corticopetal components of substantia inominata. Neuroscience, 27, 1-39.

Alheid, G.F., Heimer, L., & Switzer, R.C. (1990) Basal Ganglia. Paxinos, G. The Human Nervous System. Academic Press:Sydney. 483-582.

Altier, N., & Stewart, J. (1998) Dopamine receptor antagonists in the nucleus accumbens attenuate analgesia induced by ventral tegmental area substance P or morphine and by nucleus accumbens amphetamine. Journal of Pharmacology and Experimental Therapeutics, 285, 208-215.

Arjune, D., & Bodnar, R.J. (1990a) Inhibition of deprivation-induced feeding by naloxone and cholecystokinin in rats: effects of central alloxan. Brain Research Bulletin, 24, 375-379.

Arjune, D., & Bodnar, R.J. (1990b) Suppression of nocturnal, palatable and glucoprivic intake in rats by the κ opioid antagonist, nor-binaltorphamine. Brain Research, 534, 313-316.

Arjune, D., Bowen, W.D., & Bodnar, R.J. (1991) Ingestive behavior following central [D-Ala^2, Leu^5, Cys^6]-enkephalin (DALCE), a short-acting agonist and long-acting antagonist at the δ opioid receptor. Pharmacology Biochemistry and Behavior, 39, 429-436.

Arjune, D., Standifer, K.M., Pasternak, G.W., & Bodnar, R.J. (1990) Reduction by central β-funaltrexamine of food intake in rats under freely-feeding, deprivation and glucoprivic conditions. Brain Research, 535, 101-109.

Asakawa, A., Inui, A., Momose, K., Ueno, N., Fujino, M.A., & Kasuga, M. (1998) Endomorphins have orexigenic and anxiolytic activities in mice. Neuroreport, 9, 2265-2267.

Badiani, A., Leone, P., Noel, M.B., & Stewart, J. (1995) Ventral tegmental area opioid mechanisms and modulation of ingestive behavior. Brain Research, 670, 264-276.

Badiani, A., Leone, P., & Stewart, J. (1995) Intra-VTA injections of the μ-opioid antagonist CTOP enhance locomotor activity. Brain Research, 690, 112-116.

Bakshi, V.P., & Kelley, A.E. (1993a) Feeding induced by opioid stimulation of the ventral striatum: role of opioid receptor subtypes. Journal of Pharmacology and Experimental Therapeutics, 265, 1253-1260.

Bakshi, V.P., & Kelley, A.E. (1993b) Striatal regulation of morphine-induced hyperphagia: an anatomical mapping study. Psychopharmacology, 111, 207-214.

Bakshi, V.P., & Kelley, A.E. (1994) Sensitization and conditioning of feeding following multiple morphine microinjections into the nucleus accumbens. Brain Research, 648, 342-346.

Barbour, H.G., Gregg, D.E., & Hunter, L.G. (1930) The calorigenic action of morphine as revealed by addiction studies. Journal of Pharmacology and Experimental Therapeutics, 40, 433-465.

Basso, A.M., & Kelley, A.E. (1999) Feeding induced by GABAA receptor stimulation within the nucleus accumbens shell: Regional mapping and characterization of macronutrient and taste preference. Behavioral Neuroscience, 113, 324-336.

Beckstead, R.M., Domesick, V.B., & Nauta, W.J.H. (1979) Efferent connections of the substantia nigra and ventral tegmental area in the rat. Brain Research, 175, 191-217.

Beczkowska, I.W., & Bodnar, R.J. (1991a) Mediation of insulin hyperphagia by specific central opiate receptor antagonists. Brain Research, 547, 315-318.

Beczkowska, I.W., & Bodnar, R.J. (1991b) Naloxone and serotonin receptor subtype antagonists: interactive effects upon deprivation-induced intake. Pharmacology Biochemistry and Behavior, 38, 605-610.

Beczkowska, I.W., Bowen, W.D., & Bodnar, R.J. (1992) Central opioid receptor subtype antagonists differentially alter sucrose and deprivation-induced water intake in rats. Brain Research, 589, 291-301.

Beczkowska, I.W., Koch, J.E., Bostock, M.E., Leibowitz, S.F., & Bodnar, R.J. (1993) Central opioid receptor subtype antagonists differentially reduce intake of saccharin and maltose dextrin solutions in rats. Brain Research, 618, 261-270.

Befort, K., Tabbara, L., Bausch, S., Chavkin, C., Evans, C., Kieffer, B. (1996) The conserved aspartate residue in the third putative transmembrane domain of the δ-opioid receptor is not the anionic counterpart for cationic opiate binding but is a constituent of

the receptor binding site. Molecular Pharmacology, 49, 216-23.

Beitz, A.J. (1982) The nuclei of origin of brainstem enkephalin and substance P projections to the rodent nucleus raphé magnus. Neuroscience, 7, 2753-2768.

Berendse, H.W., Groenewegen, H.J., & Lohman, A.H.M. (1992) Compartmental distribution of ventral striatal neurons projecting to the ventral mesencephalon in the rat. The Journal of Neuroscience, 12, 2079-2103.

Bertino, M., Abelson, M.L., Marglin, S.H., Neuman, R., Burkhardt, C.A., & Reid, L.D. (1988) A small dose of morphine increases intake of and preference for isotonic saline among rats. Pharmacology Biochemistry and Behavior, 29, 617-623.

Bhakthavatsalam, P., & Leibowitz, S.F. (1986) Morphine-elicited feeding: diurnal rhythm, circulating corticosterone and macronutrient selection. Pharmacology Biochemistry and Behavior, 24, 911-917.

Bless, E.P., & Kelley, A.E. (1994) Differential effects of μ, δ and κ opiate receptor blockade in the nucleus accumbens on feeding behavior. Society for Neuroscience Abstracts, 20, 382.

Bodnar RJ, Glass MJ, Ragnauth A, Cooper ML. (1995) General, μ and κ opioid antagonists in the nucleus accumbens alter food intake under deprivation, glucoprivic and palatable conditions. Brain Research, 700, 205-12.

Bodnar, R.J. (1996) Opioid receptor subtype antagonists and ingestion. Cooper, S.J., & Clifton, P.J. Drug receptor subtypes and ingestive behaviour. Academic Press:London. 127-146.

Bodnar, R.J., Williams, C.L., Lee, S.J., & Pasternak, G.W. (1988) Role of μ_1 opiate receptors in supraspinal opiate analgesia: a microinjection study. Brain Research, 447, 25-34.

Bordi, F., Carr, D., & Meller, E. (1989) Stereotypies elicited by injection of N-propylnorapomorphine into striatal subregions and nucleus accumbens. Brain Research, 489, 205-215.

Bowen, W.D., Hellewell, S.B., Kelemen, M., Huey, R., & Steward, D. (1987) Affinity labelling of δ-opiate receptors using [D-Ala-2,Leu-5,Cys-6]-enkephalin: covalent attachment via thiosulfide exchange. Journal of Biological Chemistry, 262, 13434-13439.

Bozarth, M.A. (1994) Physical dependence produced by central morphine infusions: an anatomical mapping study. Neuroscience and Biobehavioral Reviews, 18, 373-383.

Braun, A.R., & Chase, T.N. (1986) Obligatory D-1/D-2 receptor interaction in the generation of dopamine agonist related behavior. European Journal of Pharmacology, 131, 301-306.

Breese, G.R., Duncan, G.E., Napier, T.C., Bondy, S.C., Iorio, L.C., & Mueller, R.A. (1987) 6-hydroxydopamine treatments enhance behavior response to intracerebral microinjection of D_1- and D_2-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. Journal of Pharmacology and Experimental Therapeutics, 240, 167-176.

Brog, J.S., Deutsch, A.Y., & Zahm, D.S. (1991) Afferent projections to the nucleus accumbens core and shell in the rat. Society for Neuroscience Abstracts, 17, 454.

Brog, J.S., Salyapongse, A.Y., Deutsch, A.Y., & Zahm, D.S. (1993) The patterns of afferent innevation of the core and shell of the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. Journal of Comparative Neurology, 338, 255-278.

Brown, D.R., & Holtzman, S.G. (1979) Suppression of deprivation-induced food and water intake in rats and mice by naloxone. Pharmacology Biochemistry and Behavior, 11, 567-573.

Bunzow, J.R., Saez, C., Mortrud, M., Bouvier, C., Williams, J.T., Low, M., & Grandy, D.K. (1994) Molecular cloning and tissue distribution of a putative member of the rat opioid receptor gene family that is not a μ, δ or κ receptor type. FEBS Letters, 347, 284-288.

Burdick, K., Yu, W.-Z., Ragnauth, A., Moroz, M., Pan, Y.X., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1998) Antisense mapping of opioid receptor clones: effects upon 2-deoxy-D-glucose-induced hyperphagia. Brain Research, 794, 359-363.

Cador, M., Kelley, A.E., LeMoal, M., & Stinus, L. (1986) Ventral tegmental area infusion of substance P, neurotensin and enkephalin: differential effects on feeding behavior. Neuroscience, 18, 659-669.

Carr, G.D., & White, N.M. (1986) Anatomical dissociation of amphetamines rewarding and aversive effects: an intracranial microinjection study. Psychopharmacology, 89, 340-346.

Carr, K.D., Aleman, D.O., Bak, T.H., & Simon, E.J. (1991) Effects of parabrachial opioid antagonism on stimulation-induced feeding. Brain Research, 545, 283-286.

Chen, Y., Mestek, A., Liu, J., Hurley, A., & Yu, L. (1993) Molecular cloning and functional expression of a μ-opioid receptor from rat brain. Molecular Pharmacology, 44,

8-12.

Christensen, A.V., Arnt, J., Hyttel, J., Larsen, J.J., & Svendsen, O. (1984) Pharmacological effects of a specific dopamine D-1 antagonist SCH23390 in comparison with neuroleptics. Life Sciences, 34, 1529.

Churchill, L., & Kalivas, P.W. (1992) Dopamine depletion produces augmented behavioral responses to a μ-, but not a δ-opioid receptor agonist in the nucleus accumbens: lack of a role for receptor upregulation. Synapse, 11, 47-57.

Clark, J.A., Liu, L., Price, M., Hersh, B., Edelson, M., & Pasternak, G.W. (1989) κ opiate receptor multiplicity: evidence for two U50,488H-sensitive K-1 subtypes and a novel K-3 subtype. Journal of Pharmacology and Experimental Therapeutics, 251, 461-468.

Cole, J.L., Leventhal, L., Pasternak, G.W., Bowen, W.D., & Bodnar, R.J. (1995) Reductions in body weight following chronic central opioid receptor subtype antagonists during development of dietary obesity in rats. Brain Research, 678, 168-176.

Comb, M., Herbert, E., & Crea, R. (1982) Partial characterization of the mRNA that codes for enkephalins in bovine adrenal medulla and human pheochromocytoma. Proceedings of the National Academy of Science (USA), 79, 360-364.

Cooper, S.J. (1983) Effects of opiate agonists and antagonists on fluid intake and saccharin choice in the rat. Neuropharmacology, 22, 323-328.

Cooper, S.J., Jackson, A., Kirkham, T.C., & Turkish, S. (1988) Endorphins, opiates and food intake. Rodgers, R.J., & Cooper, S.J. Endorphins, opiates and behavioral processes. John Wiley and Sons: New York. 143-186.

Corbett, A.D., Patterson, S.J., McKnight, A.T., Magnan, J., & Kosterlitz, H.W. (1982) Dynorphin (1-8) and dynorphin (1-9) are ligands for the κ subtype of opiate receptor. Nature, 299, 79-81.

Cotton, R., Giles, M.G., Miller, L., Shaw, J.S., & Timms, D. (1984) ICI 174864: a highly selective antagonist for the opioid δ receptor. European Journal of Pharmacology, 97, 331-332.

Cunningham, S.T., Finn, M., & Kelley, A.E. (1997) Sensitization of the locomotor response to psychostimulants after repeated opiate exposure: role of the nucleus accumbens. Neuropsychopharmacology, 16, 147-155.

Cunningham, S.T., & Kelley, A.E. (1992) Evidence for opiate-dopamine cross-sensitization in the nucleus accumbens: studies of conditioned reward. Brain Research

Bulletin, 29, 675-680.

Curran, E.J., & Watson, S.J. (1995) Dopamine receptor mRNA expression patterns by opioid peptide cells in the nucleus accumbens of the rat: a double in situ hybridization study. Journal of Comparative Neurology, 361, 57-76.

Czirr, S.A., & Reid, L.D. (1986) Demonstrating morphine's potentiating effects on sucrose intake. Brain Research Bulletin, 17, 639-642.

Deniau, J.M., Menetrey, A., & Thierry, A.M. (1994) Indirect nucleus accumbens input to the prefrontal cortex via the substantia nigra pars reticulata: a combined anatomical and electrophysiological study in the rat. Neuroscience, 61, 533-545.

de Olmos, J.S. (1972) The amygdaloid projection field in the rat as studied with the cupric silver method. Eleftheriou, B.E. The Neurobiology of the Amygdala. Plenum Press: New York. 145-204.

de Olmos, J.S., Alheid, G.F., & Beltramino, C.A. (1985) Amygdala. Paxinos, G. The Rat Central Nervous System. Academic Press:Sydney. 223-234.

de Olmos, J.S., & Heimer, L. (1999) The Concepts of the Ventral Striatopallidal System and Extended Amygdala. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. New York Academy of Sciences: New York. 1-32.

Deutch, A.Y., & Cameron, D.S. (1992) Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience, 46, 49-56.

Devine, D.P., Leone, P., Pocock, D., & Wise, R.A. (1993) Differential involvement of ventral tegmental μ, δ and κ opioid receptors in modulation of basal mesolimbic dopamine release: in vivo microdialysis studies. Journal of Pharmacology and Experimental Therapeutics, 266, 1236-1246.

Devine, D.P., Leone, P., & Wise, R.A. (1993) Mesolimbic dopamine neurotransmission is increased by administration of μ-opioid receptor antagonists. European Journal of Pharmacology, 243, 55-64.

DiChara, G., & Imperato, A. (1988) Opposite effects of μ and κ opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. Journal of Pharmacology and Experimental Therapeutics, 244, 1067-1080.

DiChiara, G., & Imperato, A. (1988) Opposite effects of μ and κ opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. Journal of Pharmacology and Experimental Therapeutics, 244, 1067-1080.

Di Chiara, G., Tanda, G., Bassareo, V., Pontieri, F., Acquas, E., Fenu, S., Cadoni, C., & Carboni, E. (1999) Drug Addiction as a Disorder of Associative learing; Role of Nucleus Accumbens Shell/Extended Amygdala Dopamine. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. NYAS: New york. 461-485.

Dreher, J.K., & Jackson, D.M. (1989) Role of D-1 and D-2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. Brain Research, 487, 267-277.

Echo, J., Znamensky, V., LaMonte, N., Ragnauth, A., & Bodnar, R.J. (2000) Interactions between excitatory amino acid receptors and opioid receptors in feeding elicited from the nucleus accumbens in rats. Society for Neuroscience Abstracts, 26, 993.

Eipper, B., & Mains, R. (1978) Existence of a common precursor to ACTH and endorphin in the anterior and intermediate lobes of the rat pituitary. Journal of Supramolecular Structure, 8, 247-262.

Evans, C., Keith, D., Morrison, H., Magendzo, K., & Edwards, R. (1992) Cloning of a δ-opioid receptor by functional expression. Science, 258, 1952-1955.

Evans, K.R., & Vaccarino, F.J. (1986) Intra-accumbens amphetamine: dose-dependent effects on food intake. Pharmacology Biochemistry and Behavior, 25, 1149-1151.

Evans, K.R., & Vaccarino, F.J. (1990) Amphetamine- and morphine-induced feeding: evidence for involvement of reward mechanisms. Neuroscience and Biobehavioral Reviews, 14, 9-22.

Everitt, B.J., Parkinson, J.A., Olmstead, M.S., Arroyo, M., Robledo, P., & Robbins, T.W. (1999) Associative Processes in Addiction and Reward: The Role of Amygdala-Ventral Striatal Subsystems. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. New York Academy of Sciences: New york. 412-438.

Fallon, J.H., & Leslie, F.M. (1986) Distribution of dynorphin and enkephalin peptides in the rat brain. Journal of Comparative Neurology, 249, 293-336.

Flowers, S.H., Dunham, E.S., & Barbour, H.G. (1929) Addiction edema and withdrawal edema in morphinized rats. Proceedings of the Society of Experimental and Biological Medicine, 26, 527-574.

Frenk, H., & Rogers, G.H. (1979) The suppressant effects of naloxone on food and water intake in the rat. Behavioral and Neural Biology, 26, 23-40.

Gerfen, C.R., Herkenham, M., & Thibault, J. (1987) The neostriatal mosaic: II. Patch- and matrix-directed mesostriatal dopaminergic and non-dopaminergic systems. The Journal of

Neuroscience, 7, 3915-3934.

German, D.C., Speciale, S.G., Manaye, K.F., & Sadeq, M. (1993) Opioid receptors in midbrain dopaminergic regions of the rat. I. μ receptor autoradiography. Journal of Neural Transmission, 91, 39-52.

Giraudo, S.Q., Billington, C.J., & Levine, A.S. (1998) Effects of the opioid antagonist naltrexone on feeding induced by DAMGO in the central nucleus of the amygdala and in the paraventricular nucleus in the rat. Brain Research, 782, 18-23.

Giraudo, S.Q., Kotz, C.M., Billington, C.J., & Levine, A.S. (1998) Association between the amygdala and the nucleus of the solitary tract in μ opioid induced feeding in the rat. Brain Research, 802, 184-188.

Gistrak, M.A., Paul, D., Hahn, E.F., & Pasternak, G.W. (1989) Pharmacological actions of a novel mixed opiate agonist-antagonist: naloxone benzoylhydrazone. Journal of Pharmacology and Experimental Therapeutics, 251, 469-476.

Goldman, C.K., Marino, L., & Leibowitz, S.F. (1985) Postsynaptic α-2-noradrenergic receptors in the paraventricular nucleus mediate feeding induced by norepinephrine and clonidine. European Journal of Pharmacology, 115, 11-19.

Goldstein, A., Fischli, W., Lowney, L.I., Hunkapiller, M., & Hood, L. (1981) Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. Proceedings of the National Academy of Science (USA), 74, 7219-23.

Gong, J., Strong, J.A., Zhang, S., Yue, X., DeHaven, R.N., Daubert, J.D., Cassel, J.A., Yu, G., Mansson, E., & Yu, L. (1998) Endomorphins fully activate a cloned human μ opioid receptor. FEBS Letters, 439, 152-156.

Gong, W., Neill, D.B., Lynn, M., & Justice, J.B. (1999) Dopamine D_1/D_2 agonists injected into nucleus accumbens and ventral pallidum differentially affect locomotor activity depending on site. Neuroscience, 93, 1349-1358.

Goodman, R.R., & Pasternak, G.W. (1985) Visualization of μ_1 opiate receptors in rat brain using a computerized autoradiographic subtraction technique. Proceedings of the National Academy of Science (USA), 82, 6667-71.

Gosnell, B.A. (1988) Involvement of μ opioid receptors in the amygdala in the control of feeding. Neuropharmacology, 27, 319-326.

Gosnell, B.A., Krahn, D.D., & Majchrzak, M.J. (1990) The effects of morphine on diet selection are dependent upon baseline diet preferences. Pharmacology Biochemistry and

Behavior, 37, 207-212.

Gosnell, B.A., & Levine, A.S. (1996) Stimulation of ingestive behaviour by preferential and selective opioid agonists. Cooper, S.J., & Clifton, P.G. Drug receptor subtypes and ingestive behaviour. Academic Press:London. 147-166.

Gosnell, B.A., Levine, A.S., & Morley, J.E. (1986) The stimulation of food intake by selective agonists of μ, κ and δ opioid receptors. Life Sciences, 38, 1081-1088.

Gosnell, B.A., & Majchrzak, M.J. (1989) Centrally administered opioid peptides stimulate saccharin intake in nondeprived rats. Pharmacology Biochemistry and Behavior, 33, 805-810.

Gosnell, B.A., & Majchrzak, M.J. (1990) Effects of a selective μ opioid receptor agonist and naloxone on the intake of sodium chloride solutions. Psychopharmacology, 100, 66-71.

Gosnell, B.A., Morley, J.E., & Levine, A.S. (1986) Opioid-induced feeding: localization of sensitive brain sites. Brain Research, 369, 177-184.

Gosnell, B.A., & Patel, C.K. (1993) Centrally administered μ- and δ-opioid agonists increase operant responding for saccharin. Pharmacology Biochemistry and Behavior, 45, 979-982.

Gracy, K.N., Svingos, A.L., & Pickel, V.M. (1997) Dual ultrastructural localization of μ-opioid receptors and NMDA-type glutamate receptors in the shell of the nucleus accumbens. The Journal of Neuroscience, 17, 4839-4848.

Grandison, L., & Guidotti, A. (1977) Stimulation of food intake by muscimol and β-endorphin. Neuropharmacology, 16, 533-536.

Groenewegen, H.J., Berendse, H.W., & Wouterlood, F.G. (1994) Organization of the projections from the ventral striatopallidal system to ventral mesencephalic dopaminergic neurons. Percheron, G., & McKenzie, J.S. The Basal Ganglia IV. Plenum Press: New York. 81-93.

Groenewegen, H.J., Wright, C.I., Beijer, A.V.J., & Voorn, p. (1999) Convergence and Segregation of Ventral Striatal Inputs and Ouputs. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. New York Academy of Sciences: New York. 49-63.

Gulya, K., Pelton, J.T., Hruby, V.J., & Yamamura, H.I. (1986) Cyclic somatostatin octapeptide analogues with high affinity and selectivity towards μ opioid receptors. Life

Sciences, 38, 2221-2229.

Gysling, K., & Wang, R.Y. (1983) Morphine-induced activation of A10 dopamine neurons in the rat. Brain Research, 277, 119-127.

Hahn, E.F., Carroll-Buatti, M., & Pasternak, G.W. (1982) Irreversible opiate agonists and antagonists: the 14-hydroxydihydromorphinone azines. The Journal of Neuroscience, 2, 572-576.

Hakan, R.L., & Henriksen, S.J. (1989) Opioid influences on nucleus accumbens neuronal electrophysiology: dopamine and non-dopamine mechanisms. The Journal of Neuroscience, 9, 3538-3546.

Hamilton, M.E., & Bozarth, M.A. (1988) Feeding elicited by dynorphin (1-13) microinjections into the ventral tegmental area. Life Sciences, 43, 941-946.

Handa, B.K., Lane, A.C., Lord, J.A.H., Morgan, B.A., Rance, M.J., & Smith, C.F.C. (1981) Analogs of β-LPH 61-64 possessing selective agonist activity of μ-opiate receptors. European Journal of Pharmacology, 70, 531-540.

Harris, G.C., & Aston-Jones, G. (1994) Involvement of D_2 dopamine receptors in the nucleus accumbens in the opiate withdrawal syndrome. Nature, 371, 155-157.

Heimer, L., & Alheid, G.F. (1991) Piecing together the puzzle of basal forebrain anatomy. Heimer, L., & Alheid, G.F. The Basal Forebrain: Anatomy to Function. Plenum Press:. 1-44.

Heimer, L., Alheid, G.F., & Zaborszky. (1985) Basal Ganglia. Paxinos, G. The Rat Central Nervous System. Academic Press:Sydney. 37-86.

Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., & Wohltmann, C. (1991) Specificity in the projection patterns of accumbal core and shell. Neuroscience, 41, 89-126.

Henderson, G., & McKnight, A.T. (1997) The orphan opioid receptor and its endogenous ligand - nociceptin/orphanin FQ. Trends in Pharmacological Sciences, 18, 293-300.

Herkenham, M., Moon Edley, S., & Stuart, J. (1984) Cell clusters in the nucleus accumbens of the rat, and the mosaic relationship of opiate receptors, acetylcholinesterase and subcortical afferent terminations. Neuroscience, 11, 561-593.

Hobbs, D.J., Koch, J.E., & Bodnar, R.J. (1994) Naltrexone, dopamine receptor agonists and antagonists and food intake in rats: 1. Food deprivation. Pharmacology Biochemistry and Behavior, 49, 197-204.

Hokfelt, T., Elde, R., Johansson, O., Terenius, L., & Stein, L. (1977) The distribution of enkephalin-immunoreactive cell bodies in the rat central nervous system. Neuroscience Letters, 5, 25-31.

Holtzman, S.G. (1974) Behavioral effects of separate and combined administration of naloxone and d-amphetamine. Journal of Pharmacology and Experimental Therapeutics, 189, 51-60.

Horan, P., Taylor, J., Yamamura, H.I., & Porreca, F. (1992) Extremely long-lasting antagonistic actions of nor-binaltorphamine (nor-BNI) in the mouse tail-flick test. Journal of Pharmacology and Experimental Therapeutics, 260, 1237-1243.

Hughes, J., Smith, T., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A., & Morris, H.R. (1975) Identification of two related penta-peptides from the brain with potent opiate agonist activity. Nature, 258, 577-579.

Ikemoto, S., & Panksepp, J. (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Research Reviews, 31, 6-41.

Iorio, L.C., Barnett, A., Leitz, F.H., Houser, V.P., & Korduba, C.A. (1983) SCH23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. Journal of Pharmacology and Experimental Therapeutics, 226, 462-468.

Islam, A.K., & Bodnar, R.J. (1990) Selective opioid receptor antagonist effects upon intake of a high-fat diet in rats. Brain Research, 508, 293-296.

Jackson, H.C., & Sewell, R.D.E. (1985a) Are δ opioid receptors involved in the regulation of food and water intake? Neuropharmacology, 24, 885-888.

Jackson, H.C., & Sewell, R.D.E. (1985b) Hyperphagia induced by 2-deoxy-D-glucose in the presence of the δ-opioid antagonist, ICI174864. Neuropharmacology, 24, 815-817.

Jalowiec, J.E., Panksepp, J., Zolovick, A.J., Najam, N., & Herman, B. (1981) Opioid modulation of ingestive behavior. Pharmacology Biochemistry and Behavior, 15, 477-484.

Jenck, F., Gratton, A., & Wise, R.A. (1986) Opioid receptor subtypes associated with ventral tegmental facilitation and periaqueductal gray inhibition of feeding. Brain Research, 423, 39-44.

Jenck, F., Gratton, A., & Wise, R.A. (1987) Opioid receptor subtypes associated with ventral tegmental area facilitation of lateral hypothalamic brain stimulation reward. Brain Research, 423, 34-38.

Jiang, Q., Bowen, W.D., Mosberg, H.I., Rothman, R.B., & Porreca, F. (1990) Opioid agonist and antagonist properties of [D-Ala^2, Leu^5, Cys^6]-enkephalin: selective actions at the δ-noncomplexed site. Journal of Pharmacology and Experimental Therapeutics, 255, 636-641.

Jiang, Q., Mosberg, H.I., & Porreca, F. (1990) Antinociceptive effects of [D-Ala^2]-deltorphin II, a highly selective δ agonist in vivo. Life Sciences, 47, PL43-47.

Jiang, Q., Takemori, A.E., Sultana, M., Portoghese, P.S., Bowen, W.D., Mosberg, H.I., & Porreca, F. (1991) Differential antagonism of opioid δ antinociception by [D-Ala^2, Leu^5, Cys^6]-enkephalin (DALCE) and naltrindole 5'-isothiocyanate (5'-NTII): evidence for δ receptor subtypes. Journal of Pharmacology and Experimental Therapeutics, 257, 1069-1075.

Jiang, W., Bowen, W.D., Mosberg, H.I., Rothman, R.B., & Porreca, F. (1990) Opioid agonist and antagonist properties of [D-Ala^2, Leu^5, Cys^6]-enkephalin: selective actions at the δ site. Journal of Pharmacology and Experimental Therapeutics, 225, 636-641.

Jones, D.N.C., & Holtzman, S.G. (1992) Long-term κ-opioid receptor blockade following nor-binaltorphamine. European Journal of Pharmacology, 215, 345-348.

Jôngen-Relo, A.L., Docter, G.J., Jonker, A.J., & Voorn, P. (1995) Differential localization of mRNAs encoding dopamine D_1 or D_2 receptors in cholinergic neurons in the core and shell of the rat nucleus accumbens. Molecular Brain Research, 28, 169-174.

Jôngen-Relo, A.L., Groenewegen, H.J., & Voorn, P. (1993) Evidence for a multi-compartmental histochemical organization of the nucleus accumbens in the rat. Journal of Comparative Neurology, 337, 267-276.

Joyce, E.M., & Iversen, S.D. (1979) The effect of morphine applied locally to mesencephalic cell bodies on spontaneous motor activity in the rat. Neuroscience Letters, 14, 207-212.

Kalivas, P.W., & Richardson-Carlson, R. (1986) Endogenous enkephalin modulation of dopamine neurons in the ventral tegmental area. American Journal of Physiology, 258, 243-249.

Kalivas, P.W., Widerlov, E., Stanley, D., Breese, G., & Prange, A.J. (1983) Enkephalin action on the meso-limbic system: a dopamine-dependent and a dopamine-independent increase in locomotor activity. Journal of Pharmacology and Experimental Therapeutics, 227, 229-237.

Kangawa, K., Minamino, N., Chino, N., Sakakibara, S., & Matsuo, H. (1981) The

complete amino acid sequence of α-neo-endorphin. Biochemical and Biophysical Research Communications, 99, 871-878.

Kavaliers, M., & Hirst, M. (1987) Slugs and snails and opiate tales: opioids and feeding behavior in invertebrates. Federal Proceedings, 46, 168-72.

Kelley, A.E. (1999) Functional Specificity of Ventral Striatal Compartments in Appetitive Behaviors. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. New York Academy of Sciences: New York. 71-90.

Kelley, A.E., Bless, E.P., & Swanson, C.J. (1996) Investigation of the effects of opiate antagonists infused into the nucleus accumbens on feeding and sucrose drinking in rats. Journal of Pharmacology and Experimental Therapeutics, 278, 1499-1507.

Kelley, A.E., Gauthier, A.M., & Lang, C.G. (1989) Amphetamine microinjections into distinct striatal subregions cause dissociable effects on motor and ingestive behavior. Behavioral Brain Research, 35, 27-39.

Kelley, A.E., Lang, C.G., & Gauthier, A.M. (1988) Induction of oral stereotypy following amphetamine microinjection into a discrete subregion of the striatum. Psychopharmacology, 95, 556-559.

Kelley, A.E., & Swanson, C.J. (1997) Feeding induced by blockade of AMPA and kainate receptors within the ventral striatum: a microinfusion mapping study. Behavioral Brain Research, 89, 107-113.

Khachaturian, H., Lewis, M.E., Hollt, V., & Watson, S.J. (1983) Telencephalic enkephalinergic systems in the rat brain. The Journal of Neuroscience, 3, 844-855.

Khachaturian, H., Lewis, M.E., Schaffer, K.H.M., & Watson, S. (1985) Anatomy of the CNS opioid systems. Trends in Neuroscience, 1, 10-19.

Khachaturian, H., Watson, S.J., Lewis, M.E., Coy, D., Goldstein, A., & Akil, H. (1982) Dynorphin immunocytochemistry in the rat central nervous system. Peptides, 3, 941-954.

Kieffer, B., Befort, K., Gareiaux-Ruff, C., & Hirth, C. (1992) The δ-opioid receptor: isolation of cDNA by expression cloning and pharmacological characterization. Proceedings of the National Academy of Science (USA), 89, 12048-12052.

Kimura, S., Lewis, R.V., Stern, A.S., Rossier, J., Stein, S., & Undenfriend, S. (1980) Probable precursors of (leu) and (met)- enkephalin in adrenal medulla: Peptides of 3-5 kilodaltons. Proceedings of the National Academy of Science (USA), 77, 1681-85.

Kirkham, T.C., & Blundell, J.E. (1984) Dual action of naloxone on feeding revealed by behavioral analysis: separate effects on initiation and termination of eating. Appetite, 5, 45-52.

Kirkham, T.C., & Cooper, S.J. (1988a) Attenuation of sham feeding by naltrexone is stereospecific: evidence for opioid mediation of orosensory reward. Physiology and Behavior, 43, 845-847.

Kirkham, T.C., & Cooper, S.J. (1988b) Naloxone attenuation of sham feeding is modified by manipulation of sucrose concentration. Physiology and Behavior, 44, 491-494.

Kiyatkin, E.A., Wise, R.A., & Gratton, A. (1993) Drug- and behavior-associated changes in dopamine-related electrochemical signals during intravenous heroin self-administration in rats. Synapse, 14, 60-72.

Koch, J.E., & Bodnar, R.J. (1993) Involvement of μ_1 and μ_2 opioid receptor subtypes in tail-pinch feeding in rats. Physiology and Behavior, 53, 603-605.

Koch, J.E., & Bodnar, R.J. (1994) Selective alterations in macronutrient intake of food-deprived or glucoprivic rats by centrally-administered opioid receptor subtype antagonists in rats. Brain Research, 657, 191-201.

Koch, J.E., Glass, M.J., Cooper, M.L., & Bodnar, R.J. (1995) Alterations in deprivation, glucoprivic and sucrose intake following general, μ and κ opioid antagonists in the hypothalamic paraventricular nucleus of rats. Neuroscience, 66, 951-957.

Koch, J.E., Pasternak, G.W., Arjune, D., & Bodnar, R.J. (1992) Naloxone benzoylhydrazone, a κ_3 opioid agonist, stimulates food intake in rats. Brain Research, 581, 311-314.

Koene, P., Prinssen, E.P.M., & Cools, A.R. (1993) Involvement of the nucleus accumbens in oral behavior in the freely moving rat. European Journal of Pharmacology, 233, 151-156.

Koob, G.F. (1992) Drugs of abuse: anatomy, pharmacology and function. Trends in Pharmacological Sciences, 13, 177-184.

Koob, G.F. (1999) The Role of the Striatopallidal and Extended Amygdala Systems in Drug Addiction. McGinty, J.F. Advancing from the Ventral Striatum to the Extended Amygdala. New York Academy of Sciences: saNew York. 445-460.

Koob, G.F., & Bloom, F.E. (1988) Cellular and molecular mechanisms of drug dependence. Science, 242, 715-723.

Koob, G.J., Riley, S.J., Smith, S.C., & Robbins, T.W. (1978) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi on feeding, locomotor activity and amphetamine anorexia in rats. Journal of Comparative and Physiological Psychology, 92, 917-927.

Kopp, J., Lindefors, N., Brene, S., Hall, H., Persson, H., & Sedvall, G. (1992) Effect of raclopride on dopamine D_2 receptor mRNA expression in rat brain. Neuroscience, 47, 771-779.

Koshikawa, N., Kitamura, M., Kobayashi, M., & Cools, A.R. (1996) Contralateral turning elicited by unilateral stimulation of dopamine D_2 and D_1 receptors in the nucleus accumbens of rats is due to the stimulation of these receptors in the shell, but not the core, of this nucleus. Psychopharmacology, 126, 185-190.

Kotz, C.M.., Billington, C.J., & Levine, A.S. (1997) Opioids in the nucleus of the solitary tract are involved in feeding in the rat. American Journal of Physiology, 272, R1028-R1032.

Kotz, C.M.., Grace, M.K., Briggs, J., Levine, A.S., & Billington, C.J. (1995) Effects of opioid antagonists naloxone and naltrexone on neuropeptide Y-induced feeding and brown fat thermogenesis in the rat. Journal of Clinical Investigations, 96, 163-170.

Kotz, C.M.., Glass, M.J., Levine, A.S. & Billington, C.J. (2000) Regional effect of naltrexone in the nucleus of the solitary tract in blockade of NPY-induced feeding. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology, 272, 499-503.

Kreil, G., Barra, D., Simmaco, M., Erspamer, V., Falconari-Erspamer, G., Negri, L., Severini, C., Corsi, R., & Melchiorri, P. (1989) Deltorphan, a novel amphibian skin peptide with high selectivity and affinity for δ opioid receptors. European Journal of Pharmacology, 162, 123-128.

Kyrkouli, S.E., Stanley, B.G., & Leibowitz, S.F. (1986) Galanin: stimulation of feeding induced by medial hypothalamic injection of this novel peptide. European Journal of Pharmacology, 122, 159-160.

Latimer, L.G., Duffy, P., & Kalivas, P.W. (1987) μ opioid receptor involvement in enkephalin activation of dopamine neurons in the ventral tegmental area. Journal of Pharmacology and Experimental Therapeutics, 241, 328-337.

Leibowitz, S.F. (1978) Paraventricular nucleus: a primary site mediating adrenergic stimulation of feeding and drinking. Pharmacology Biochemistry and Behavior, 8, 163-175.

Leibowitz, S.F., & Hor, L. (1982) Endorphinergic and α-noradrenergic systems in the paraventricular nucleus: effects on eating behavior. Peptides, 3, 421-428.

LeMagnen, J., Marfaing-Jallat, P., Micelli, D., & Devos, M. (1980) Pain modulating and reward systems: a single brain mechanism. Pharmacology Biochemistry and Behavior, 12, 729-733.

LeMoine, C., & Bloch, B. (1996) Expression of the D-3 dopamine receptor in peptidergic neurons of the nucleus accumbens: comparison with the D-1 and D-2 dopamine receptors. Neuroscience, 73, 131-143.

Leone, P., Pocock, D., & Wise, R.A. (1991) Morphine-dopamine interaction: ventral tegmental morphine increases nucleus accumbens dopamine release. Pharmacology Biochemistry and Behavior, 39, 469-472.

Leventhal, L., Cole, J.L., Rossi, G.C., Pan, Y.X., Pasternak, G.W., & Bodnar, R.J. (1996) Antisense oligodeoxynucleotides against the MOR-1 clone alter weight and ingestive responses in rats. Brain Research, 719, 78-84.

Leventhal, L., Kirkham, T.C., Cole, J.L., & Bodnar, R.J. (1995) Selective actions of central μ and κ opioid antagonists upon sucrose intake in sham-feeding rats. Brain Research, 685, 205-210.

Leventhal, L., Mathis, J.P., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1998) Orphan opioid receptor antisense probes block orphanin FQ-induced hyperphagia. European Journal of Pharmacology, 349, R1-R3.

Leventhal, L., Silva, R.M., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1998) Morphine-6-β-glucuronide-induced hyperphagia: characterization of opioid action by selective antagonists and antisense mapping in rats. Journal of Pharmacology and Experimental Therapeutics, 287, 538-544.

Leventhal, L., Stevens, L.B., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1997) Antisense mapping of the MOR-1 opioid receptor clone: modulation of hyperphagia induced by DAMGO. Journal of Pharmacology and Experimental Therapeutics, 282, 1402-1407.

Levine, A.S., Grace, M., & Billington, C.J. (1991) B-funaltrexamine (β-FNA) decreases deprivation and opioid-induced feeding. Brain Research, 562, 281-284.

Levine, A.S., Grace, M., Billington, C.J., & Portoghese, P.S. (1990) Nor-binaltorphamine decreases deprivation and opioid-induced feeding. Brain Research, 534, 60-64.

Levine, A.S., Grace, M., Welch, W., Billington, C.J., & Portoghese, P.S. (1989) Injection of opioid antagonists into the paraventricular nucleus: effects on deprivation-induced feeding. Society for Neuroscience Abstracts, 15, 896.

Levine, A.S., & Morley, J.E. (1981) Peptidergic control of insulin-induced feeding. Peptides, 2, 261-264.

Levine, A.S., Morley, J.E., Gosnell, B.A., Billington, C.J., & Bartness, T.J. (1985) Opioids and consummatory behavior. Brain Research Bulletin, 14, 663-672.

Lewis, M.E., Khachaturian, H., Akil, H., & Watson, S.J. (1984) Anatomical relationship between opioid peptides and receptors in the rhesus monkey brain. Brain Research Bulletin, 13, 801-812.

Lewis, M.E., Khachaturian, H., & Watson, S.J. (1985) Combined autoradiographic-immunocytochemical analysis of opioid receptors and opioid peptide neuronal systems in brain. Peptides, 6, 37-47.

Ling, G.S.F., Simantov, R., Clark, J.A., & Pasternak, G.W. (1986) Naloxonazine action in vivo. European Journal of Pharmacology, 129, 33-38.

Longoni, R., Spina, L., Mulas, A., Carboni, E., Garau, L., Melchiorri, P., & DiChiara, G. (1991) (D-Ala2) Deltorphin II: D_1-dependent stereotypies and stimulation of dopamine release in the nucleus accumbens. The Journal of Neuroscience, 11, 1565-1576.

Lord, J.A.H., Waterfield, A.A., Hughes, J., & Kosterlitz, H. (1977) Endogenous opioid peptides: multiple agonists and receptors. Nature, 267, 495-499.

Lowy, M.T., Maickel, R.P., & Yim, G.K.W. (1980) Naloxone reduction of stress-related feeding. Life Sciences, 26, 2113-2118.

Lu, X.Y., Ghasemzadeh, M.B., & Kalivas, P.W. (1998) Expression of D-1 receptor, D-2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. Neuroscience, 82, 767-780.

Lynch, W.C. (1986) Opiate blockade inhibits saccharin intake and blocks normal preference acquisition. Pharmacology Biochemistry and Behavior, 24, 833-836.

Lynch, W.C., & Burns, G. (1990) Opioid effects on intake of sweet solutions depend both on prior drug experience and on prior ingestive experience. Appetite, 15, 23-32.

Mains, R.E., Eipper, B.A., & Ling, N. (1977) Common precursor to corticotropins and endorphin. Proceedings of the National Academy of Science (USA), 1974, 3014-3018.

Majeed, N.H., Przewlocka, B., Wedzony, K., & Przewlocki, R. (1986) Stimulation of food intake following opioid microinjection into the nucleus accumbens septi in rats. Peptides, 7, 711-716.

Maldonado-Irizarry, C.S., & Kelley, A.E. (1994) Differential behavioral effects following microinjection of an NMDA antagonist into nucleus accumbens subregions. Psychopharmacology, 116, 65-72.

Maldonado-Irizarry, C.S., Swanson, C.J., & Kelley, A.E. (1995) Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. The Journal of Neuroscience, 15, 6779-6788.

Mann, P.E., Arjune, D., Romero, M.T., Pasternak, G.W., Hahn, E.F., & Bodnar, R.J. (1988) Differential sensitivity of opioid-induced feeding to naloxone and naloxonazine. Psychopharmacology, 94, 330-341.

Mansour, A., Fox, C.A., Akil, H., & Watson, S.J. (1995) Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends in Neuroscience, 18, 22-29.

Mansour, A., Fox, C.A., Thompson, R.C., Akil, H., & Watson, S.J. (1994) μ-opioid receptor mRNA expression in the rat CNS: comparison to μ-receptor binding. Brain Research, 643, 245-265.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., & Watson, S.J. (1988) Anatomy of CNS opioid receptors. Trends in Neuroscience, 11, 308-314.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H., & Watson, S.T. (1987) Autoradiographic differentiation of μ, δ and κ opioid receptors in the rat forebrain and midbrain. The Journal of Neuroscience, 7, 2445-64.

Marks-Kaufman, R. (1982) Increased fat consumption induced by morphine administration in rats. Pharmacology Biochemistry and Behavior, 16, 949-955.

Marks-Kaufman, R., & Kanarek, R. (1990) Diet selection following a chronic morphine and naloxone regimen. Pharmacology Biochemistry and Behavior, 35, 665-669.

Marks-Kaufman, R., Plager, A., & Kanarek, R. (1985) Central and peripheral contributions of endogenous opioid systems to nutrient selection in rats. Psychopharmacology, 85, 414-418.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., & Gilbert, P.E. (1976) The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-

dependent chronic spinal dog. Journal of Pharmacology and Experimental Therapeutics, 197, 517-532.

Martin, W.R., Wikler, A., Eades, C.G., & Pescor, F.T. (1963) Tolerance to and physical dependence on morphine in rats. Psychopharmacology, 4, 247-260.

Martin-Schild, S., Zadina, J.E., Gerall, A.A., Vigh, S., & Kastin, A.J. (1997) Localization of endomorphin-2-like immunoreactivity in the rat medulla and spinal cord. Peptides, 18, 1641-1649.

Mattia, A., Farmer, S.C., Takemori, A.E., Sultana, M., Portoghese, P.S., Mosberg, H.I., Bowen, W.D., & Porreca, F. (1992) Spinal opioid δ antinociception in the mouse: mediation by a 5'-NTII-sensitive δ receptor subtype. Journal of Pharmacology and Experimental Therapeutics, 260, 518-525.

Mattia, A., Vanderah, T., Mosberg, H.I., & Porreca, F. (1991) Lack of antinociceptive cross-tolerance between [D-Pen2, D-Pen5]-enkephalin and [D-Ala2]-deltorphan II in mice: evidence for δ receptor subtypes. Journal of Pharmacology and Experimental Therapeutics, 258, 583-587.

McLean, S., & Hoebel, B.G. (1983) Feeding induced by opiates injected into the paraventricular hypothalamus. Peptides, 4, 287-292.

Meredith, G.E., Pennartz, C.M., & Groenewegen, H.J. (1993) The cellular framework for chemical signalling in the nucleus accumbens. Progress in Brain Research, 99, 3-24.

Meunier, J.C., Mollereau, C., Toll, L., Suadeau, C., Moisand, C., Alvinerie, P., Butour, J.L., Guillemot, J.C., Ferrara, P., Monsarrat, B., Mazargull, H., Vassart, G., Parmentier, M., & Constentin, J. (1995) Isolation and structure of the endogenous agonist of the opioid receptor like ORL1 receptor. Nature, 377, 532-535.

Mogenson, G.J. (1987) Limbic-motor integration. Progress in Physiological Psychology, 12, 117-170.

Mogenson, G.J., Jones, D., & Yim, C.Y. (1980) From motivation to action: functional interface between the limbic system and the motor system. Progress in Neurobiology, 14, 69-97.

Mogenson, G.J., & Wu, M. (1982) Neuropharmacological and electrophysiological evidence implicating the mesolimbic dopamine system in feeding responses elicited by electrical stimulation of the medial forebrain bundle. Brain Research, 253, 243-251.

Mollereau, C., Parmentier, M., Mailleux, P., Butour, J.L., Moisand, C., Chalon, P., Caput, D., Vassart, G., & Meunnier, J.C. (1994) ORL-1, a novel member of the opioid family:

cloning, functional expression and localization. FEBS Letters, 341, 33-38.

Moore, R.Y., & Bloom, F.E. (1978) Central catecholamine neuron systems: anatomy and physiology of the dopamine systems. Annual Review of Neuroscience, 1, 129-169.

Morley, J.E., & Levine, A.S. (1983) Involvement of dynorphin and the κ opioid receptor in feeding. Peptides, 4, 797-800.

Morley, J.E., Levine, A.S., Grace, M., & Kneip, J. (1982) An investigation of the role of κ opiate receptors in the initiation of feeding. Life Sciences, 31, 2617-2626.

Morley, J.E., Levine, A.S., Yim, G.K.W., & Lowy, M.T. (1983) Opioid modulation of appetite. Neuroscience and Biobehavioral Reviews, 7, 281-305.

Mosberg, H.I., Hurst, R., Hruby, V.J.K., Galligan, J.J., Burks, T.F., Gee, K., & Yamamura, H.I. (1983) Conformationally constrained cyclic enkephalins show pronounced δ receptor selectivity. Life Sciences, 32, 2565-69.

Mosberg, H.I., Hurst, R., Hruby, V.J., Gee, K., Yamamura, H.I., Galligan, J.J., & Burks, T.F. (1983) Bis-penicillamine enkephalins possess highly improved specificity towards δ opioid receptors. Proceedings of the National Academy of Science (USA), 80, 5871-5874.

Moskowitz, A.S., & Goodman, R.R. (1985) Autoradiographic analysis of μ_1 and μ_2 and δ opioid binding in the central nervous system of C57BL6BY and CXBK (opioid receptor deficient) mice. Brain Research, 360, 108-116.

Mucha, R.F., & Iversen, S.D. (1986) Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. Brain Research, 397, 214-224.

Nakajima, S., Xinhe, L., & Lau, C.L. (1993) Synergistic interaction of D-1 and D-2 dopamine receptors in the modulation of the reinforcing effect of brain stimulation. Behavioral Neuroscience, 107, 161-165.

Negri, L., Potenza, R.L., Corsi, R., & Melchiorri, P. (1991) Evidence for two subtypes of δ receptors in rat brain. European Journal of Pharmacology, 196, 335-336.

Nencini, P., & Stewart, J. (1990) Chronic systemic administration of amphetamine increases food intake to morphine, but not to U50,488H, microinjected into the ventral tegmental area in rats. Brain Research, 527, 254-258.

Nicola, S.M., Surmeier, D.J., & Malenka, R.C. (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. Annual Review of Neuroscience, 23, 185-215.

Noel, M.B., & Gratton, A. (1995) Electrochemical evidence of increased dopamine transmission in prefrontal cortex and nucleus accumbens elicited by ventral tegmental μ receptor activation in freely behaving rats. Synapse, 21, 110-122.

Noel, M.B., & Wise, R.A. (1993) Ventral tegmental injections of morphine but not U50,488H enhance feeding in food-deprived rats. Brain Research, 632, 68-73.

Noel, M.B., & Wise, R.A. (1995) Ventral tegmental injections of a selective μ or δ opioid enhance feeding in food-deprived rats. Brain Research, 673, 304-312.

Okuda-Ashitake, E., Minami, T., Tachibana, S., Yoshihara, Y., Nishiuchi, Y., Kimura, T., & Ito, S. (1998) Nocistatin, a peptide that blocks nociceptin action in pain transmission. Nature, 392, 286-289.

Ostrowski, N.L., Rowland, N., Foley, T.L., Nelson, J.L., & Reid, L.D. (1981) Morphine antagonists and consummatory behaviors. Pharmacology Biochemistry and Behavior, 14, 549-559.

Papadouka, V., & Carr, K.D. (1994) The role of multiple opioid receptors in the maintenance of stimulation-induced feeding. Brain Research, 639, 42-48.

Paronis, C.A., Waddell, A.B., & Holtzman, S.G. (1993) Naltrexone in vivo protects μ receptors from inactivation by β-funaltrexamine, but not κ receptors from inactivation by nor-binaltorphamine. Pharmacology Biochemistry and Behavior, 46, 813-817.

Pasternak, G.W., & Hahn, E.F. (1980) Long acting opiate agonists and antagonists: 14-hydroxydiphydro-morphinone hydrazone. Journal of Medicinal Chemistry, 23, 674-677.

Pasternak, G.W., & Standifer, K.M. (1995) Mapping of opioid receptors using antisense oligodeoxynucleotides: Correlating their molecular biology and pharmacology. Trends in Pharmacological Sciences, 16, 344-350.

Pasternak, G.W., & Wood, P.L. (1986) Multiple μ opiate receptors. Life Sciences, 38, 1889-1896.

Paul, D., Bodnar, R.J., Gistrak, M.A., & Pasternak, G.W. (1989) Different μ receptor subtypes mediate spinal and supraspinal analgesia in mice. European Journal of Pharmacology, 168, 307-314.

Paul, D., Levison, J.A., Howard, D.H., Pick, C.G., Hahn, E.F., & Pasternak, G.W. (1990) Naloxone benzoylhydrazone (NalBxoH) analgesia. Journal of Pharmacology and Experimental Therapeutics, 255, 769-774.

Pentney, R.J., & Gratton, A. (1991) Effects of local δ and μ opioid receptor activation on basal and stimulated dopamine release in striatum and nucleus accumbens of rat: an in vivo electrochemical study. Neuroscience, 45, 95-102.

Pert, C.B., Kuhar, M.J., & Snyder, S.H. (1976) Opiate receptor: autoradiographic localization in rat brain. Proceedings of the National Academy of Science (USA), 73, 3729-3733.

Pert, C.B., & Snyder, S.H. (1973) Opiate receptor: demonstration in nervous tissue. Science, 179, 1011-1014.

Phillips, G.D., Howes, S.R., Whitelaw, R.B., Robbins, T.W., & Everitt, B.J. (1995) Analysis of the effects of intra-accumbens SKF38393 and LY171555 upon the behavioral satiety sequence. Psychopharmacology, 117, 82-90.

Pick, C.G., Paul, D., & Pasternak, G.W. (1991) Comparison of naloxonazine and β-funaltrexamine antagonism of μ_1 and μ_2 opioid actions. Life Sciences, 48, 2005-2011.

Pomonis, J.D., Billington, C.J., & Levine, A.S. (1996) Orphanin FQ, agonist of orphan opioid receptor ORL1, stimulates feeding in rats. Neuroreport, 8, 369-371.

Portoghese, P.S., Larson, D.L., Sayre, L.M., Fries, D.S., & Takemori, A.E. (1980) A novel opioid receptor site directed alkylating agent with irreversible narcotic antagonistic and reversible agonistic activities. Journal of Medicinal Chemistry, 23, 233-234.

Portoghese, P.S., Lipkowski, A.W., & Takemori, A.E. (1987) Binaltorphamine and nor-binaltorphamine, potent and selective K-opioid receptor antagonists. Life Sciences, 40, 1287-1292.

Portoghese, P.S., Sultana, M., & Takemori, A.E. (1988) Naltrindole, a highly selective and potent non-peptide δ opioid receptor antagonist. European Journal of Pharmacology, 146, 185-186.

Portoghese, P.S., Sultana, M., & Takemori, A.E. (1990) Naltrindole 5'-isothiocyanate: a non-equilibrium, highly selective δ opioid receptor antagonist. Journal of Medicinal Chemistry, 33, 1547-1548.

Prinssen, E.P.M., Balestra, W., Bemelmans, F.F.J., & Cools, A.R. (1994) Evidence for a role of the shell of the nucleus accumbens in oral behavior of freely moving rats. The Journal of Neuroscience, 14, 1555-1562.

Protais, P., Chagrauoui, A., Arbaoui, J., & Mocaer, E. (1994) Dopamine receptor antagonist properties of S 14506, 8-OH-DPAT, raclopride and clozapine in rodents. European Journal of Pharmacology, 271, 167-177.

Quirion, R., & Weiss, A.S. (1983) Peptide E and other proenkephalin-derived peptides are potent κ opiate receptor agonists. Peptides, 4, 445-449.

Raffa, R., Martinez, R.P., & Porreca, F. (1992) Lack of antinociceptive efficacy of intracerebroventricular [D-Ala2, Glu4]-Deltorphin, but not [D-Pen2, D-Pen5]-enkephalin, in the μ-opioid receptor deficient CXBK mouse. European Journal of Pharmacology, 216, 453-456.

Ragnauth, A, Moroz, M, Bodnar, R.J. (2000) Multiple opioid receptors mediate feeding elicited by μ and δ opioid receptor subtype agonists in the nucleus accumbens shell in rats. Brain Research 876, 76-87.

Ragnauth, A, Ruegg, H, Bodnar, R.J. (1997) Evaluation of opioid receptor subtype antagonist effects in the ventral tegmental area upon food intake under deprivation, glucoprivic and palatable conditions. Brain Research, 767, 8-16.

Ragnauth, A, Znamensky, V, Moroz, M, Bodnar, R.J. (2000) Analysis of dopamine receptor antagonism upon feeding elicited by μ and δ opioid agonists in the shell region of the nucleus accumbens. Brain Research, 877, 65-72.

Ramirez, I. (1990) Why do sugars taste good? Neuroscience and Biobehavioral Reviews, 14, 125-134.

Reinscheid, R.K., Nothacker, H.P., Bourson, A., Ardati, A., Henningsen, R.A., Bunzow, J.R., Grandy, D.K., Langen, H., Monsma, F.J., & Civilli, O. (1995) Orphanin FQ: a neuropeptide that activates an opioid-like G protein-coupled receptor. Science, 270, 792-794.

Rizvi, T.A., Ennis, M., Behbehani, M.M., & Shipley, M.T. (1991) Connections between the central nucleus of the amygdala and the midbrain periaqueductal gray: topography and reciprocity. Journal of Comparative Neurology, 303, 121-131.

Roberts, J.L., Seeburg, P.H., Shine, J., Herbert, E., Baxter, J.D., & Goodman, H.M. (1979) Corticotropin and β-endorphin: Construction of analysis of recominant DNA complementary to mRNA for the common precursor. Proceedings of the National Academy of Science (USA), 76, 2153-2157.

Robinson, T.E., & Berridge, K.C. (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Reviews, 18, 247-291.

Rockwood, G.A., & Reid, L.D. (1982) Naloxone modifies sugar-water intake in rats drinking with open gastric fistulas. Physiology and Behavior, 29, 1175-1178.

Rossi, G.C., & Pasternak, G.W. (1997) Establishing the molecular biology of opioid behavior through antisense approaches. Weiss, B. Antisense Oligodeoxynucleotides and Antisense RNA. CRC Press:Boca Raton, FL. 115-130.

Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (1994) μ and δ opioid synergy between the periaqueductal gray and the rostro-ventral medulla. Brain Research, 665, 85-93.

Ruegg, H., Yu, W.-Z., & Bodnar, R.J. (1997) Opioid receptor subtype agonist-induced enhancements of sucrose intake are dependent upon sucrose concentration. Physiology and Behavior, 62, 121-128.

Salamone, J.D. (1994) The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. Behavioral Brain Research, 61, 117-133.

Salamone, J.D., Cousins, M.S., & Snyder, B.J. (1997) Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. Neuroscience and Biobehavioral Reviews, 21, 341-359.

Salamone, J.D., Mahan, K., & Rogers, S. (1993) Ventrolateral striatal dopamine depletions impair feeding and food handling in rats. Pharmacology Biochemistry and Behavior, 44, 605-610.

Sanger, D.J., & McCarthy, P.S. (1980) Differential effects of morphine on food and water intake in food deprived and freely feeding rats. Psychopharmacology, 72, 103-106.

Sanger, D.J., & McCarthy, P.S. (1981) Increased food and water intake produced by rats by opiate receptor agonists. Psychopharmacology, 74, 217-220.

Sar, M., Stumpf, W.E., Miller, R.J., Chang, K.J., & Cuatrecasas, P. (1978) Immunohistochemical localization of enkephalin in rat brain and spinal cord. Journal of Comparative Neurology, 182, 17-37.

Sawynok, J., Pinsky, C., & LaBella, F.S. (1979) On the specificity of naloxone as an opiate antagonist. Life Sciences, 25, 1621-1632.

Schroeder, R.L., Weinger, M.B., Vakassian, L, Koob, G.F. (1991) Methylnaloxonium diffuses out of the rat brain more slowly than naloxone after direct intracerebral injection. Neurosci Letters, 121, 173-177.

Schaefer, L.A., Koch, J.E., & Bodnar, R.J. (1994) Naltrexone, dopamine receptor agonists and antagonists and food intake in rats: 2. 2-deoxy-D-glucose. Pharmacology Biochemistry and Behavior, 49, 205-211.

Schulz, R., Wilhelm, A., & Dirlich, G. (1984) Intracerebral microinjection of different antibodies against the endogenous opioids suggests α-neoendorphin participation in control of feeding behavior. Naunyn Schmiedebergs Archives of Pharmacology, 326, 222-226.

Schwartz, J.C., Diaz, J., Bordet, R., Griffon, N., & Perachon, S. (1998) Functional implications of multiple dopamine receptor subtypes: the D-1/D-3 receptor co-existence. Brain Research Reviews, 26, 236-242.

Segall, M.A., & Margules, D.L. (1989) Central mediation of naloxone-induced anorexia in the ventral tegmental area. Behavioral Neuroscience, 103, 857-864.

Seizinger, B.R., Hollt, V., & Herz, A. (1981) Evidence of the occurrence of the opioid octapeptide dynorphin (1-8) in the neurointermediate pituitary of rats. Biochemical and Biophysical Research Communications, 102, 197-205.

Self, D.W., & Stein, L. (1992) Receptor subtypes in opioid and stimulant reward. Pharmacology and Toxicology, 70, 87-94.

Sherman, T.G., Akil, H., & Watson, S.J. (1989) The molecular biology of neuropeptides. Discussions in Neurosciences, 6, 11-22.

Shetreat, M.E., Lin, L., Wong, A.C., & Rayport, S. (1996) Visualization of D-1 dopamine receptors on living nucleus accumbens neurons. Journal of Neurochemistry, 66, 1475-1482.

Shor-Posner, G., Azar, A.P., Filart, R., Tempel, D., & Leibowitz, S.F. (1986) Morphine-stimulated feeding: analysis of macronutrient selection and paraventricular nucleus lesions. Pharmacology Biochemistry and Behavior, 24, 931-939.

Shor-Posner, G., Grinker, J.A., Marinescu, C., Brown, O., & Leibowitz, S.F. (1986) Hypothalamic serotonin in the control of meal patterns and macronutrient selection. Brain Research Bulletin, 17, 663-671.

Sidhu, A., VanOene, J.C., Dandridge, P., Kaiser, C., & Kebabian, J.W. (1986) [125-I]SCH23390: the ligand of choice for identifying the D-1 dopamine receptor. European Journal of Pharmacology, 128, 213.

Sills, T.L., Baird, J.P., & Vaccarino, F.J. (1993) Individual differences in feeding effects of amphetamine: role of nucleus accumbens and circadian factors. Psychopharmacology, 112, 211-218.

Sills, T.L., & Vaccarino, F.J. (1996) Individual differences in sugar consumption following

systemic or intraccumbens administration of amphetamine in non-deprived rats. Pharmacology Biochemistry and Behavior, 54, 665-670.

Simon, E.J., & Hiller, J.M. (1994) Opioid peptides and opioid receptors. Siegel, G.J., Agranoff, B.W., Albers, R.W., & Molinoff, P.B. Basic Neurochemistry. Raven:New York. 321-339.

Simon, E.J., Hiller, J.M., & Edelman, I. (1973) Stereospecific binding of the potent narcotic analgesic (3H)etorphine to rat brain homogenate. Proceedings of the National Academy of Science (USA), 70, 1947-49.

Simone, D.A., Bodnar, R.J., Goldman, E.J., & Pasternak, G.W. (1985) Involvement of opioid receptor subtypes in rat feeding behavior. Life Sciences, 36, 829-833.

Siviy, S.M., & Reid, L.D. (1983) Endorphinergic modulation of acceptability of putative reinforcers. Appetite, 4, 249-257.

Smith, G.P., & Epstein, A.N. (1969) Increased feeding response to decreased glucose utilization in the rat and monkey. American Journal of Physiology, 217, 1083-1087.

Sofuoglu, M., Portoghese, P.S., & Takemori, A.E. (1991) Differential antagonism of δ opioid agonists by naltrindole and its benzofuran analogue (NTB) in mice: evidence for δ receptor subtypes. Journal of Pharmacology and Experimental Therapeutics, 257, 676-680.

Spanagel, A., Herz, A., & Shippenberg, T.S. (1990) The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vitro microdialysis study. Journal of Neurochemistry, 55, 1734-1740.

Spanagel, R., Almeida, O.F.X., & Shippenberg, T.S. (1994) Evidence that nor-binaltorphamine can function as an antagonist at multiple opioid receptor subtypes. European Journal of Pharmacology, 264, 157-162.

Spanagel, R., Herz, A., & Shippenberg, T.S. (1990) The effects of opioid peptides on dopamine release in the nucleus accumbens: an in vivo microdialysis study. Journal of Neurochemistry, 55, 1734-1739.

Speciale, S.G., Manaye, K.F., Sadeq, M., & German, D.C. (1993) Opioid receptors in midbrain dopaminergic regions of the rat. II. κ and δ receptor autoradiography. Journal of Neural Transmission, 91, 53-66.

Stanley, B.G., Daniel, D.R., Chin, A.S., & Leibowitz, S.F. (1985) Paraventricular nucleus injections of peptide YY and neuropeptide Y preferentially enhance carbohydrate

ingestion. Peptides, 6, 1205-1211.

Stanley, B.G., Hoebel, B.G., & Leibowitz, S.F. (1983) Neurotensin: effects of hypothalamic and intravenous injections on feeding and drinking in rats. Peptides, 4, 493-500.

Stanley, B.G., Lanthier, D., & Leibowitz, S.F. (1989) Multiple brain sites sensitive to feeding stimulation by opioid agonists: a cannula-mapping study. Pharmacology Biochemistry and Behavior, 31, 825-832.

Stanley, B.G., & Leibowitz, S.F. (1984) Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sciences, 35, 2635-2642.
Stein, J.A., Znamensky, V., Baumer, F., Rossi, G.C., Pasternak, G.W., & Bodnar, R.J. (2000) Mercaptoacetate induces feeding through central opioid-mediated mechanisms in rats. Brain Research, in press, .

Stratford, T.R., Holahan, M.R., & Kelley, A.E. (1997) Injections of nociceptin into nucleus accumbens shell or ventromedial hypothalamic nucleus increase food intake. Neuroreport, 8, 423-426.

Stratford, T.R., & Kelley, A.E. (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. The Journal of Neuroscience, 17, 4434-4440.

Stratford, T.R., & Kelley, A.E. (1999) Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. The Journal of Neuroscience, 19, 11040-11048.

Stratford, T.R., Swanson, C.J., & Kelley, A.E. (1998) Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. Behavioral Brain Research, 93, 43-50.

Stefano, G.B., & Salzet, M. (1999) Invertebrate opioid precursors: evolutionary conservation and the significance of enzymatic processing. International Reviews in Cytology 187, 261-86.

Suda, T., Tozawa, F., Tachibana, S., Demura, S., & Shizume, K. (1982) Multiple forms of immunoreactive dynorphin in rat pituitary and brain. Life Sciences, 31, 51-57.

Surmeier, D.J., Song, W.J., & Yan, Z. (1996) Coordinated expression of dopamine receptors in neostriatal medium spiny neurons. The Journal of Neuroscience, 16, 6579-6591.

Svingos, A.L., Clarke, C.L., & Pickel, V.M. (1998) Cellular sites for activation of δ-

opioid receptors in the rat nucleus accumbens shell: relationship with met5-enkephalin. The Journal of Neuroscience, 18, 1923-1933.

Svingos, A.L., Clarke, C.L., & Pickel, V.M. (1999) Localization of the δ-opioid receptor and dopamine transporter in the nucleus accumbens shell: implications for opiate and psychostimulant cross-sensitization. Synapse, 34, 1-10.

Svingos, A.L., Moriwaki, A., Wang, J.B., Uhl, G.R., & Pickel, V.M. (1996) Ultrastructural immunocytochemical localization of μ-opioid receptors in rat nucleus accumbens: extrasynaptic plasmalemmal distribution and association with Leu5-enkephalin. The Journal of Neuroscience, 16, 4162-4173.

Swanson, C.J., Heath, S., Stratford, T.R., & Kelley, A.E. (1997) Differential behavioral responses to dopaminergic stimulation of nucleus accumbens subregions in the rat. Pharmacology Biochemistry and Behavior, 58, 933-945.

Swanson, L.W. (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Research Bulletin, 9, 321-353.

Takemori, A.E., Larson, D.L., & Portoghese, P.S. (1981) The irreversible narcotic antagonist and reversible agonistic properties of the fumarate methyl ester derivative of naltrexone. European Journal of Pharmacology, 70, 445-451.

Tempel, A., & Zukin, R.S. (1987) Neuroanatomical patterns of μ, δ and κ opioid receptors of rat brain as determined by quantitative in vitro autoradiography. Proceedings of the National Academy of Science (USA), 84, 4308-4312.

Tempel, D.L., Leibowitz, K.J., & Leibowitz, S.F. (1988) Effects of PVN galanin on macronutrient selection. Peptides, 9, 309-314.

Tempel, D.L., & Leibowitz, S.F. (1989) PVN steroid implants: effect on feeding patterns and macronutrient selection. Brain Research Bulletin, 23, 553-560.

Tempel, D.L., McEwen, B.S., & Leibowitz, S.F. (1992) Effects of adrenal steroid agonists on food intake and macronutrient selection. Physiology and Behavior, 52, 1161-1166.

Tepperman, F.S., & Hirst, M. (1982) Concerning the specificity of the hypothalamic opiate receptor responsible for food intake in the rat. Pharmacology Biochemistry and Behavior, 17, 1141-1144.

Tepperman, F.S., & Hirst, M. (1983) Effects of intrahypothalamic injection of D-Ala-2,D-

Leu-5-enkephalin on feeding and temperature in the rat. European Journal of Pharmacology, 96, 243-249.

Tepperman, F.S., Hirst, M., & Gowdey, C.W. (1981) Hypothalamic injection of morphine: feeding and temperature responses. Life Sciences, 28, 2459-2467.

Terenius, L. (1973) Stereospecific interaction between narcotic analgesia and a synaptic plasma membrane fraction of rat cerebral cortex. acta pharmacologica et toxicilogica, 32, 317-320.

Thornhill, J.A., Hirst, M., & Gowdey, C.W. (1976) Disruption of diurnal feeding patterns of rats by heroin. Pharmacology Biochemistry and Behavior, 4, 129-135.

Uhl, G.R., Childers, S.R., & Pasternak, G.W. (1994) An opiate receptor gene family reunion. Trends in Neuroscience, 17, 89-93.

Ukai, M., & Holtzman, S.G. (1987) Suppression of deprivation-induced water intake by opioid antagonists: central sites of action. Psychopharmacology, 91, 279-284.

Ukai, M., & Holtzman, S.G. (1988) Effects of β-funaltrexamine on ingestive behaviors in the rat. European Journal of Pharmacology, 153, 161-165.

Unterwald, E.M., & Cuntapay, M. (2000) Dopamine-opioid interactions in the rat striatum: a modulatory role for dopamine D_1 receptors in δ opioid receptor-mediated signal transduction. Neuropharmacology, 39, 372-381.

Usada, I., Tanaka, K., & Chiba, T. (1998) Efferent projections of the nucleus accumbens in the rat with special reference to subdivisions of the nucleus - biotinylate dextran amine study. Brain Research, 797, 73-93.

Van Bockstaele, E.J., Gracy, K.N., & Pickel, V.M. (1995) Dynorphin-immunoreactive neurons in the rat nucleus accumbens: ultrastructure and synaptic input from terminals containing substance P and/or dynorphin. Journal of Comparative Neurology, 351, 117-133.

Van Bockstaele, E.J., Sesack, S.R., & Pickel, V.M. (1994) Dynorphin-immunoreactive terminals in the rat nucleus accumbens: cellular sites for modulation of target neurons and interactions with catecholamine afferents. Journal of Comparative Neurology, 341, 1-15.

VanVoigtlander, P.F., Lahti, R.A., & Ludens, J.H. (1983) U50,488H: a selective and structurally novel non-μ (κ) opioid agonist. Journal of Pharmacology and Experimental Therapeutics, 224, 7-11.

Vaupel, B. (1983) Naloxone fails to antagonize the δ effects of PCP and SKF 10,047 in the dog. European Journal of Pharmacology, 92, 269-274.

Voorn, P., Gerfen, C.R., & Groenewegen, H.J. (1989) The compartmental organization of the ventral striatum of the rat: immunohistochemical distribution of enkephalin, substance P, dopamine, and calcium binding protein. Journal of Comparative Neurology, 289, 189-201.

Voorn, P., Jorritsma-Byham, B., Van Dijk, C., & Buijs, R.M. (1986) The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscope study with antibodies against dopamine. Journal of Comparative Neurology, 251, 84-99.

Walters, J.R., Bergstrom, D.A., Carlson, J.H., Chase, T.N., & Braun, A.R. (1987) D-1 dopamine receptor activation required for postsynaptic expression of D-2 agonist effects. Science, 236, 719-722.

Ward, S.J., Portoghese, P.S., & Takemori, A.E. (1982) Pharmacological profiles of β-funaltrexamine (β-FNA) and β-chlornaltrexamine (B-CNA) on the mouse vas deferens preparation. European Journal of Pharmacology, 80, 377-384.

Watson, S.J., Akil, H., Fischili, W., Goldstein, A., Zimmerman, E.A., & Nilaver, G. (1982) Dynorphin and vasopressin: Common localization and expression in magnocellular neurons. Science, 216, 85-87.

Watson, S.J., Akil, H., Richard, C.W., & Barchas, J.D. (1978) Evidence for two separate opiate peptide neuronal systems and the coexistence of β-lipotropin, β-endorphin and ACTH immunoreactivities in the same hypothalamic neurons. Nature, 275, 226-228.

Weiss, G.F., Papadakos, P., Knudson, K., & Leibowitz, S.F. (1986) Medial hypothalamic serotonin: effects on deprivation and norepinephrine-induced eating. Pharmacology Biochemistry and Behavior, 25, 1223-1230.

Welch, C.C., Kim, E.-M., Grace, M.K., Billington, C.J., & Levine, A.S. (1996) Palatability-induced hyperphagia increases hypothalamic dynorphin peptide and mRNA levels. Brain Research, 721, 126-131.

White, F.J., Bednarz, L.M., Wachtel, S.R., Hjorth, N., & Brooderson, R.J. (1988) Is stimulation of both D-1 and D-2 receptors necessary for the expression of dopamine-mediated behaviors? Pharmacology Biochemistry and Behavior, 30, 189-193.

Wise, R.A. (1982) Neuroleptics and operant behavior: the anhedonia hypothesis. Behavioral and Brain Sciences, 5, 39-87.

Wise, R.A., & Bozarth, M.A. (1987) A psychomotor stimulant theory of addiction.

Psychological Review, 94, 469-492.

Wise, R.A., & Hoffman, D.C. (1992) Localization of drug reward mechanisms by intracranial injections. Synapse, 10, 247-263.

Wise, R.A., & Rompre, P.P. (1989) Brain dopamine and reward. Annual Reviews of Psychology, 40, 191-225.

Woods, J.S., & Leibowitz, S.F. (1985) Hypothalamic sites sensitive to morphine and naloxone: effects on feeding behavior. Pharmacology Biochemistry and Behavior, 23, 431-438.

Yasuda, K., Raynor, K., Kong, H., Breder, C., Takeda, J., Reisine, T., & Bell, G. (1993) Cloning and functional comparison of κ and δ opioid receptors from mouse brain. Proceedings of the National Academy of Science (USA), 90, 6736-6740.

Yim, C.Y., & Mogenson, G.J. (1980) Electrophysiological studies of neurons in the ventral tegmental area of Tsai. Brain Research, 181, 301-313.

Yoshida, Y., Koide, S., Hirose, N., Takada, K., Tomiyama, K., Koshikawa, N., & Cools, A.R. (1999) Fentanyl increases dopamine release in rat nucleus accumbens: involvement of μ- and δ_2 opioid receptors. Neuroscience, 92, 1357-1365.

Yu, W.-Z., Ruegg, H., & Bodnar, R.J. (1997) δ and κ opioid receptor subtypes and ingestion: antagonist and glucoprivic effects. Pharmacology Biochemistry and Behavior, 56, 353-361.

Zaborszky, L., Alheid, G.F., Bienfeld, M.C., Eiden, L.E., Heimer, L., & Palkovits, M. (1985) Cholecystokinin innervation of the ventral striatum: a morphological and immunological study. Neuroscience, 14, 427-453.

Zadina, J.E., Hackler, L., Ge, L.-J., & Kastin, A.J. (1997) A potent and selective endogenous agonist for the μ-opiate receptor. Nature, 386, 499-502.

Zagon, I.S., Sassani, J.W., Allison, G., & McLaughlin, P.J., (1995) Conserved expression of the opioid growth factor, [Met^5]enkephalin, and the zeta (ζ) opioid receptor in vertebrate cornea. Brain Research 671, 105-11.

Zahm, D.S. (1998) Is the Caudomedial Shell of the Nucleus Accumbens part of the Extended Amygdala? A Consideration of Connections. Critical Reviews in Neurobiology, 12, 245-265.

Zahm, D.S., & Brog, J.S. (1992) On the significance of subterritories in the "accumbens"

part of the rat ventral striatum. Neuroscience, 50, 751-767.

Zahm, D.S., & Heimer, L. (1990) Two transpallidal pathways originating in rat nucleus accumbens. Journal of Comparative Neurology, 302, 437-446.

Zahm, D.S., & Heimer, L. (1992) The efferent projections of the rostral pole of the nucleus accumbens in the rat: Comparison with the core and shell projection patterns. Journal of Comparative Neurology, 327, 220-232.

Zamir, N., Palkovits, M., & Brownstein, M.J. (1983) Distribution of immunoreactive dynorphin in the central nervous system of the rat. Brain Research, 280, 81-93.

Zamir, N., Palkovits, M., & Brownstein, M.J. (1984) Distribution of immunoreactive dynorphin A1-8 in discrete nuclei of the rat brain: comparison with dynorphin A. Brain Research, 307, 61-68.

Zhang, M., Gosnell, B.A., & Kelley, A.E. (1998) Intake of high-fat food is selectively enhanced by μ opioid receptor stimulation within the nucleus accumbens. Journal of Pharmacology and Experimental Therapeutics, 285, 908-914.

Zhang, M., & Kelley, A.E. (1997) Opiate agonists microinjected into the nucleus accumbens enhance sucrose drinking in rats. Psychopharmacology, 132, 350-360.

Znamensky, V., Echo, J., La Monte, N., Christian, G., Ragnauth, A., & Bodnar, R.J. (2000) Interactions between GABA receptors and opioid receptors in feeding elicited from the nucleus accumbens in rats. Society for Neuroscience Abstracts, 26, 992. submitted.

Zukin, R.S., Eghbalai, M., Olive, D., Unterwald, E.M., & Tempel, A. (1988) Characterization and visualization of rat and guinea pig brain K opioid receptors: evidence for K-1 and K-2 opioid receptors. Proceedings of the National Academy of Science (USA), 85, 4061-4065.

Zukin, R.S., & Zukin, S.R. (1981) Multiple opiate receptors: emerging concepts. Life Sciences, 29, 2681-2690.