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**The Role of Excitatory and Inhibitory Neurotransmitters Upon Opioid-Induced
Feeding in the Nucleus Accumbens Shell and Ventral Tegmental Area in Rats.**

by

Joyce A. Echo

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

2002

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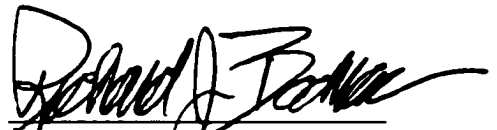
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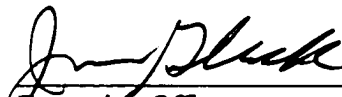
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This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the Dissertation requirements for the degree of Doctor of Philosophy.

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Abstract**THE ROLE OF EXCITATORY AND INHIBITORY NEUROTRANSMITTERS UPON
OPIOID-INDUCED FEEDING IN THE NUCLEUS ACCUMBENS SHELL AND
VENTRAL TEGMENTAL AREA**

by

Joyce A. Echo

Advisor: Dr. Richard J. Bodnar

The reciprocal connections between the Nacc shell and ventral tegmental area are implicated in reward processes related to ingestive behavior. The Nacc shell and VTA support μ agonist induced feeding. The present study examined whether excitatory amino acid (EAA) NMDA and AMPA receptor subtypes, as well as GABA_A and GABA_B receptor subtypes are involved in opioid-mediated food intake in the Nacc shell and VTA. Pretreatment in the Nacc shell with the GABA_A-selective antagonist, bicuculline, potentiated feeding induced by the μ -selective agonist, DAMGO without altering baseline intake; this enhancement in feeding was reduced by pretreatment with general (naltrexone) or μ -selective (β -FNA) opioid antagonists. In contrast, pretreatment in the Nacc shell with the GABA_B-selective antagonist, saclofen, reduced DAMGO-induced feeding without altering baseline intake. On the other hand, pretreatment in the VTA with either bicuculline or saclofen reduced DAMGO-induced feeding that could not be accounted for

by changes in baseline intake. The GABA_B-selective agonist, baclofen, increased food intake when administered into the Nacc shell or the VTA; this effect was reduced by saclofen, but not bicuculline. Pretreatment with naltrexone reduced feeding elicited by baclofen in the VTA, but not in the Nacc shell. The significant feeding response produced by the GABA_A agonist, muscimol, in the Nacc shell was reduced by bicuculline or naltrexone, but not by saclofen pretreatment. However, muscimol failed to produce feeding when administered into the VTA. The EAA agonists, AMPA or NMDA administered into the Nacc shell each increased food intake, effects that were blocked by naltrexone pretreatment. DAMGO-induced feeding elicited from the Nacc shell was significantly enhanced by AMPA co-treatment, which in turn was blocked by pretreatment with either naltrexone or β -FNA. In contrast, co-treatment with NMDA and DAMGO in the Nacc shell reduced food intake. These data demonstrate receptor selective actions of the GABA and EAA receptor subtypes, and the site-specificity of these receptor subtypes upon μ opioid agonist-induced feeding in the Nacc shell and VTA. Further, the EAA and GABA receptor subtypes thereby modulate the opioid mediation of the reward value of food.

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Glossary and Definitions of Abbreviated Terms

Brain Sites

Nacc= nucleus accumbens
VTA= ventral tegmental area
LH=lateral hypothalamus
NTS= nucleus tractis solitarius
PAG= periaqueductal gray
CNA= central nucleus of the amygdala
SNC= substantia nigra compacta
SNR= substantia nigra reticulata
PVN= paraventricular nucleus of the hypothalamus
VMH= ventromedial hypothalamus
VP= ventral pallidum
MFB= medial forebrain bundle
PFC= prefrontal cortex
i.c.v.= intracerebroventricular

Opioid Peptides

POMC=proopiomelanocortin, endogenous opioid peptide
OFQ/N= orphanin/nociceptin, endogenous opioid peptide

Opioid Receptors and Selective Agonists and Antagonists

μ= mu receptor
δ= delta receptor
κ= kappa receptor
DAMGO= [D-Ala², NMe⁴, Gly-ol⁵]-enkephalin, μ-selective opioid agonist
DPDPE= D-Pen², D-Pen⁵-enkephalin; delta₁ agonist
Deltorphin= D-Ala², Glu⁴-deltorphin, delta₂ agonist
Dynorphin= kappa agonist
β-FNA= beta-funaltrexamine; μ-selective antagonist
CTOP= Cys²-Tyr³-Orn⁵-Pen⁷, μ-selective antagonist
DALCE= D-Ala², Leu⁵, Cys⁶-enkephalin, delta₁ antagonist
NTII= naltrindole-5' -isothiocyanate, delta₂ selective antagonist

Nor-BNI= nor-binaltorphamine, kappa antagonist
Naloxone= general opioid antagonist
Naltrindole = general δ antagonist
Naloxonazine= μ_1 -selective antagonist
M6G= morphine-6 β -glucuronide, morphine metabolite
NalBzOH= naloxone benzoylh-drazone, κ_3 receptor agonist
NTX= naltrexone, general opioid antagonist
U50, 488H= trans-(+)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclo-hexyl]-benzeneacetamide methanesulfonate hydrate, kappa₁ agonist
DALA= D-Ala², Met⁵ -enkephalinamide, met-enkephalin analogue
DSLET= [³H] D-Ala², D-Leu⁵ -enkephalin, general delta agonist
DADLE= [³H] D Ser², Leu⁵, Thr⁶ -enkephalin, general delta agonist

Opioid Receptor Clones

MOR= μ opioid receptor clone
DOR= δ opioid receptor clone
KOR= κ opioid receptor clone
ORL-1= orphan opioid receptor clone

Excitatory Amino Acid Receptors and Selective Agonists and Antagonists

EAA= excitatory amino acids
NMDA= -methyl-D-aspartate, ionotropic glutamate receptor subtype
AMPA= D,L- α -amino-3-hydroxy-5-methylisoxazole, non-NMDA ionotropic glutamate receptor subtype
KA= kainic acid, non-NMDA ionotropic glutamate receptor subtype
DNQX= 6,7-dinitroquinoxaline-2,3-dione, selective AMPA/kainate antagonist
CNQX= 6-cyano-7-nitroquinoxaline, selective AMPA/kainate antagonist
NBQX= 2,3-dihydroxy-6-nitro-7-sulfamoylbenzo-(F) quinoxaline, selective AMPA/kainate antagonist
D-AP5= D-(-)-2-amino-5-phosphonopentanoic acid, selective NMDA antagonist
AP-7= D-(-)-2-amino-7-phosphonoheptanoic acid, selective NMDA antagonist
APV= 2-amino-5-phosphonovaleric acid, selective NMDA antagonist
MK-801= (5*R*,10*S*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a, d'*]cyclo-hepten-5,10-imine, non-competitive NMDA antagonist

GABA Receptor Subtypes and Selective Agonists and Antagonists

GABA= γ -aminobutyric acid, major inhibitory amino acid neurotransmitter

Muscimol= (5-Aminomethyl-3-hydroxyisoxazole, GABA_A receptor subtype selective agonist

Baclofen= ($\pm$)- β -(Aminomethyl)-4-chlorobenzenepropanoic acid, GABA_B receptor subtype selective agonist

Bicuculline= (-)-Bicuculline methiodide, GABA_A receptor subtype selective antagonist

Picrotoxin= GABA_A receptor subtype antagonists

Saclofen= β -(Aminomethyl)-4-chloro-benzeneethanesulfonic acid, GABA_B receptor subtype selective antagonist

GVG= γ -vinyl-GABA, irreversible GABA-transaminase inhibitor

Dopamine receptor subtypes and antagonists

DA= dopamine

D₁, D₂= dopamine receptor subtypes

SCH23390= D₁ receptor antagonist

SKF-83566= D₁ Receptor antagonist

Raclopride= D₂ receptor antagonist

Sulpiride= D₂ receptor antagonist

Fluphenazine= fluphenazine HCL, dopamine antagonist

Other neurotransmitters, peptides

NPY= neuropeptide Y

5-HT= 5-hydroxytryptamine, serotonin

ACh= acetylcholine

Dosages

ng= nanogram

μ g= microgram

nmoles= nanomoles

Reward-related Terms

CPP= conditioned place preference

ICSS= intracranial self-stimulation

ICSA= intracranial self-administration

BSR= brain stimulation reward

Other Terms

2DG= 2-deoxy-D-glucose, antimetabolic agent

6-OH-DA= 6-hydroxydopamine, neurotoxin that selectively degenerates DA neurons

HVA= homovanillic acid, metabolite of dopamine

I. 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. The endogenous opioid system has been identified, primarily through pharmacological studies, to play a role in the elicitation of ingestive behavior under a variety of environmental and homeostatic conditions. General opioid antagonists typically reduce food intake under a wide range of ingestive situations, including spontaneous intake, as well as intake under conditions of food deprivation, glucoprivation, and exposure to palatable substances (see review: Bodnar, 1996). Moreover, general opioid agonists stimulate food intake under a variety of situations (i.e. see reviews: Morley et al., 1983; Levine et al., 1985; Cooper et al., 1988; Gosnell and Levine, 1996a). 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, have been implicated in feeding behavior since selective opioid receptor subtype agonists each increase food intake (Uhl et al., 1994; Mollereau et al., 1994; Bunzow et al., 1994; Pan et al., 1995; Gong et al., 1998; Leventhal et al., 1997; Leventhal et al., 1998a).

The neural sites at which opioid-induced activation of ingestive behavior occurs have also been identified. These include limbic, homeostatic and gustatory nuclei, including the hypothalamic paraventricular and ventromedial nuclei, the amygdala, the

nucleus tractus solitarius, and the parabrachial region (Tepperman and Hirst, 1982; Stanley et al., 1989; Gosnell, 1988; Carr et al., 1991; Kotz et al, 1997; Giraudo et al., 1998a; 1998b) . The reciprocal connections between the ventral tegmental area (VTA) and nucleus accumbens (Nacc) shell have been implicated in reward and reinforcement processes in general (Alheid and Heimer, 1988; Evans and Vaccarino, 1990), and in ingestive behavior in particular (Stanley et al., 1989; Bakshi and Kelley, 1993a). A distinction can be made between the Nacc shell and the Nacc core based on morphological characteristics and afferent and efferent connections. Whereas the Nacc core is primarily involved in motor processes, the Nacc shell is implicated in ingestive behavior and is thus the focus of the current study (see review: Kelley, 1999a). The involvement of the ventral tegmental area and the Nacc shell in the opioid modulation of ingestive behavior has been demonstrated by their ability to elicit feeding responses following opioid agonist microinjections (Stanley et al., 1989; Cador et al., 1986; Mucha and Iverson, 1986; Noel and Wise, 1995; Majeed et al., 1986; Bakshi and Kelley, 1993a; Stratford et al., 1997).

Indeed, opioid agonist-induced feeding elicited from the Nacc shell is dependent upon local opioid and dopaminergic receptors for their full expression. Recent studies have elucidated the differential roles in ingestive behavior of the opioid receptor subtypes in the Nacc shell. Ragnauth et al (2000a) showed that feeding elicited by μ or δ agonists in the Nacc shell are dependent on multiple opioid receptors. This was demonstrated by the ability of μ , δ_1 , δ_2 , and κ_1 receptor antagonists to significantly reduce feeding elicited by the μ opioid agonist, DAMGO, in the Nacc shell. A similar pattern of antagonist

effects was shown for δ_1 (DPDPE) opioid agonist-induced feeding. In contrast to the efficacy of opioid antagonists to reduce δ_1 opioid agonist feeding, these selective opioid antagonists failed to significantly reduce δ_2 (deltorphan) opioid agonist-induced feeding. A recent study has also indicated the role of dopamine in the opioid-mediation of ingestive behavior in the Nacc shell (Ragnauth et al., 2000b). This study demonstrated the differential actions of D_1 and D_2 receptors upon μ (DAMGO) and δ_2 opioid-induced feeding elicited from the Nacc shell, such that the selective D_1 antagonist SCH23390 but not the D_2 antagonist, raclopride, significantly reduced DAMGO-induced feeding elicited from the Nacc shell. Further, D_1 and D_2 antagonists produced mild and inconsistent effects upon deltorphan-induced feeding elicited from the Nacc shell. The results from this study suggest that δ_2 opioid agonist feeding elicited from the Nacc shell is not dependent on the dopamine system, whereas μ opioid agonist feeding in the Nacc shell is dependent in part upon D_1 receptors. Previous studies (Bakshi and Kelley, 1993a, 1993b; Bodnar et al., 1995; Kelley et al., 1996; Ragnauth et al., 2000a; Ragnauth et al., 2000b) have indicated that the μ receptor in particular plays a more critical role in ingestive behavior in the Nacc as compared to the δ and κ opioid receptor subtypes. This is based on evidence of the greater consistency and magnitude of effects of μ agonists and antagonists.

The goal of this dissertation is to elucidate the role of other important neurochemical systems that are involved in the full expression of opioid-mediated ingestive behavior in the Nacc shell and in the VTA. The excitatory amino acid NMDA and AMPA receptor subtypes, as well as the $GABA_A$ and $GABA_B$ receptor subtypes are of particular

interest. NMDA receptors have been localized in the shell compartment of the Nacc (Gracy and Pickel, 1996; Gracy et al., 1997) and intense feeding responses occur following microinjections of DNQX, an AMPA/kainate receptor antagonist into this nucleus (Maldonado-Irizarry et al., 1995). Therefore, if excitatory amino acid (EAA) antagonists induce feeding, it would be expected that (EAA) agonists would produce the opposite effect. Thus, they should reduce feeding when administered into the Nacc shell. However, the ingestive actions of EAA agonists and antagonists are dependent upon the site and duration of action. Stanley et al (1993a; 1993b) has demonstrated that lateral hypothalamic infusions of either glutamate, kainic acid, AMPA or NMDA stimulate feeding over 1 h with adjacent hypothalamic, thalamic and amygdaloid sites displaying minimal effects. Therefore, an alternative hypothesis suggests that increases in food intake may occur following EAA administration in the Nacc shell. GABA receptors have also been localized in the Nacc shell (Meredith et al., 1993), and GABA receptor agonists have been shown to elicit feeding following microinjection into this nucleus (Stratford and Kelley, 1997). The ingestive effects of GABA agonists are receptor-selective since GABA_A agonist (muscimol)-induced feeding was blocked by pretreatment with GABA_A (bicuculline) but not GABA_B (saclofen) antagonists in the Nacc shell, and GABA_B (baclofen)-induced feeding was blocked by pretreatment with GABA_B but not GABA_A antagonists (Stratford and Kelley, 1997). A series of reciprocal inputs between the Nacc shell and the VTA have been identified, and autoradiographic studies have demonstrated the localization of GABA receptors in the VTA. Furthermore, GABA neurons in the VTA

project to the Nacc (Churchhill et al., 1992; Van Bockstaele and Pickel, 1995).

This dissertation attempted to elucidate the role of the excitatory amino acid receptor subtypes, NMDA and AMPA, and GABA receptor subtypes in the μ opioid-mediation of ingestive behavior in the Nacc shell. In addition, given that there are reciprocal GABAergic connections between the Nacc shell and the VTA (Churchhill et al., 1992; Van Bockstaele and Pickel, 1995), the effects of GABA receptor subtypes upon μ opioid-mediated ingestive behavior in the VTA were also explored. The following section presents an overview of the specific aims of this dissertation as well as hypotheses, which will be explored further in the *Rationale* section.

The **first aim** of this dissertation examined whether opioid agonist-induced feeding is dependent upon GABA synapses in the Nacc shell. Since food intake is stimulated by either GABA or opioid receptor subtype agonists administered into the Nacc shell (Bakshi and Kelley, 1993a, 1993b; Bodnar et al., 1995; Kelley et al., 1996; Stratford and Kelley, 1997; Stratford and Kelley, 1999), it was hypothesized that GABA receptor antagonists should reduce DAMGO-induced feeding. It was further hypothesized that GABA agonist-induced feeding is dependent upon opioid synapses, and therefore it was expected that opioid receptor antagonism would reduce GABA agonist-induced feeding elicited from the Nacc shell by the respective GABA_A and GABA_B agonists, muscimol and baclofen. Thus, pretreatment with the general opioid antagonist naltrexone would most likely reduce muscimol or baclofen-induced feeding. This aim also confirmed the ability of GABA receptor subtypes to elicit feeding following administration in the Nacc shell, as well as the

selective blockade of feeding by the GABA_A and GABA_B antagonists, bicuculline and saclofen (Stratford and Kelley, 1997).

Given that potent feeding responses occur following microinjections of DNQX, an AMPA/kainate receptor antagonist into the the Nacc shell (Maldonado-Irizarry et al., 1995), the **second aim** of this dissertation tested the hypothesis that pretreatment with either AMPA or NMDA would reduce μ opioid-induced feeding in the Nacc shell. In addition, it was also explored whether AMPA or NMDA alone would alter spontaneous food intake. Again, as a result of the work of Kelley and co-workers (Maldonado-Irizarry et al., 1995) which showed that EAA antagonists increase feeding in the Nacc shell, it was hypothesized that EAA agonists, NMDA and AMPA, alone would not increase spontaneous food intake in the Nacc shell, but would reduce DAMGO-induced feeding. An alternative hypothesis is based upon the stimulatory actions of EAA agonists to induce food intake elicited from the hypothalamus, and predicted that these agonists alone would stimulate spontaneous intake when administered into the Nacc shell. Furthermore, based on this alternative hypothesis, co-treatment of NMDA or AMPA with DAMGO in the Nacc shell would potentiate DAMGO-induced feeding.

Given that the VTA is proposed as a major component of the afferent and efferent circuitry of the Nacc shell, and given the presence of GABA-opioid connectivity in the VTA (Kalivas et al., 1993), the **third aim** examined whether feeding elicited by the μ opioid agonist DAMGO in the VTA would be altered by pretreatment with GABA antagonists administered into the VTA. This aim elucidated whether the GABA antagonist

effects upon μ opioid agonist-induced feeding are site-specific. Given the strong GABAergic connections between the VTA and Nacc shell, it was hypothesized that similar effects would be found in each nucleus; such that GABA antagonists would reduce DAMGO-induced feeding. In addition, it was also explored whether the GABA agonists muscimol- and baclofen-induced feeding when administered in the VTA. It was hypothesized that the administration of GABA agonists into the VTA would increase food intake confirming previous studies (Wirtshafter et al., 1993; Klitenick and Wirtshafter, 1988; Arnt and Scheel-Kruger, 1979). Furthermore, it was hypothesized that GABA agonist-induced feeding elicited from the VTA would be reduced by pretreatment with general opioid antagonist based upon evidence of GABA-opioid connections within the VTA (Kalivas et al., 1993).

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 genes, *IV.* Systemic and ventricular studies of 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, *VIII.* Anatomy and projections of the VTA, *IX.* Amino acid neurotransmitters, and *X.* a rationale for the present experiments.

II. INTRODUCTION

A. Opioid peptides

For centuries extracts from the poppy seed, opium, have been used as analgesics as well as to induce a “dream-like” state of euphoria. Morphine, named after the Greek god of dreams, Morpheus, was identified 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 preparation in the dog, 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), ketocyclazocine (κ) and SKF 10047 (σ). This was soon followed by the description of another (δ : deferens) opioid receptor subtype using the mouse vas deferens bioassay, while the μ receptor was further characterized in the guinea pig ileum assay in which morphine was more potent than the enkephalins (Lord et al., 1977). In 1975, the identification of met- and leu-enkephalin as endogenous opioid pentapeptides (Hughes et al., 1975) led to 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-orphanin/pro-nociceptin and e) pre-pro-endomorphin. 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 et al., 1989; Mansour et al., 1995; Meunier et al., 1995; Reinscheid et al., 1995; Zadina et al., 1997). These opioid peptides are briefly reviewed in the following section (see Table 1).

1. POMC. The C-terminus of POMC contains the 31-amino acid peptide β -endorphin, and its 91 amino acid precursor β -lipotropin (Eipper and Mains, 1978; Mains et al., 1977; Roberts et al., 1979). 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). 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).

2. Pro-enkephalin. Proenkephalin 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. (Hokfelt et al., 1977; Khachaturian et al., 1983, 1985; Sar et al., 1978).

3. Pro-dynorphin. Prodynorphin 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). Immunoreactive dynorphin perikarya are located in all major areas

of the brain as well as the dorsal and ventral horns of the spinal cord.

4. Pro-orphanin/Pro-nociceptin. Orphanin (OFQ)/nociceptin is a recently discovered (Meunier et al., 1995; Reinscheid et al., 1995) heptadecapeptide which is structurally similar to dynorphin A. It was first thought to merely have hyperalgesic properties (Meunier et al., 1995), however others have shown its ability to produce spinal and supraspinal analgesia (Rossi et al., 1998; Shane et al., 2001; Zhu et al., 1999). Others have even shown anti-opiate properties of OFQ/nociceptin (Kest et al., 2001). Unlike traditional opioid peptides, it binds with very low affinity to classical opioid receptor subtypes. However, studies have shown that the opiate antagonist naloxone blocks OFQ/nociceptin analgesia (Rossi et al., 1998). Immunohistochemical and autoradiographic studies have identified OFQ/nociceptin in the bed nucleus of the stria terminalis, medial pre-optic area, lateral septum, amygdala and median eminence (Shane et al., 2001; Olszewski et al., 2000; Letchworth et al., 2000).

5. Pre-pro-endomorphins. In 1997 Endomorphin-1 and Endomorphin-2 were isolated from the brain and identified as the endogenous ligands for the μ opioid receptor (Zadina et al., 1997). The N-terminal sequence of the endomorphins differs from the classical opioid peptides as follows: endomorphin-1 (Try-Pro-Trp-Phe-NH₂) and endomorphin-2 (Try-Pro-Phe-Phe-NH₂). 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.,

Table 1. Endogenous Opioid Peptides and Gene Precursor Molecules

<u>5 Gene Precursor Molecules</u>	<u>Peptides</u>	<u>Brain Sites</u>
1. POMC	β -endorphin	1. Arcuate nucleus and periarculate nucleus of hypothalamus 2. NTS
2. Pro-Enkephalin	leu-enkephalin, met-enkephalin	throughout CNS
3. Pro-dynorphin	α and β -neoendorphin, dynorphin A and B	throughout CNS
4. Pro-orphanin/pro-nociceptin	Orphanin	bed nucleus of stria terminalis, medial preoptic area, lateral septum, amygdala, median eminence
5. Pre-pro-endorphin	Endomorphin 1 Endomorphin 2	thalamus, hypothalamus, cortex, striatum medulla, spinal cord

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.

B. Opioid receptors and selective agonists and antagonists

Although both endogenous opioid peptides and receptors have been localized, there is relatively poor anatomical correlation between them (see review: Akil et al., 1984). Further, there appears to be cross-reactivity between opioid peptides and receptors in binding assays (Meucci et al., 1989; Russell et al., 1987). β -endorphin selectively binds to 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 prodynorphin peptides can bind to μ , κ and δ opioid receptors depending on the peptide product and species (Corbett et al., 1982; Quirion et al., 1983). Endomorphin-1 and endomorphin-2 have a high affinity and selectivity for the μ receptor, and it has been suggested that they are the 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 with emphasis on the μ receptor since this receptor subtype is the focus of the current study (see Table 2).

1. 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, VTA,

NTS, spinal trigeminal nucleus and dorsal horn. Moderate binding is observed in the PAG and raphe nuclei (Mansour et al., 1988). The μ receptor has been characterized pharmacologically using the μ -selective agonist (i.e., D-Ala², Met-Phe⁴, Gly(ol)⁵-enkephalin, DAMGO: Handa et al., 1981) and antagonists (i.e, β -funaltrexamine, β -FNA: Portoghesi et al., 1980; Takemori et al., 1981) and (Cys²-Tyr³-Orn⁵-Pen⁷, CTOP: Gulya et al., 1986).

1a. 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 and Hahn, 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 and Pasternak, 1985; Moskowitz and Goodman, 1985). μ binding is denser in the frontal cortex, striatum, ventral palladium, Nacc, medial thalamus, interpeduncular nucleus, median raphe, VTA 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_1 and μ_2 receptors in spinal and supraspinal analgesia (Bodnar et al., 1988; Paul et al., 1989; Pick et al., 1991).

Table 2. Opioid receptor subtypes and agonists and antagonists

Receptor	Peptides	Agonist	Antagonist	Clone	Primary Brain Sites
μ Subtypes: μ_1 , μ_2	β -endorphin Endo-1 Endo-2	DAMGO	β -FNA; CTOP Naloxonazine	MOR	neocortex, caudate-putamen, Nacc, thalamus, hippocampus, VTA
δ Subtypes: δ_1 δ_2	β -endorphin Enkephalin dynorphin	DADL DSLET DPDPE Deltorphin	Naltrindole DALCE NTII	DOR	neocortex, caudate-putamen, Nacc, amygdala, VTA
κ Subtypes: κ_1 κ_2 κ_3	Enkephalin dynorphin	dynorphin U50, 488H NalBZOH	 Nor-BNI LY255582	KOR	NTS, caudate-putamen, Nacc, amygdala, hypothalamus

2. δ opioid receptors. Autoradiographic studies indicate that δ receptor binding is densest in the olfactory-related neural areas, neocortex, caudate-putamen, Nacc, and amygdala. The development of selective δ receptor agonists and antagonists led to the classification of δ_1 and δ_2 receptor subtypes. The δ_1 receptor has been pharmacologically characterized by the agonist D-Pen², D-Pen⁵-enkephalin (DPDPE: Mosberg et al., 1983) and long-term actions of the antagonist D-Ala², Leu⁵, Cys⁶-enkephalin (DALCE) (Bowen et al., 1987; Jiang et al., 1990a). The δ_2 receptor has been pharmacologically characterized by the agonist D-Ala², Glu⁴-deltorphin (Jiang et al., 1991) and the antagonist naltrindole-5'-isothiocyanate (NTII) (Portoghese et al., 1990).

3. κ opioid receptors. κ receptor binding is densest in the caudate-putamen, Nacc, amygdala, hypothalamus, neural lobe of the pituitary, median eminence, and NTS (Mansour et al., 1988). Selective agonists and antagonists have also distinguished multiple κ receptor subtypes. The κ_1 receptor subtype has been characterized using the agonist U50,488H (VanVoigtlander 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). 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).

Summary: Thus, at least three classes of opioid receptor families exist. The characterization of the opioid receptor subtypes using selective agonists and antagonists

has been employed. The localization of the opioid receptor subtypes throughout various neural sites has been demonstrated. The roles of endogenous opioid peptides and their receptors in eliciting feeding responses are reviewed in the next section (see Table 3).

C. Systemic and Ventricular Opioids and Ingestion

That opioids may be involved in ingestion was first noted by Flowers et al., (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 et al., 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.

1. 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

Table 3. Intracerebroventricular studies on opioids and ingestion

Receptor	Agonist	Feeding Conditions	Antagonist effects
Endogenous Opioid Peptides: β -endorphin, OFQ, endomorphins		↑ Feeding when given i.c.v.	
μ μ_1	DAMGO-induced feeding ↓ by β -FNA and Nor-BNI	↑ spontaneous intake ↑ palatable intake (sucrose, glucose, saccharin, sodium chloride)	β -FNA = ↓ spontaneous intake/body weight; intake following food-deprivation, 2-DG, lipoprivation, and palatable intake; intake following stress-related conditions Naloxonazine = ↓ spontaneous intake/body weight, intake following stress-related conditions, BUT NOT glucoprivic/ Palatable intake
δ	DSLET (general) DPDPE (δ_1) Deltorphin (δ_2)	↑ feeding under spontaneous, palatable, and challenge situations	DSLET-induced feeding ↓ by μ/κ antagonists DPDPE-induced feeding ↓ by NTII (δ_2) BUT NOT by DALCE (δ_1) Deltorphin-induced feeding ↓ by NTII and DALCE
κ	U50,488H (κ_1) and NalBZOH (κ_3)	↑ spontaneous feeding and sucrose intake	U50,488H-induced intake ↓ by Nor-BNI Nor-BNI ↓ in nocturnal, sucrose, high-fat and 2DG intake

(Majeed et al., 1986). Both met- and leu-enkephalin into the VMH elicit feeding (Tepperman and Hirst, 1983) and dynorphin induces feeding in nondeprived rats (Morley and Levine, 1983). While dynorphin₁₋₁₇ increases feeding in the Nacc (Majeed, et al, 1986), VMH and PVN (Gosnell et al., 1986), dynorphin₁₋₁₃ 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).

2. 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. However, the emphasis will be on the μ receptor subtype as this is the subject of the current study.

A. 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 normal 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 nondeprived rats (Bhakthavatsalam and Leibowitz, 1986), which led to the idea that opioid, and particularly μ , agonists stimulate intake of the preferred macronutrient (Gosnell and Majchrzak, 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-induced and insulin-induced glucoprivation as well as 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 LH (Koch and Bodnar, 1993; Papadouka and Carr, 1994). Likewise, antisense probes

directed against the MOR-1 clone reduce feeding under spontaneous, glucoprivic and lipoprivic conditions (Leventhal et al., 1996; Burdick et al., 1998; 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-induced 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-1 gene, significantly

reduced DAMGO-induced feeding. In contrast, AS probes directed against exons 2 and 3 of the cloned MOR failed to alter DAMGO-induced feeding (Leventhal et al., 1997). Further, AS directed against the morphine metabolite, morphine-6 β -glucuronide (M6G), itself a potent inducer of feeding, showed the opposite pattern of effects to that of DAMGO-induced feeding. Whereas AS probes directed against exons 2 and 3 of the MOR-1 gene significantly reduced M6G-induced feeding, AS directed against exons 1 and 4 failed to significantly alter M6G-induced feeding (Leventhal et al., 1998b).

B. 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 agonists also stimulate intake under palatable and challenge situations. The ability of selective δ antagonists to block selective δ agonist-induced feeding is somewhat less consistent. Thus, 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) antagonists (Yu, et al., 1997), yet deltorphin-induced feeding was blocked by either DALCE or NTII (Yu et al., 1997). Further, AS probes directed against exon 3 of the DOR-1 clone reduced deltorphin II-induced feeding, but failed to block M6G-induced feeding (Leventhal et al., 1998b). 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 antagonist studies. The ability of δ agonists, but not δ antagonists, to alter intake is

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

C. 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, 1983; Morley et al., 1982; Morley and Levine, 1983; Sanger and McCarthy, 1981). Central injections of both selective κ_1 (U50,488H) and κ_2 (NaIBzOH) agonists increase spontaneous food intake (Gosnell et al., 1986; Koch et al., 1992), and stimulate sucrose intake (Lynch and Burns, 1990; Ruegg et al., 1997). Feeding induced by U50488H 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., 1998b). The κ receptor has been implicated in ingestive behavior in antagonist 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).

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

Table 4. Endogenous opioid peptides and opioid receptor subtypes central sites of action upon feeding.

Receptors	Brain Sites
Endogenous Opioids: β -endorphin	PVN, VMH, Nacc
morphine, DADL, nociceptin, dynorphin	VMH, PVN
Met and Leu-enkephalin	VMH
dynorphin ₁₋₁₇	Nacc, VMH, PVN
dynorphin ₁₋₁₃	VTA
OFQ	VMH, Nacc
μ (DAMGO)	VMH, PVN, amygdala (central nucleus), Nacc, VTA, NTS, perifornical area, lateral parabrachial area, ventral striatum
δ (DSLET, DPDPE, deltorphin)	VMH, PVN, Nacc, amygdala (central nucleus), perifornical area, lateral parabrachial area, ventral striatum
κ (U50,488H)	plays a lesser role in central sites of action
General, μ and κ , BUT NOT antagonists	↓ deprivation-induced feeding in PVN

at which these agonists and antagonists exert their ingestive effects (see Table 4).

D. 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 occurs in 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). Interestingly, neither the VTA nor Nacc produce U50,488H-induced feeding (Bakshi and Kelley, 1993a; 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), and ventral striatum (Bakshi and Kelley, 1993a, 1993b). Amygdala microinjections of DAMGO, morphine and D-Ala², Met⁵-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).

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., 1986; 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).

A further recent investigation (Kotz et al. 2000) showed that the medial NTS is the region most sensitive to naltrexone blockade of PVN NPY-induced feeding. In addition, Giraudo et al (1999) presented evidence that opioids in the PVN are responsible for the rewarding aspects of foods, whereas NPY is involved in energy metabolism. DAMGO-injected rats consumed most of their calories from a sucrose solution. This is in contrast to the effects on consumption of NPY in the PVN; NPY-injected rats consumed

an equal amount of calories from chow and a sucrose solution. However, a recent review (Glass et al., 1999) suggests that opioids in different brain sites may be involved in multiple aspects of ingestive behavior, including energy needs. Specifically, it is suggested that opioids in the hindbrain may be involved in both sensory and metabolic aspects of food intake, opioids in the amygdala may be involved in processing the emotional properties of food, and opioids in the hypothalamus may have a role in energy metabolism.

Levine and co-workers (Giraudou 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 (Giraudou 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 (Giraudou et al., 1998b). Glass et al (2000) demonstrated the differential roles of the PVN and CNA in diet selection. This recent study examined the role of the PVN and CNA in preferred and nonpreferred food consumption. Consumption of both preferred and nonpreferred diets was suppressed after

PVN naltrexone administration, whereas only preferred diet intake was reduced after CNA naltrexone treatment.

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 (see Table 5).

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

Feeding is elicited following opioid microinjections along the mesolimbic dopaminergic pathway, including the VTA and NAcc. Thus, VTA microinjections of either morphine, DPDPE, DAMGO or D-Ala²-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 U50488H 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.,

Table 5. Summary of opioid receptor subtype agonists and antagonists feeding responses along the Nacc-VTA axis

<u>Nacc</u>	<u>Feeding Situations</u>
μ , δ and κ agonists; DADL, DPDPE, β -endorphin, dynorphin, DAMGO, nociceptin	↑ food intake
<u>Antagonist Actions</u>	
μ_1 , δ_1 , δ_2 , and κ receptor antagonists	↓ feeding elicited by DAMGO (μ) and DPDPE (δ_1); Antagonists DO NOT reduce deltorphin (δ_2) feeding
NTX (general), β -FNA (μ)	↓ deprivation, glucoprivic and palatable intake
Nor-BNI (κ_1)	↓ deprivation, glucoprivic BUT NOT palatable intake
Naloxonazine	Naloxonazine (μ_1) failed to block ingestive effects
Dopamine	D ₁ (SCH23390) BUT NOT D ₂ (raclopride) antagonists ↓ DAMGO-induced feeding; effects less consistent with DPDPE-induced feeding

<u>VTA</u>	<u>Feeding Situations</u>
μ and δ agonists: morphine, DPDPE, DAMGO, DALA κ agonists play a lesser role	↑ food intake
<u>Antagonists Actions</u>	
NTX	NTX ONLY at high doses ↓ DAMGO-induced, deprivation and glucoprivic feeding
β -FNA (μ), Nor-BNI (κ_1)	↓ DAMGO-induced feeding; DID NOT ↓ feeding under various conditions
Naltrindole (δ)	DID NOT ↓ DAMGO-induced feeding
DALCE (δ_1)	DID NOT ↓ deprivation, glucoprivic and palatable intake
NTII (δ_2)	↓ deprivation, glucoprivic and palatable intake

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; Jongen-Relo et al., 1993; Khachaturian et al., 1982; Lewis et al., 1984, 1985; VanBockstaele 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 microinjections of either morphine, DADL, DPDPE, β -endorphin, dynorphin, DAMGO, 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.

Recent studies (Bodnar et al., 1995; Ragnauth et al., 1997; 2000a; 2000b) have further indicated the integral role the opioid system in the Nacc plays in ingestive behavior. Studies (Bodnar et al. 1995; Kelley et al., 1996) demonstrated that the opioids in the Nacc are involved in regulating food intake under a variety of conditions. This was shown by the ability of general, μ , and κ opioid antagonists to significantly reduce food intake under deprivation, glucoprivic and palatable conditions. Since the VTA has reciprocal connections with the Nacc and it is recognized as a neural site supporting μ and δ opioid agonist feeding, it is important to note the various conditions under which the opioid system plays a role in the VTA. Ragnauth et al (1997) suggested a minimal role for the VTA in the elicitation of deprivation, glucoprivic and palatable intake because of the failure of selective opioid antagonists to significantly reduce feeding under these conditions. The general opioid antagonist naltrexone reduced deprivation and glucoprivic feeding elicited from the VTA only at high doses that are comparable to those employed in ventricular studies. Furthermore, the μ selective opioid antagonist, β -FNA and κ antagonist Nor-BNI failed to reduce feeding elicited from the VTA under the various environmental conditions. The selective δ_2 antagonist NTII, but not the selective δ_1 antagonist DALCE significantly reduced deprivation, glucoprivic and palatable-induced intake elicited from the VTA, which is consistent with the finding of moderate densities of δ receptors in the VTA. Furthermore, Ragnauth et al (1997) suggest that the effects of δ

agonists and antagonists in the VTA play a modulatory rather than a direct role in ingestion.

Another recent study (Lamonte et al., 2001) examined whether DAMGO-induced feeding elicited from the VTA was altered by VTA pretreatment with an equimolar dose range of general (naltrexone), as well as selective μ (β -FNA), κ_1 (Nor-BNI) or δ (naltrindole) opioid receptor subtype antagonists. Consistent with a receptor-selective antagonist effect upon agonist-induced feeding, β -FNA pretreatment in the VTA significantly decreased VTA DAMGO-induced feeding, indicating a role for μ opioid receptors in this response. Interestingly, pretreatment with κ (Nor-BNI), but not δ (naltrindole) opioid antagonists in the VTA significantly and dose-dependently decreased DAMGO-induced feeding. Therefore, DAMGO-induced feeding elicited from the VTA is similar to its parallel ingestive response elicited from the Nacc shell (Ragnauth et al., 2000) in its dependence upon both intrinsic μ and κ opioid receptors, but differs from the Nacc shell in its insensitivity to δ opioid antagonism. The fact that δ opioid antagonism failed to affect DAMGO-induced feeding elicited from the VTA differs markedly from the ability of δ opioid antagonism in the VTA to produce small, but significant antagonism of feeding elicited by either deprivation, glucoprivation or exposure to a palatable substance (Ragnauth et al., 1997). Additionally, systemic pretreatment with naltrexone dose-dependently reduced DAMGO-induced feeding elicited from the VTA, but only at rather high doses (50-100 ug). These patterns of dose-dependent, and less potent general opioid antagonist effects within the VTA are consistent with the limited ability of VTA naltrexone to reduce or eliminate intake under deprivation, glucoprivic, and palatable conditions

(Ragnauth et al., 1997) as compared to quite potent general antagonist effects upon these measures following microinjection into the Nacc (Bodnar et al., 1995; Kelley et al., 1996).

Ragnauth et al (2000a) demonstrated that feeding elicited by μ or δ agonists in the Nacc are dependent on multiple opioid receptors. This was demonstrated by the ability of μ , δ_1 , δ_2 , and κ_1 receptor antagonists to significantly reduce feeding elicited by the μ opioid agonist, DAMGO, in the Nacc. A similar pattern of antagonists effects was shown for δ_1 (DPDPE) opioid agonist feeding. However, the selective opioid subtype antagonists failed to significantly reduce δ_2 (deltorphan) opioid agonist feeding. These previous studies (Bodnar et al., 1995; Ragnauth et al., 2000a) suggest that the μ receptor in particular plays a more critical role in ingestive behavior in the Nacc as compared to the δ and κ opioid receptors based on the greater magnitude of effects on food intake elicited from the μ receptor in the Nacc. Specifically, deprivation, glucoprivic and palatable intake was reduced by general (naltrexone) and μ (β -FNA) opioid antagonists, whereas the κ (Nor-BNI) receptor antagonist reduced deprivation and glucoprivic feeding, but failed to reduce palatable intake, suggesting a more limited role for the κ opioid receptor in the Nacc in ingestive behavior (Bodnar et al., 1995; Kelley et al., 1996). Furthermore, a particular role for the μ_2 receptor in these ingestive responses has been suggested based on evidence that the selective μ_1 opioid antagonist, naloxonazine, failed to block ingestive responses under a variety of conditions (Bodnar et al., 1995).

Since the general aim of the current study is to ascertain whether other neurochemical systems play a role in the elicitation of opioid-induced feeding in the Nacc it is important to review studies that demonstrate the interaction between the opioid

system in the Nacc and other neurotransmitter systems. One study of particular interest is the one conducted by Ragnauth et al (2000b) that demonstrated the role of the dopamine system in opioid-induced feeding in the Nacc shell. This current study showed that D₁ and D₂ receptors have differential actions upon μ (DAMGO) and δ_2 (deltorphin) opioid agonist feeding. The selective D₁ antagonist SCH23390 significantly reduced DAMGO-induced feeding elicited from the Nacc shell, whereas the selective D₂ antagonist raclopride produced less consistent effects upon DAMGO-induced feeding elicited from the Nacc shell. Additionally, D₁ and D₂ antagonists produced mild and inconsistent effects upon deltorphin-induced feeding. The results from this study suggest that δ_2 opioid agonist feeding elicited from the Nacc shell is not dependent on the dopamine system, whereas μ opioid agonist feeding is partially dependent upon the integrity of D₁ receptors in the Nacc shell.

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 receptor subtypes. How these structures support opioid-induced feeding is a major goal of the present series of studies. The following sections provide a detailed examination of the morphology of the Nacc and VTA, as well as their afferent and efferent projections (see Table 6).

F. Anatomy and Projections of the Nacc

1. 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

Table 6. Major Afferent and Efferent Connections of the Nacc and VTA

Nacc	Afferent	Efferent
Core	dorsal subiculum of the hippocampus, VP, VTA	basal ganglia circuitry, including ventrolateral globus pallidus, entopeduncular nucleus, VP, substantia nigra, subthalamic nucleus
Shell	amygdala, infra-limbic cortex, PVN, VTA, VP, ventral subiculum of hippocampus, prefrontal cortex, brainstem autonomic areas, NTS, median raphe nucleus	ventromedial globus pallidus, ventral bed nucleus of stria terminalis, lateral preoptic area, LH, VP, VTA
VTA (nucleus paranigralis, nucleus parabrachialis pigmentosus, nucleus linearis raphe caudalis)	The VTA has reciprocal connections with the following areas: prefrontal cortex, Nacc core and shell, VP, lateral habenular nucleus, posterior hypothalamic nucleus, amygdala, bed nucleus of stria terminalis, nucleus of the diagonal band, parabrachial nuclei, locus coeruleus, dorsal raphe nucleus Additional afferent connections: substantia innominata, cerebellar nuclei, superior colliculus, LH, preoptic area	Additional efferent connections: lateral septal nucleus, mediodorsal nucleus, median raphe nucleus

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 the calcium-binding protein 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 LH (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, LH 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, 1999b).

The pioneering work of Curt Richter and Eliot Stellar (see review: Kelley, 1999a) 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 neurotransmitter systems including GABA, EAA and opioids (see review: Kelley, 1999a). Opioid mediation of feeding within the Nacc, and particularly 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, 1999b; Koob, 1992, 1999; Robinson and Berridge, 1993; Salamone et al., 1994; Wise and Bozarth, 1987).

2. 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; Jongen-Rêlo 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 raphe 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 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. The connections with the ventral mesencephalon are more complicated. Generally, the shell connects to sub-cortical areas such as the LH, 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., 1994; Heimer et al., 1991; Zahm and Heimer 1990; Usada et al, 1998; Groenewegen et al., 1994; 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, 1994; 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 focus of the dissertation is on the shell region of the Nacc since this area has been shown to be the critical locus for opioid-induced feeding.

G. Anatomy and projections of the VTA

The VTA is a midbrain region that has connections to various neural sites, including cortical, limbic, striatal and brainstem areas. Investigations of the cytoarchitecture of the VTA shows the existence of distinct subgroups and cell types. The three subgroups, nucleus paranigralis, nucleus parabrachialis pigmentosus, and nucleus linearis raphe caudalis, are distinguished by various factors, including cell types, projection sites, and differential neurochemical densities (Phillipson, 1979a; 1979b). Histochemical procedures have elucidated the anatomical localization of these distinct subgroups. The nucleus parabrachialis pigmentosus lies dorsally, and the nucleus paranigralis is situated ventrally in the VTA, while the nucleus linearis continues posteriorly and medially from the VTA and extends dorsally in the midline towards the dorsal raphe nucleus (Phillipson, 1979a). Furthermore, there is a distinction in cell types among the areas of the VTA. A Golgi study of the VTA showed the following characteristics of two morphological cell types within each area. Type 1 were small to medium and had two to four primary dendrites and type 2 were medium sized, with two to five primary dendrites (Phillipson, 1979b). Nucleus paranigralis neurons were small to medium in size and their dendritic fields were oriented in an anteromedial direction while slanting dorsally over the

interpeduncular nucleus and fossa (Phillipson, 1979b). While the neurons of the parabrachial group were also small to medium sized, they did not exhibit any preferred orientation (Phillipson, 1979b; 1979c). The neurons in the nucleus linearis raphe caudalis were small and were strongly oriented in a dorso-ventral direction (Phillipson, 1979b). All three regions have a significant number of dopamine-containing cell groups (Phillipson, 1979a). However, with regard to GABA receptors, a higher density of GABA_A receptors have been shown in the paranigral region as compared to the parabrachial region of the VTA (Churchill et al., 1992).

Distinctions have also been made between rostral and caudal divisions of the VTA based on pharmacological and behavioral data. Pharmacological studies have demonstrated differential feeding and locomotor responses when pharmacological agents were administered in rostral and caudal divisions of this nucleus. Arnt and Scheel-Kruger (1979) showed that injections of the GABA_A agonist, muscimol, produced feeding and hypermotility when administered in the caudal portion of the VTA, and a decrease in spontaneous activity with no effect on food intake when administered in the rostral portion of the VTA. Furthermore, the involvement GABA_A antagonists in reinforcement behavior has been observed in the anterior, but not the posterior VTA (Ikemoto et al., 1997). A differentiation between rostral and caudal divisions of the VTA can also be made based on projection sites. Rostral VTA projects to frontal cortex whereas the caudal VTA projects to the entorhinal cortex (Arnt and Scheel-Kruger, 1979).

The VTA has extensive afferent and efferent connections along the neuroaxis. The existence of reciprocal connections between the prefrontal, Nacc and ventral pallidum

and VTA has been shown (Churchill et al., 1992). In terms of neurochemical distribution, there is a reciprocal GABAergic innervation between the Nacc and VTA (Van Bockstaele and Pickel, 1995). Studies have investigated specifically GABAergic and enkephalin projections from the Nacc to the VTA. A projection from the Nacc to the VTA via the ventral pallidum has been suggested (Kalivas et al., 1993). There is a topographic organization to these connections in that the ventromedial portion of the ventral pallidum projects to the VTA (Kalivas et al., 1993). The diagonal band also projects to the VTA, and this projection is partly GABAergic and enkephalinergic (Kalivas et al., 1993). The distinction between the nucleus paranigralis and parabrachial region of the VTA is also demonstrated by their differential connections. Kalivas et al. (1993) showed a projection from the shell of the Nacc to specifically the paranigralis region of the VTA.

Recent studies employing anterograde and retrograde tracing as well as immunocytochemistry have demonstrated a significant reciprocal connections between the VTA and the prefrontal cortex (Carr and Sesack, 2000a; Carr and Sesack, 2000b). In addition to a dopamine component which had been established from previous studies (Pirot et al., 1992), evidence for the existence of GABA-containing neurons in the VTA that project to the prefrontal cortex, as well as prefrontal projections to GABA-containing neurons in the VTA has been shown. In addition, some of the prefrontal dendrites that were contacted by GABA VTA terminals were also GABA labeled (Carr and Sesack, 2000a; Carr and Sesack, 2000b).

In addition to the reciprocal connections between the VTA and various neural sites elucidated in this section, other significant reciprocal connections of the VTA include

the following areas: lateral habenular nucleus; the posterior hypothalamic nucleus; the amygdala; the bed nucleus of the stria terminalis, the nucleus of the diagonal band; parabrachial nuclei; and locus coeruleus (Beckstead et al., 1979; Philipson, 1979c). Reciprocal connections between the VTA and the dorsal raphe nucleus have been established (Vertes, 1991; Beckstead et al., 1979). Utilizing the retrograde transport of horseradish peroxidase (HRP), it was demonstrated that many of the afferent connections to the VTA project specifically to dopamine-containing neurons, including stria terminalis, amygdala, diagonal band of Broca, Nacc, dorsal bank of rhinal sulcus, substanta innominata, dorsal raphe nucleus, locus coeruleus, cerebellar nuclei, superior colliculus, medial and lateral preoptic areas, and lateral hypothalamus (Phillipson, 1979c). Additional efferent connections of VTA include the medial zone of the mediodorsal nucleus, the medial half of the lateral septal nucleus; and anterior portion of the median raphe nucleus (Churchill et al., 1992). Specific neuronal populations of the VTA project to the inferior olive (Fallon et al., 1984).

Based on afferent and efferent connections, the ventrolateral portion of the VTA has been viewed as a distinct subgroup. Retrograde and anterograde studies have provided evidence of significant afferent connections to the ventrolateral tegmental area (VLT), including the lateral substantia nigra, the intercollicular nucleus, the inferior colliculus, the oral and caudal pontine reticular nucleus, the gigantocellular and lateral paragigantocellular reticular nuclei, the spinal trigeminal nuclei, and the intermediate layers of the spinal cord (Herbert et al., 1997). Significant efferent connections to the VLT include the following: superior colliculus; intercollicular nucleus and the inferior colliculus;

oral and caudal pontine reticular nucleus; the gigantocellular and lateral paragigantocellular reticular nuclei; and the medial column of the facial nucleus (Herbert et al., 1997).

Summary: The widespread efferent and afferent connections of the VTA, including cortical, subcortical, brainstem and spinal nuclei, suggests the integrative role that this structure plays in various behavioral functions. Furthermore, the reciprocal connections between the Nacc shell and VTA, along with pharmacological studies, indicate that the VTA is an integral site for ingestive behavior in addition to its role in other functions, including motor behavior.

The focus of the current study is to elucidate the underlying neuroanatomical and neurochemical circuitry that supports μ opioid agonist feeding in the Nacc shell and VTA. The specific concern of this study is to understand the roles that the excitatory, particularly NMDA and AMPA, and inhibitory, particularly GABA, amino acid neurotransmitters play in the μ opioid-mediation of ingestive behavior in these neural sites. Therefore, the following section will review the pharmacology of the EAA receptor subtypes, NMDA and AMPA, and the major inhibitory amino acid neurotransmitter, GABA since these receptor subtypes are a particular focus of the current study.

H. Amino Acid Neurotransmitters

Studies have demonstrated that there are several amino acids for which neurotransmitters roles have been proven or considered probable. Some amino acids neurotransmitters play a more direct neurotransmitter role while other types play more diffuse modulatory roles. γ -Aminobutyric acid and glycine are considered the principal

inhibitory neurotransmitters, whereas glutamate and to a lesser degree aspartate are the presumed excitatory neurotransmitters. These amino acid neurotransmitters can be found throughout various neural sites and pathways (McGeer et al., 1987a; 1987b; Roberts, 1986a; 1986b; Nagai et al., 1984).

γ-Aminobutyric Acid: GABA was discovered in brain tissue in 1950 by independent investigations of Eugene Roberts and Jorge Awapara (Awapara et al., 1950; Roberts and Frankel, 1950). Further studies in a number of laboratories suggested the role of GABA in inhibitory neurotransmission (Kravitz et al., 1963). Studies demonstrated the presence of GABA in the fourth ventricle when Purkinje cells were stimulated (Obata and Takeda, 1969), and was further elucidated by Roberts with immunohistochemical methods, which showed the localization of GABA to specific neuronal elements (Roberts, 1986a). Currently, GABA is established as an inhibitory transmitter in the nervous system (Roberts, 1986a; McCormack, 1989).

GABA Receptors: Pharmacological studies of mammalian synapses have identified two distinct GABA receptor subtypes, GABA_A and GABA_B receptors (see Table 7). The GABA_A receptor and the GABA_B receptor have been shown to exhibit differential pharmacological actions (Paul, 1995). The differential actions of these receptor subtypes are indicated by their differential antagonists and agonists (see review: Sieghart, 1995), which will be elucidated in the following description of each receptor subtype. There is also a differentiation of these receptor subtypes based on distribution in the central nervous system (see review: Sieghart, 1995). For example, autoradiographic studies have

Table 7. GABA receptor subtypes and agonists and antagonists

GABA Receptor Subtypes	Agonists	Antagonists	Receptor Families	Binding Sites
GABA _A	Muscimol	Bicuculline Picrotoxin	5 subunits: α , β , γ , δ , ρ	Cl ⁻ channel, benzo- diazepines, barbiturates, picrotoxin, steroids, and separate binding sites for agonists/ antagonists
GABA _B	Baclofen	Saclofen		

shown an abundance of GABA_A sites in the granular cell layers of the cerebellum where no GABA_B sites are present (Paul, 1995). The concentration of GABA_B sites in the interpeduncular nucleus is much greater than the concentration of GABA_A sites (Paul, 1995). Both receptor subtypes have been shown to have pre- and postsynaptic locations (Paul, 1995).

1. GABA_A Receptors: The GABA_A receptor is the most prevalent of the two established GABA receptors in the mammalian CNS and has been extensively characterized (Schofield et al., 1987; Sieghart et al., 1995; Luddens et al., 1995). The GABA_A receptor contains a transmembrane ion channel that predominantly conducts chloride ions (Sieghart, 1995). Research in the late 1980's showed that the GABA_A receptor is a multi-subunit receptor-channel complex (Luddens and Wisden, 1991; Sieghart, 1995). Besides the binding site for GABA that mediates the effects of agonists and competitive antagonists, this receptor complex possesses a number of other sites. The other sites include a Cl⁻ channel, and modulatory binding sites for benzodiazepines, barbiturates, picrotoxin and steriods (Sieghart, 1995; Luddens and Wisden, 1991). The most well established competitive antagonist for the GABA_A receptor is bicuculline (Sieghart, 1995). GABA_A sites are sensitive to muscimol (agonist), 3-aminopropanesulfonic acid(agonist), and isoguvacine (agonist). Muscimol has been the most extensively studied and is a GABA-mimetic agent in that it interacts directly with GABA receptors (Sieghart, 1995). Evidence for differentiation from the GABA_B receptor is the insensitivity of the GABA_A receptor to the agonist baclofen and the antagonist

saclofen (see review: Bowery, 1989; Bowery, 1993).

The application of molecular biological techniques has led to the progress in the structural analysis of the GABA_A receptor complex. Five different families of receptor subunits have been identified (Luddens et al., 1995; Macdonald and Olsen, 1994). The five subunits and their number of isoforms is as follows: α , 6 isoforms; β , 4 isoforms; γ , 3 isoforms; δ , 1 isoform; and ρ , 2 isoforms (Luddens et al., 1995; Macdonald and Olsen, 1994). With the understanding of the structural components of the GABA_A receptor, the application of agonists and antagonists can be utilized in pharmacological studies. Thus, muscimol has been utilized as a GABA_A agonist tool and bicuculline has been employed as a GABA_A antagonist tool (Sieghart, 1995).

2. GABA_B Receptor: Hill and Bowery (1981) identified GABA sites that were insensitive to bicuculline and demonstrated binding sites for baclofen. Autoradiographic mapping has shown that there is a differential distribution of the two receptor subtypes in the brain, with the heaviest concentrations of GABA_B receptors in the molecular layer of the cerebellum, and the interpeduncular nucleus (Chu et al., 1990; Gehlert et al., 1985). This receptor subtype has been found both pre and post-synaptically and current studies indicate that the GABA_B receptor operates through the activation of one or more G proteins (Bowery, 1989; Bowery, 1993). In contrast to GABA_A receptors, which are directly associated with a Cl⁻ channel, binding to GABA_B sites is dependent on Ca²⁺ or Mg²⁺. GABA_B sites are sensitive to saclofen (antagonist) and baclofen (agonist), but insensitive to bicuculline and muscimol (Bowery, 1993; Wojcik and Holopainen, 1992).

Therefore, receptor selectivity is indicated by the following lines of evidence. The GABA_A receptor is sensitive to muscimol (agonist) and bicuculline (antagonist), and insensitive to baclofen (agonist) and saclofen (antagonist) whereas the GABA_B receptor is sensitive to baclofen and saclofen and insensitive to muscimol and bicuculline. Behavioral studies have also confirmed this receptor selectivity. Kelley and co-workers (Stratford and Kelley, 1997) demonstrated that muscimol-induced feeding elicited from the Nacc shell was blocked by pretreatment with bicuculline but not saclofen, and baclofen-induced feeding was blocked by pretreatment with saclofen but not bicuculline. The current study will also focus on the receptor selectivity of the GABA_A and GABA_B receptors. One part of the current proposal will attempt to replicate the findings by Kelley, and furthermore, the study will also explore whether μ opioid agonist feeding is dependent upon the GABA_A and GABA_B antagonists.

Summary: GABA is the most important inhibitory neurotransmitter in the CNS. GABA's involvement in inhibitory control is mediated at the postsynaptic as well as the presynaptic level. The presence of GABAergic neurons at all levels of the neuroaxis suggests that neural inhibition is essential in many different behavioral and physiological mechanisms. The role of GABA has been strongly implicated in locomotor activity, feeding behavior, aggression, sexual behavior, mood and regulation of pain sensitivity (see review: Matsumoto, 1989; Paredes and Agmo, 1992).

Excitatory Amino Acids: Glutamate

The excitatory potency of glutamate was first demonstrated in crustacean muscle

and later by direct topical application to mammalian brain (Hayashi, 1954; Van Harreveld, 1959). Studies by Rustioni and co-workers (Tracey et al., 1991) employing immunocytochemistry have identified primary afferent fibers terminating in the lumbar spinal cord with nerve terminals enriched in glutamate. Cotman and co-workers' studies on the dentate gyrus of the hippocampal formation have provided evidence that glutamate may be an important neurotransmitter (Cotman et al., 1995).

Receptor Classes: Pharmacological studies have demonstrated the existence of at least five different subtypes of EAA receptors, AMPA, NMDA, kainate, AP_4 , and AP5, by the employment of various agonists and antagonists (see review: Gasic and Hollman, 1992; Sommer and Seeburg, 1992; Seeburg, 1993). AMPA, kainate, and NMDA receptors are characterized as ionotropic receptors, which indicates that they are ligand-gated channel receptors. In addition, they are named for the pharmacological agonists for which they respond to selectively (Perl et al., 1990; Stewart et al., 1990). Additional agonists for these receptors include quisqualic acid, which preferentially activates AMPA receptors, and ibotenic acid, which is particularly selective for NMDA receptors (Perl et al., 1990; Stewart et al., 1990). Receptor binding studies have led to the discovery of antagonists for EAA receptors. Competitive antagonists for the NMDA receptors include CPP, D-AP5 and D-AP7. CNQX, NBQX, and DNQX are considered competitive non-NMDA antagonists with particular selectivity for AMPA receptors. YM900, LY215490 and GYKI52466 have been identified recently as non-NMDA antagonists (Cunningham et al., 1993; Ornstein et al., 1993; Rogawski, 1993).

Metabotropic receptors: In 1985 Sladeczek and co-workers (Sladeczek et al., 1985) revealed the existence of a novel glutamate receptor, which was pharmacologically distinct from the ionotropic receptor family. These receptors are coupled to G proteins and operate through second messenger systems (Sladeczek et al., 1985). Genetic studies indicated the existence of seven different metabotropic receptors designated as mGluR1 through mGluR7. There are three isoforms of mGluR1 (α , β , and γ) and two isoforms of mGluR5, which include a and b (see review: Bockaert et al., 1993; Schoepp, 1994). However, the lack of specific antagonists has limited the characterization of the role of individual mGluRs in glutamatergic transmission, and understanding the role of these receptors in behavioral functions (Schoepp and Conn, 1993).

The current study focused on the role of the AMPA and NMDA EAA receptor subtypes, and therefore more detail will be provided on the mechanisms of these receptor subtypes in the following sections (see Table 8).

AMPA receptors: AMPA receptors are nonselective to Na^+ and K^+ ions but for the most part are impermeable to Ca^{2+} . Cloning studies have led to the characterization of these receptors. Sixteen different subunits for ionotropic glutamate receptors have been cloned and four of these subunits, GluR1-GluR4 are related to the AMPA subtype (Seeburg, 1993; Bettler and Mülle, 1995). AMPA and quisqualate are selective agonists for the AMPA receptor.

NMDA receptors: In contrast to AMPA/kainate receptors, the NMDA receptor channels exhibit significant permeability to Ca^{2+} , as well as Na^+ and K^+ ions (McBain and

Table 8. EAA receptor subtypes and agonists and antagonists

EAA Receptor Classes - Ionotropic Receptors	Agonists	Antagonists	Receptor Clones	Binding Sites
1. AMPA	AMPA, quisqualate	DNQX, CNQX, NBQX, YM900, LY2145490, GYK152466	GluR1-GluR4	
2. NMDA	NMDA	D-AP5, D-AP7, APV, CPP, MK-801	NMDAR1-NMDAR2A-2D	glutamate and related agonists, glycine, PCP, MK-801, ketamine, magnesium, zinc

Mayer, 1994; Scatton, 1993). An important aspect of the NMDA receptor is that this receptor is subject to a voltage-dependent blockade by low concentrations of Mg^{2+} . Another important property of the NMDA receptor is that the amino acid glycine binds to a separate recognition site on the receptor complex and acts as a co-agonist (Johnson and Ascher, 1987). It is suggested that extracellular glycine levels could serve as a modulatory influence on NMDA receptor function (Kemp and Leeson, 1993). In addition, there are binding sites on the NMDA receptor complex for polyamines, such as spermine and spermidine, and PCP and related drugs (Anis et al., 1983; Lodge and Anis, 1982). Thus, there are at least six pharmacologically distinct sites on the NMDA receptor: a binding site for L-glutamate and related agonists, allowing the opening of a high conductance channel that permits entry of Na and Ca; a strychnine-insensitive glycine modulatory site; a site that binds PCP and related noncompetitive antagonists, including MK-801 and ketamine; a voltage-dependent magnesium (Mg^{2+}) binding site; an inhibitory cation site that binds zinc (Zn^{2+}); and a polyamine regulatory site (Scatton, 1993; Mayer et al., 1989). In addition, two distinct binding sites are associated with the transmitter recognition site, one that preferentially binds agonists and one that preferentially binds antagonists (Scatton, 1993).

Genetic studies have led to the cloning of the NMDA receptor. The first NMDA receptor subunit to be cloned was designated NMDAR1, and further studies demonstrated at least nine different isoforms of this subunit (see review: McBain and Mayer, 1994; Seeburg, 1993). The existence of four additional subunits, NMDAR2A-2D, are known which are structurally similar to each other, but exhibit different sequences from

NMDAR1 (McBain and Mayer, 1994; Seeburg, 1993).

The NMDA receptor is widely distributed in the mammalian brain and spinal cord, with particular high receptor densities found in the hippocampus and cerebral cortex (Monaghan et al., 1989; Petralia et al., 1994). NMDA receptors appear to have an essential role in long-term depression (LTD), long-term potentiation (LTP), and developmental plasticity (Monaghan et al., 1989; Petralia et al., 1994).

A focus of the current study is on the role of the EAA receptor agonists NMDA and AMPA in the μ opioid-mediation of ingestive behavior in the Nacc shell. Previous studies have demonstrated potent feeding responses with the intracerebral administration of EAA agonists and antagonists into various neural sites. Kelley and co-workers demonstrated that EAA antagonists elicited food intake from the the Nacc shell (Maldonado-Irizarry et al., 1995). Stanley et al (1993a; 1993b) has demonstrated that EAA agonists increase feeding in certain neural sites. Intracerebral administration of either glutamate, kainic acid, AMPA or NMDA into the lateral hypothalamus stimulated feeding over 1 h. The ionotropic receptors, including AMPA and NMDA, are a focus of this study rather than the metabotropic receptors because of their role in ingestive behavior (Maldonado-Irizarry et al., 1995; Stanley et al., 1993a; Stanley et al., 1993b; Khan et al., 1999; Stanley et al., 1997; Stratford et al., 1998; Stanley et al., 1996).

III. Rationale

The Nacc shell has been identified as a neural site involved in ingestive behavior. Potent feeding responses are elicited following microinjection of μ and δ opioid agonists, but not κ agonists, into this structure. Multiple opioid receptor subtypes mediate feeding elicited by specific opioid agonists in the Nacc (Ragnauth et al., 2000a) such that μ , δ_1 , δ_2 and κ_1 , but not μ_1 opioid antagonists block feeding elicited by either DAMGO or DPDPE. Further, general, μ , δ and κ opioid receptor antagonists in the Nacc differentially affect feeding elicited under either deprivation, glucoprivic or palatable conditions (Bodnar et al., 1995, Kelley et al., 1996). Dopamine receptors in the Nacc differentially mediate opioid-induced feeding since D_1 (SCH23390), but not D_2 (raclopride) dopaminergic receptor antagonists reduce DAMGO-induced feeding elicited from the Nacc (Ragnauth et al., 2000b).

This dissertation examined the neurochemical and neuroanatomical circuitry involved in the opioid mediation of ingestive behavior in the Nacc. Specifically, the role of excitatory (NMDA, AMPA) and inhibitory (GABA) neurotransmitters in the Nacc were examined in **Aims 1 and 2** so as to ascertain the role of these neurotransmitters in the μ opioid-mediation of ingestive behavior in the Nacc shell. **Aim 3** examined the role of GABA in the μ opioid-mediation of food intake in the VTA. The rationale for the third aim is that the VTA has reciprocal GABAergic connections with the Nacc, and therefore may be a major component of the afferent circuitry to the Nacc in the mediation of opioid-induced feeding.

Aim 1: The role of the inhibitory neurotransmitter, GABA, upon opioid-induced feeding in the Nacc shell: GABA, localized in medium-sized spiny projection neurons in the Nacc (Meredith et al., 1993), is involved in the modulation of feeding responses in this structure. Increases in food intake, but not water intake occur following Nacc administration of either GABA_A (muscimol) or GABA_B (baclofen) agonists, or by increasing local GABA levels with γ -vinyl-GABA (Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Ward et al., 2000). Moreover, these ingestive effects were receptor-selective since muscimol-induced feeding was blocked by pretreatment with GABA_A (bicuculline) but not GABA_B (saclofen) antagonists in the Nacc, and since baclofen-induced feeding was blocked by pretreatment with GABA_B but not GABA_A antagonists (Stratford and Kelley, 1997). Muscimol, like opioid agonists in the Nacc shell (Zhang et al., 1998; Zhang and Kelley, 1997), stimulates intake of palatable carbohydrate and fat diets, but not of water, saline or saccharin solutions (Basso and Kelley, 1999). Muscimol in the Nacc activated Fos-like immunoreactivity in the lateral hypothalamus, lateral septum, paraventricular hypothalamus, VTA, substantia nigra and nucleus of the solitary tract (Stratford and Kelley, 1999). GABA-mediated actions upon feeding are site-specific since GABA and its agonists also stimulate food intake following administration into the hypothalamic ventromedial and paraventricular nuclei, the dorsal and median raphe nuclei, the VTA, and the substantia nigra (Arnt and Scheel-Kruger, 1979; Bishop et al., 2000; Grandison and Guidotti, 1977; Hodge et al., 1996; Kelly et al., 1977; Kelly and Grossman, 1979; Kelly et al., 1979; Klitenick and Wirtshafter, 1988; Klitenick and

Wirtshafter, 1989; Przewlocka et al., 1979; Redgrave et al., 1984; Wirtshafter et al., 1993), yet decrease food intake following administration into the amygdala (Minano et al., 1992). In contrast, the GABA_A antagonist, bicuculline stimulates palatable intake following administration into the lateral hypothalamus and chow intake following administration into the ventral pallidum, and decreases food intake following administration into the ventromedial hypothalamus (Hansen and Ferreira, 1986; Kelly et al., 1977; Stratford et al., 1999). The first aim examined whether μ opioid synapses are dependent upon GABA synapses in the shell region of the Nacc, and thus the the following hypotheses were tested:

1. Since food intake is stimulated by GABA and opioid receptor subtype agonists administered into the Nacc, and specifically its shell region (e.g., Bakshi and Kelley, 1993a; Bakshi and Kelley, 1993b; Basso and Kelley, 1999; Stratford and Kelley, 1997), it was hypothesized that GABA receptor antagonists would reduce μ -opioid agonist-induced feeding. Thus, the first experiment examined whether feeding elicited by DAMGO in the Nacc shell was reduced by pretreatment with an equimolar dose range of either GABA_A (bicuculline) or GABA_B (saclofen) antagonists, and whether these antagonists altered spontaneous food intake.

2. The second experiment confirmed whether GABA_A and GABA_B antagonists specifically and differentially reduced feeding elicited by GABA_A (muscimol) and GABA_B (baclofen) agonists in the Nacc shell as shown previously (Stratford and Kelley, 1997). Furthermore, it was hypothesized that GABA feeding is dependent upon opioid synapses,

and therefore it was expected that pretreatment with the general opioid antagonist naltrexone would reduce feeding induced by muscimol or baclofen.

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Aim 2: The role of the excitatory neurotransmitters NMDA and AMPA upon opioid-induced feeding in the Nacc shell: EAA agonists and antagonists have been implicated in the mediation of food intake. Systemic administration of the non-competitive NMDA receptor antagonist, MK-801 significantly increases food intake in satiated and deprived rats, and under normal and palatable conditions (Burns and Ritter, 1997) by delaying meal termination (Burns et al., 1998). MK-801 produces this ingestive effect through increases in gastric emptying (Covasa et al., 2000), and through activation of vagal afferents (Burns and Ritter, 1998). The nucleus tractus solitarius (NTS) and dorsal vagal complex (DVC) are implicated in MK-801-induced increases in food intake since MK-801 microinjections directly into the NTS stimulate food intake (Treece et al., 1998), while lesions placed in the NTS and DVC block this ingestive response (Treece et al., 2000). Kainate/quisqualate receptor antagonists also produce short-term increases in food intake following administration into the median raphe nucleus (Wirtshafter and Krebs, 1990).

It must be noted, however that EAA receptor subtype agonists also are capable of producing site-specific stimulation of food intake. Thus, lateral hypothalamic infusions of either glutamate, kainic acid, AMPA or NMDA selectively stimulate feeding over a 1 h test (Stanley et al., 1993a; Stanley et al., 1993b). When these agonists are administered into adjacent hypothalamic, thalamic and amygdaloid sites, they produced minimal effects (Stanley et al., 1993a, Stanley et al., 1993b). NMDA antagonists in the lateral hypothalamus selectively decrease feeding elicited by NMDA, but not AMPA or kainic

acid, and reduce feeding under deprivation and nocturnal conditions (Stanley et al., 1996). NMDA-induced feeding elicited from the lateral hypothalamus is dependent upon a coagonist glycine site (Stanley et al., 1996) and the NR2A and NR2B subunits of the NMDA receptor (Khan et al., 1999).

Kelley (see review: Kelley, 1999a) has recently proposed a specific and important role for EAA receptors in the Nacc, and particularly its shell region, in the mediation of feeding. Microinfusions of the AMPA/kainate antagonists, DNQX, CNQX or NBQX, into the Nacc shell, but not into adjacent areas such as the Nacc core and ventral striatum, produced short-term (30 min) and intense increases in feeding (Kelley and Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998). NMDA antagonists were ineffective in the Nacc shell (Kelley and Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998). In contrast, if AMPA itself was administered into the Nacc shell, it reduced both deprivation-induced intake and sucrose intake in 30-min feeding tests (Stratford et al., 1998). DNQX-induced feeding elicited from the Nacc shell was reduced by pretreatment in the Nacc shell with either AMPA or D₁/D₂ antagonists, but not with opioid antagonists. Kelley (1999a) has proposed anatomical links between the Nacc shell and lateral hypothalamus in mediating the local ingestive actions of GABA (Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Stratford and Kelley, 1999; Ward et al., 2000) and EAA. Pretreatment with the GABA_A agonist, muscimol in the lateral hypothalamus blocked feeding elicited by DNQX in the Nacc shell (Maldonado-Irizarry et al., 1995). In turn, muscimol-induced feeding elicited from the Nacc shell was dose-

dependently decreased by pretreatment with NMDA antagonists in the lateral hypothalamus (Stratford and Kelley, 1999). On the basis of this evidence, Kelley and co-workers postulate that removal of tonic excitatory inputs in the Nacc shell with selective AMPA antagonists disinhibits neural circuitry involved in the initiation of ingestive behavior (Kelley, 1999a).

The Nacc is one of a number of intracerebral sites in which significant increases in food intake occur following administration of general, μ , δ , but not κ opioid receptor subtype agonists (Bakshi and Kelley, 1993a; Bakshi and Kelley, 1993b; Evans and Vaccarino, 1990; Majeed et al., 1986; Mucha and Iversen, 1986; Ragnauth et al., 2000a; Ragnauth et al., 2000b). Feeding elicited by the μ -selective opioid agonist, DAMGO in the Nacc is blocked by Nacc pretreatment with μ , δ_1 , δ_2 and κ_1 , but not μ_1 opioid antagonists (Ragnauth et al., 2000a). Moreover, general, μ , δ and κ opioid receptor antagonists in the Nacc differentially reduce feeding elicited under either deprivation, glucoprivic or palatable conditions (Bodnar et al., 1995; Kelley et al., 1996). Opioid agonist-induced feeding in the Nacc shell is modulated by other neurochemical systems as well. For example, D_1 (SCH23390), but not D_2 (raclopride) dopamine receptor antagonists administered into the Nacc shell reduce subsequent DAMGO-induced feeding (Ragnauth et al., 2000b). Also, feeding elicited by Nacc administration of $GABA_A$ (muscimol) or $GABA_B$ (baclofen) receptor subtype agonists (Basso and Kelley, 1999; Soderpalm and Berridge, 2000; Ward et al., 2000) is respectively blocked by Nacc pretreatment with $GABA_A$ (bicuculline) and $GABA_B$ (saclofen) antagonists (Stratford and Kelley, 1997).

Further, whereas pretreatment with the GABA_A antagonist, bicuculline, significantly and dose-dependently enhanced DAMGO-induced feeding elicited from the Nacc shell, pretreatment with equimolar doses of the GABA_B antagonist, saclofen, significantly and dose-dependently reduced DAMGO-induced feeding elicited from the Nacc shell (Znamensky et al., 2001). Therefore, dopaminergic and GABAergic receptor systems appear to interact with opioid receptors in the Nacc shell to modulate the latter's ability to elicit feeding.

The present study was designed to examine whether EAA and opioid agonists interact with each other in modulating food intake elicited from the Nacc shell. The model proposed by Kelley (1999a) would lead to the hypothesis that AMPA pretreatment in the Nacc shell would fail to affect feeding itself, but should reduce feeding elicited by DAMGO in the Nacc shell by producing local tonic inhibition. However, the studies demonstrating that EAA agonists stimulate feeding in specific brain sites such as the lateral hypothalamus (Khan et al., 1999; Stanley et al., 1993a; Stanley et al., 1996; Stanley et al., 1993b) lead to an alternative hypothesis that EAA agonists would exert time-dependent stimulatory effects upon feeding behavior elicited from the Nacc shell. Therefore, to test these possibilities, the first experiment examined whether AMPA administered into the Nacc shell would dose-dependently and time-dependently increase food intake in nondeprived rats tested during a portion of the light cycle when minimal amounts of intake occurred. The dose-dependent effects of naltrexone pretreatment in the Nacc shell upon AMPA-induced feeding were then examined. We did not examine whether DNQX, an

AMPA-selective antagonist, would block AMPA-induced feeding since DNQX produces intense stimulation of feeding elicited from the Nacc shell (Kelley and Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998), and an experiment designed to examine whether two potential orexigenic stimuli (AMPA and DNQX) would eliminate each other's effects was not considered feasible based upon interpretive problems. The second experiment followed an identical protocol in examining whether NMDA administered into the Nacc shell altered food intake, and whether naltrexone pretreatment was effective in blocking this response. Again, we did not examine whether NMDA antagonists would block NMDA-induced feeding since MK-801, a non-competitive NMDA antagonist, also produces intense stimulation of feeding following systemic and central administration (Burns et al., 1998; Burns and Ritter, 1997; Treece et al., 1998). To examine potential interactions between opioid and EAA agonists in the Nacc shell directly, the third experiment examined whether cotreatment with either AMPA or NMDA administered into the Nacc shell altered DAMGO-induced feeding elicited from the Nacc shell, and then whether pretreatment with either general or μ -selective (β -funaltrexamine; β FNA) opioid antagonists administered into the Nacc shell altered enhanced feeding responses elicited by combined AMPA and DAMGO cotreatment.

This aim of the dissertation has been published in the journal Brain Research. (921 (2001) 86-97).

Aim 3: The role of GABA upon opioid-induced feeding in the VTA: The VTA is involved in the opioid modulation of ingestive behavior. Hence, microinjections of the μ opioid agonist, DAMGO, in the VTA produce potent feeding and other oral responses (Badiani et al., 1995a; Cador et al., 1986; Mucha and Iversen, 1986; Nencini and Stewart, 1990; Noel and Wise, 1993; Noel and Wise, 1995). Both μ opioid agonists and antagonists administered in the VTA increase both locomotor activity and dopamine release in the nucleus accumbens (Nacc) (Devine et al., 1993a), and the reciprocal pathways between the VTA and Nacc shell have been implicated in the mediation of reward and reinforcement (Kelley, 1999a; Salamone, 1994; Self and Stein, 1992; Wise and Bozarth, 1987). However, the VTA is a far less effective site at which general and selective μ , κ and δ opioid antagonists act to inhibit food intake under deprivation, glucoprivic or palatable conditions (Ragnauth et al., 1997; Segall and Margules, 1989) relative to either the hypothalamic paraventricular nucleus (Koch et al., 1995) or the Nacc shell (Bodnar et al., 1995; Kelley et al., 1996). Further, whereas general and selective μ , δ and κ opioid antagonists potently eliminate DAMGO-induced feeding elicited from the Nacc shell (Ragnauth et al., 2000a), these same opioid antagonists produce less marked and less potent effects upon DAMGO-induced feeding elicited from the VTA (Lamonte et al., 2001).

GABA is one of a number of other neurochemical systems involved in the mediation of both reward processes and food intake responses elicited from the VTA. Thus, VTA administration of the GABA transaminase inhibitor, γ -vinyl-GABA, blocked

VTA heroin self-administration, an effect reversed by GABA_B (saclofen), but not GABA_A (bicuculline) antagonism (Xi and Stein, 2000). Whereas VTA microinjections of bicuculline reduced alcohol intake (Nowak et al., 1998), intra-VTA injections of the GABA_B agonist, baclofen, reduced cocaine self-administration (Brebner et al., 2000). GABA microinjected into the VTA disrupted appetitive approach of a sucrose reward (Ikemoto and Panksepp, 1996), and administration of the GABA_A agonist, muscimol, into the VTA produced potent feeding and drinking responses (Arnt and Scheel-Kruger, 1979; Klitenick and Wirtshafter, 1988). Such responsiveness appears to be mediated by the autoradiographic localization of GABA receptors within the VTA (Churchill et al., 1992). Further, neurons containing GABA and enkephalin neurons project from the Nacc, ventral pallidum and diagonal band of Broca to the VTA (Kalivas et al., 1993) with close appositions observed between GABA and enkephalin neurons (Sesack and Pickel, 1995), suggesting that enkephalin and GABA are released from convergent terminals and therefore may be involved in the co-regulation of common neurons. Indeed, microinjections of either GABA_A (muscimol) or GABA_B (baclofen) into the Nacc shell elicit feeding responses which are GABA receptor subtype-selective (Soderpalm and Berridge, 2000; Stratford and Kelley, 1997; Ward et al., 2000). In turn, GABA receptor subtype antagonist pretreatment in the Nacc shell differentially alters DAMGO-induced feeding such that the GABA_A antagonist, bicuculline, enhances DAMGO-induced feeding elicited from the Nacc shell, whereas the GABA_B antagonist, saclofen, reduces this opioid-mediated response (Znamensky et al., 2001). Further, naltrexone pretreatment in the Nacc

shell is more effective in preventing feeding responses elicited by GABA_A relative to GABA_B agonists (Znamensky et al., 2001).

Since muscimol in the Nacc shell activates c-fos-like immunoreactivity in a number of structures including the VTA (Stratford and Kelley, 1999), it appears that the VTA would be a logical site of action at which opioid and GABAergic systems modulate each other's ingestive effects. Thus, the following hypotheses were tested:

1. The first experiment of the present study examined whether VTA pretreatment with either the GABA_A antagonist, bicuculline or the GABA_B antagonist, saclofen altered feeding elicited by intra-VTA DAMGO in the same differential manner as observed in the Nacc shell (Znamensky et al., 2001). Given the strong GABAergic connections between the VTA and Nacc shell and evidence for GABA-opioid connectivity in the VTA (Kalivas et al., 1993), it was hypothesized that μ opioid feeding is dependent upon GABA synapses in the VTA, and therefore pretreatment with GABA antagonists would reduce DAMGO-induced feeding elicited from the VTA.

2. The second experiment of the present study examined whether the GABA_A (muscimol) and GABA_B (baclofen) agonists displayed feeding elicited from the VTA as previously described (Arnt and Scheel-Kruger, 1979; Klitenick and Wirtshafter, 1988), whether each response displayed the GABA receptor subtype selectivity previously observed in the Nacc shell (Stratford and Kelley, 1997), and whether each response was sensitive to general opioid antagonist pretreatment previously observed in the Nacc shell (Znamensky et al., 2001). It was hypothesized that the GABA agonists would increase

food intake in the VTA, and furthermore muscimol and baclofen-induced food intake would be reduced by their respective antagonists, bicuculline and saclofen. Based on evidence for potential co-regulation of enkephalins and GABA in the VTA (Kalivas et al., 1993), as in the Nacc shell, it was hypothesized that GABA synapses are dependent upon opioid synapses and therefore the general opioid antagonist naltrexone would reduce feeding induced by GABA agonists.

This aim of the dissertation has been accepted pending revision to the journal, Physiology and Behavior.

IV. GENERAL METHODS

A. Subjects, Surgery and Histology: Adult male albino Sprague-Dawley rats (Charles River Laboratories, Kingston, NY, 80-120 days of age) were individually housed in wire-mesh cages and maintained on a 12h light/12 h dark cycle with Purina rat chow and water available *ad libitum*. Each rat was pretreated with chlorpromazine (3mg/kg, i.p.) and anesthetized with Ketamine Hcl (120 mg/kg, i.p.). Bilateral stainless steel guide cannulae (26 gauge, Plastics One, Roanoke, VA) were aimed stereotaxically (Kopf Instruments, Tujunga, CA) at the Nacc shell using the following coordinates: incisor bar (+5), 3.1 mm anterior to the bregma suture, 1.7 mm lateral and angled 10° towards the sagittal suture, and 6.8 mm from the top of the skull. VTA coordinates were: incisor bar (+5 mm), 5.6 mm posterior to the bregma suture, 2.2 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 4 anchor screws with dental acrylic. To allow full drug clearance, all animals were allowed at least 2 weeks to recover from stereotaxic surgery before behavioral testing began since ketamine, a NMDA antagonist (Davidson and Carlton, 1998; Pallares et al., 1995) was employed as the anesthetic. It should be noted however that the typical interval between ketamine and subsequent NMDA administration in Protocols 3, 4 and 5 of Experiment 2 was close to one month, thereby minimizing any potential ketamine-induced NMDA antagonism. At the completion of testing, all animals received an overdose of Euthasol (Del Marva Laboratories, 390 mg/ml sodium pentobarbital; 50 mg/ml sodium phenytoin; 0.05 ml/kg), and were injected transcardially with potassium chloride (15 mg/ml, 0.9% saline). Transcardiac perfusions

were performed with 0.9% normal saline followed by 10% buffered formalin. Coronal 40- μ m sections, stained with Cresyl violet, were examined by light microscopy by an observer unfamiliar with the behavioral data; only animals with confirmed cannulae placements were included in the experimental groups described in the protocols which were subject to data analysis. In Experiment 1, four animals with misplaced cannulae did not show DAMGO-induced feeding, and were not included in the data analysis.

B. Food Intake Measures: In all experiments, each of the rats was initially tested for spontaneous food intake over a 4 h time course at 3-10 h into the light cycle during which minimal amounts of intake are typically observed. Cumulative intakes were assessed 1, 2 and 4 h following the placement of preweighed food pellets onto the floor of the cage to allow easy access to the food. All intakes were adjusted for spillage which was collected by paper towels placed under the wire mesh cages. Spillage was subtracted from the remaining chow to calculate the final intake. Rats were tested over at least four baseline sessions to establish consistently low intakes, and minimize the occurrence of novelty-induced feeding. Each rat was tested at a maximum of three dose conditions. Thus, most of the rats were utilized for three protocols for a given experiment. Additionally, there was at least one week between conditions.

C. Drugs: Muscimol, bicuculline, baclofen, saclofen, AMPA, NMDA and naltrexone were purchased from Sigma Chemical Company (St. Louis, MO). DAMGO was purchased from Peninsula Laboratories (Belmont, CA), and β -funaltrexamine was purchased from Research Biochemicals International (Natick, MA). All drugs were dissolved in distilled water, except for saclofen and baclofen, which were dissolved in

0.1M NaOH. All microinjections were administered bilaterally in 1.0. μ l 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. Particular microinjection conditions were presented in counterbalanced order at weekly intervals to minimize potential order and carry-over effects in all experimental protocols.

D. Statistical Analyses: Separate one-way repeated measures randomized-block analyses of variance were performed at each cumulative intake point. Tukey corrected comparisons ($P < 0.05$) were used to assess significant alterations in agonist-induced feeding relative to corresponding vehicle control conditions. Dunnett comparisons ($P < 0.05$) were employed to examine significant alterations induced by antagonist pretreatment upon agonist-induced feeding.

V. EXPERIMENT 1. Role of GABA Receptor Subtype Antagonists in the Nacc Shell**In Mediating Feeding Induced by the μ Selective Opioid Agonist DAMGO.****A. Specific Procedures:**

Protocol 1: In assessing GABA_A receptor antagonism upon μ -opioid agonist-induced feeding, the first group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n= 15), b) DAMGO (2.5 μ g (4.8 nmol), 1.25 μ g each side, n= 15), c) bicuculline (75 ng (1.62 nmol), 37.5 ng each side, n=7), and bicuculline at doses of either d) 7.5 ng (0.162 nmol, 3.75 ng each side, n= 7) or e) 75 ng (n=7) paired with DAMGO (2.5 μ g). Subgroups of rats receiving different antagonist conditions were matched on the basis of both intake following vehicle and agonist treatments in this and all subsequent protocols. GABA antagonist treatments preceded agonist treatments by 20 min in this and all subsequent protocols, and cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 2: In assessing GABA_B receptor antagonism upon μ -opioid agonist-induced feeding, the second group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n= 16), b) DAMGO (2.5 μ g, n= 16), c) saclofen (1500 ng (6 nmol), 750 ng each side, n=7), and saclofen at doses of either d) 500 ng (2 nmol, 250 ng each side, n= 7), e) 1000 ng (4 nmol, 500 ng each side, n=7) or f) 1500 ng (n=7) paired with DAMGO (2.5 μ g). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 3: In assessing opioid antagonism of interactions between GABA_A

receptor antagonism and the μ -opioid agonist, the third group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n= 17), b) DAMGO (2.5 μ g, n= 17), c) bicuculline (75 ng) paired with DAMGO (2.5 μ g) (n=7), naltrexone at doses of d) 1 μ g (2.6 nmol, 0.5 μ g each side, n= 7) or e) 5 μ g (13.2 nmol, 2.5 μ g each side, n= 6) paired with bicuculline (75 ng) and DAMGO (2.5 μ g), and f) β -funaltrexamine (4 μ g (8.2 nmol), 2 μ g each side, n= 6) paired with bicuculline (75 ng) and DAMGO (2.5 μ g). To optimize opioid antagonism, naltrexone and β -funaltrexamine were respectively administered 20 min and 24 h prior to bicuculline and DAMGO combinations with cumulative intakes assessed 1, 2 and 4 h following the last microinjection.

Protocol 4: In assessing GABA and opioid antagonism of GABA_A induced-feeding, the fourth group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n=7), b) muscimol (50 ng, 25 ng each side, 2.6 nmol, n=7), and either c) naltrexone (5 μ g, n=7), d) bicuculline (75 ng, n=6) or e) saclofen (500 ng, n=7) paired with muscimol (50 ng). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 5: In assessing GABA and opioid antagonism of GABA_B induced-feeding, the fifth group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n=9), b) baclofen (200 ng, 100 ng each side, 9 nmol, n=9), and either c) naltrexone (5 μ g, n=6), d) bicuculline (75 ng, n=7) or e) saclofen (500 ng, n=6) paired with baclofen (200 ng). Cumulative intakes were assessed 1, 2 and 4 h following

the last microinjection.

B. Results:

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; these placements were similar to those reported previously by this laboratory (Bodnar et al., 1996; Ragnauth et al., 2000a; Ragnauth et al., 2000b). Four animals with misplaced cannula placements ventral and medial to the Nacc shell failed to display agonist-induced feeding and were not included in any of the following data analyses.

Nacc GABA_A receptor antagonist and Nacc opioid-induced feeding:

Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F = 56.27, P < 0.0001$), 2 ($F = 49.50, P < 0.0001$), and 4 ($F = 12.12, P < 0.0001$) h. DAMGO significantly increased food intake relative to control treatment after 1, 2, and 4 h. Pretreatment in the Nacc shell with the selective GABA_A receptor subtype antagonist, bicuculline, significantly and dose-dependently increased DAMGO-induced feeding with the higher (75 ng) but not lower (7.5 ng) dose effective after 1 and 2 h (Figure 1, upper panel). Administration of bicuculline into the Nacc shell failed to alter food intake over the time course. The higher, but not lower bicuculline dose in the presence and absence of DAMGO produced seizure behavior (barrel rolls, body rotation, back flip and twitching of forepaws, vibrissae and mouth) in about 60% of rats that occurred within 1 min after injection and persisted for at most 5 min. Animals appeared normal after 20 min when food was introduced (see Table 9).

Figure 1. Alterations in spontaneous food intake (g, $\pm$ S.E.M.) following administration of the μ opioid agonist, DAMGO (2.5 μ g) in the Nacc shell relative to control treatment in rats pretreated with either the GABA_A antagonist, bicuculline (7.5-75 ng, upper panel) or the GABA_B antagonist, saclofen (500-1500 ng, lower panel). The asterisks (*) indicate significant increases in food intake following opioid agonist treatment relative to control treatment (Tukey comparisons, $P < 0.05$). The crosses (+) indicate significant alterations (increases or decreases) in food intake following GABA antagonist pretreatment in the Nacc shell relative to opioid agonist treatment alone (Dunnett comparisons, $P < 0.05$).

FIGURE 1

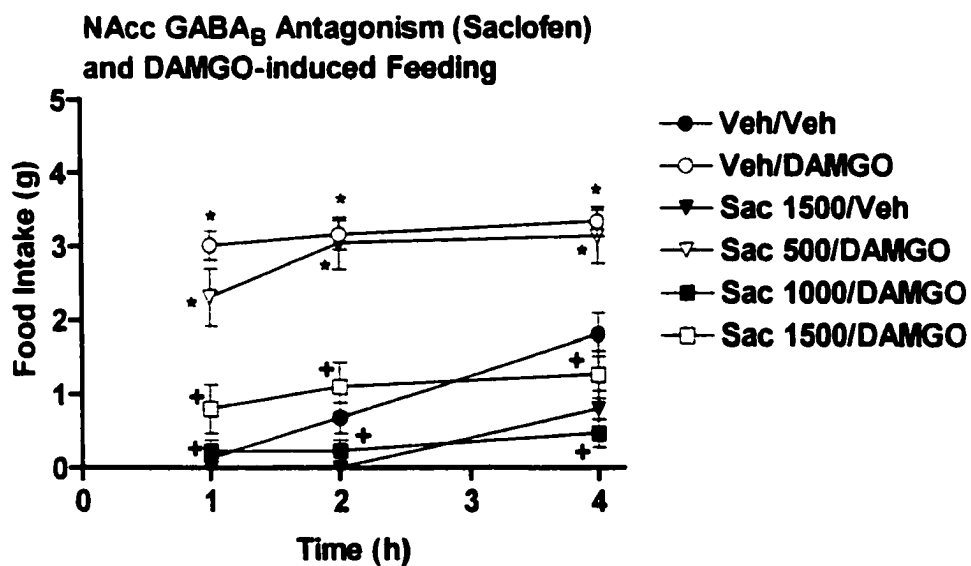
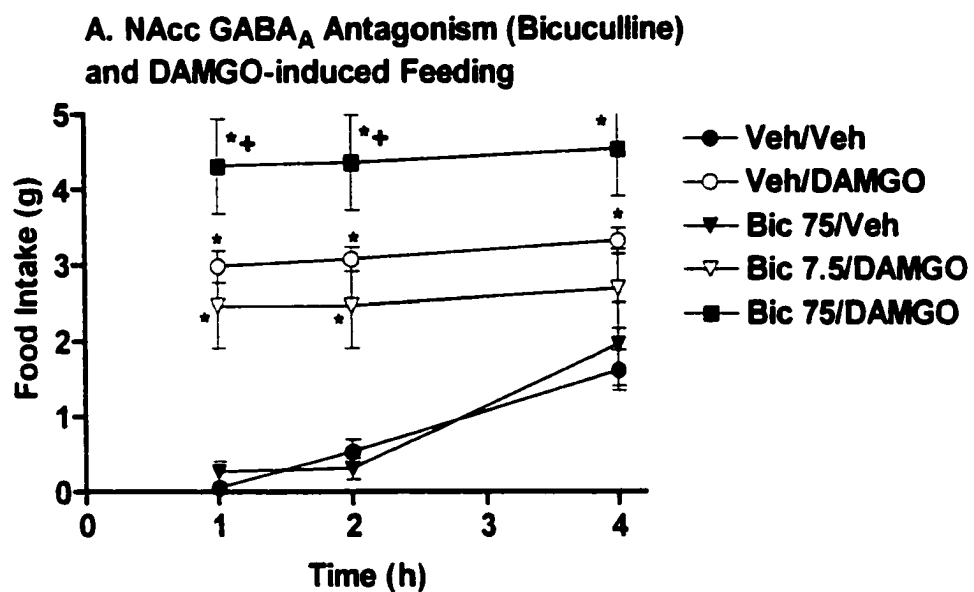


Table 9. GABA Antagonists - Alterations in DAMGO-induced Feeding in the Nacc Shell and VTA

Receptor Subtypes	Antagonist	Nacc Shell	VTA
GABA _A	Bicuculline	↑ DAMGO-induced feeding	↓ DAMGO-induced feeding
GABA _B	Saclofen	↓ DAMGO-induced feeding	↓ DAMGO-induced feeding
General and μ -selective opioid antagonists	NTX and β -FNA	↓ combination of DAMGO-Bicuculline feeding	N/A

Nacc GABA_B receptor antagonist and Nacc opioid-induced feeding:

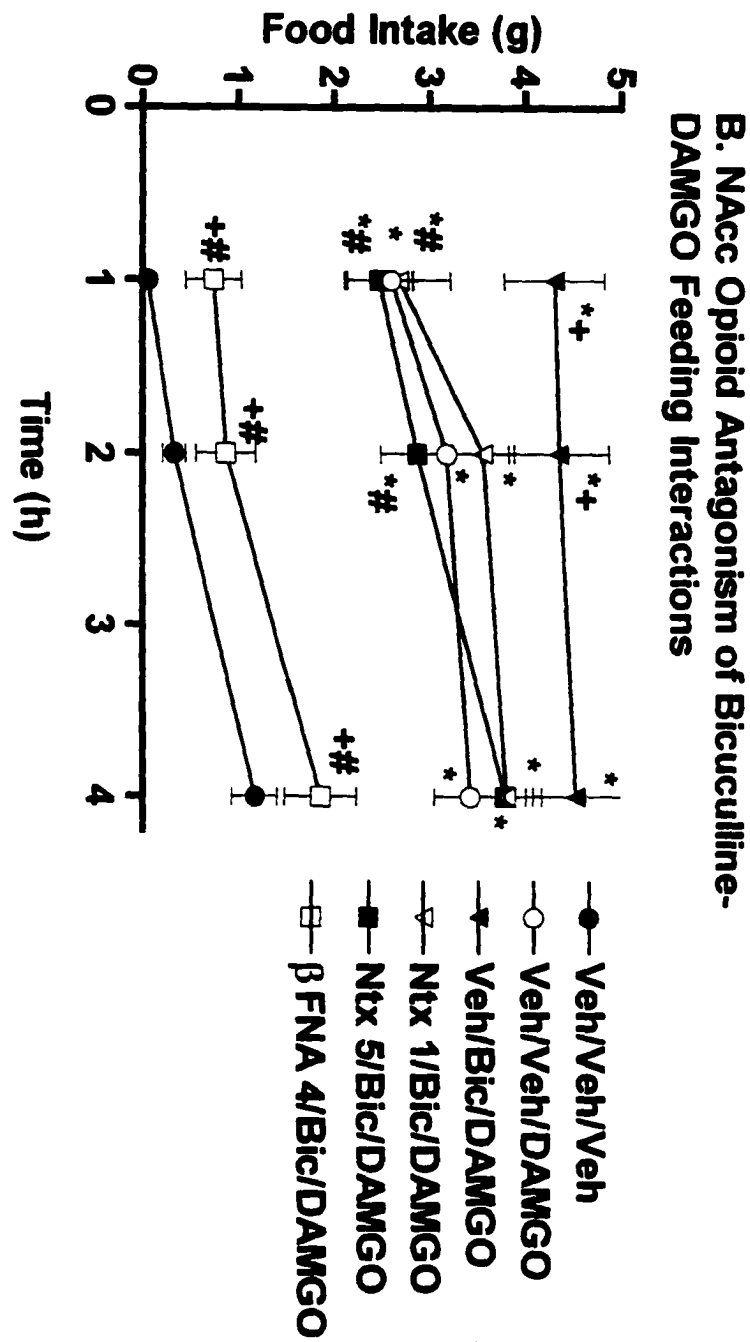
Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F=57.18$, $P<0.0001$), 2 ($F=58.16$, $P<0.0001$), and 4 ($F=34.08$, $P<0.0001$) h. DAMGO significantly increased food intake relative to control treatment after 1, 2, and 4 h. Pretreatment in the Nacc shell with the selective GABA_B receptor subtype antagonist, saclofen, significantly and dose-dependently decreased DAMGO-induced feeding with the higher (1000 and 1500 ng), but not lowest (500 ng) doses effective over the 4 h time course (Figure 1, lower panel). Administration of saclofen into the Nacc shell failed to alter food intake over the time course.

Nacc Opioid Antagonism of GABA_A antagonism/opioid-induced feeding:

Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F=41.93$, $P<0.0001$), 2 ($F=42.82$, $P<0.0001$), and 4 ($F=23.61$, $P<0.0001$) h. Paired injections of bicuculline and DAMGO in the Nacc shell significantly increased food intake relative to DAMGO alone after 1 and 2 h. Pretreatment in the NAcc shell with the selective μ opioid receptor subtype antagonist, β -funaltrexamine (4 μ g: 1–4 h), or the general opioid antagonist, naltrexone (5 μ g: 1–2 h), significantly and dose-dependently reduced feeding elicited by bicuculline and DAMGO (Figure 2). Bicuculline produced the same pattern of seizure activity in rats pretreated with the opioid antagonists.

Figure 2. Alterations in spontaneous food intake (g, $\pm$ S.E.M.) following either administration of the μ opioid agonist, DAMGO (2.5 μ g) or administration of combined DAMGO (2.5 μ g) and bicuculline (75 ng) in the Nacc shell relative to control treatment in rats pretreated with either the general opioid antagonist, naltrexone (1-5 μ g) or the μ opioid antagonist, β -funaltrexamine (4 μ g). The asterisks (*) indicate significant increases in food intake following either opioid agonist treatment or combined opioid agonist and GABA_A antagonist treatment relative to control treatment (Tukey comparisons, $P < 0.05$). The crosses (+) indicate significant increases in food intake following combined GABA_A antagonist and opioid agonist treatments in the Nacc shell relative to opioid agonist treatment alone (Dunnett comparisons, $P < 0.05$). The sign (#) indicates significant opioid antagonist-induced decreases in food intake relative to combined GABA_A antagonist and opioid agonist treatment in the Nacc shell (Dunnett comparisons, $P < 0.05$).

FIGURE 2



Nacc GABA and opioid antagonism of GABA_A agonist feeding: Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F=5.89$, $P<0.0019$), and 2 ($F=3.70$, $P<0.018$) h. Muscimol in the Nacc shell significantly increased food intake relative to control treatment after 1 and 2, but not after 4 h (see Table 10). Further, pairing the GABA_B antagonist, saclofen with muscimol in the Nacc shell significantly increased food intake after 1 and 2h. However when muscimol was paired with either the GABA_A antagonist bicuculline or the general opioid antagonist, naltrexone in the Nacc shell, food intake failed to significantly increase over control levels (Figure 3, upper panel).

Figure 3. Alterations in spontaneous food intake (g, $\pm$ S.E.M.) following administration of either GABA_A (muscimol, 50 ng, upper panel) or GABA_B (baclofen, 200 ng, lower panel) agonists in the Nacc shell relative to control treatment in rats pretreated with either the general opioid antagonist, naltrexone (5 μ g), the GABA_A antagonist, bicuculline (75 ng) or the GABA_B antagonist, saclofen (500 ng). The asterisks (*) indicate significant increases in food intake following GABA agonist treatment relative to control treatment (Tukey comparisons, $P < 0.05$). The crosses (+) indicate significant decreases in food intake following pretreatment with either opioid, GABA_A or GABA_B antagonists in the Nacc shell relative to GABA agonist treatment alone (Dunnett comparisons, $P < 0.05$).

FIGURE 3

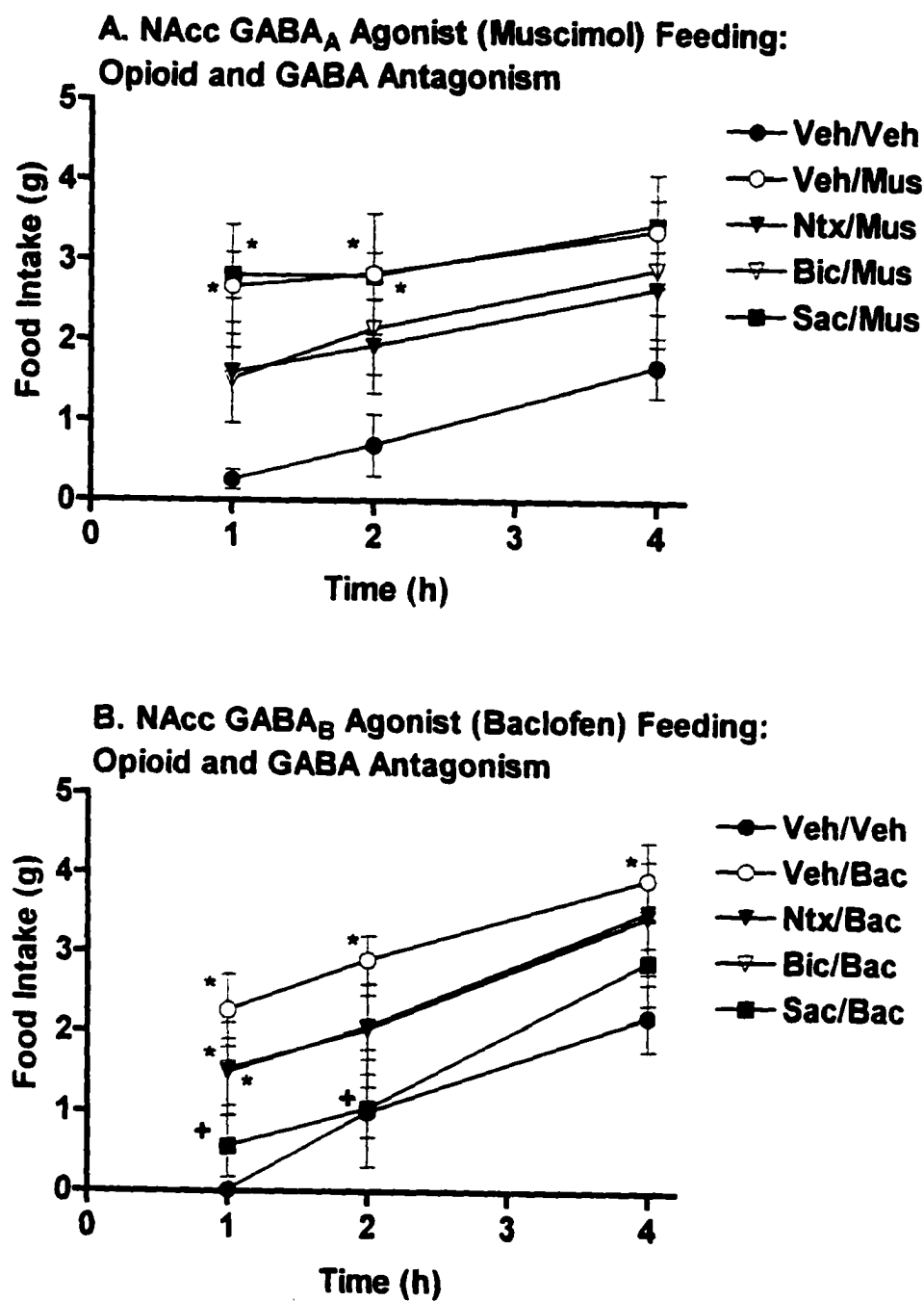


Table 10. GABA Agonists - Feeding Responses in the Nacc Shell and VTA

Receptor Subtype	Agonist	Nacc Shell	VTA
GABA _A	Muscimol	↑ Feeding; Musimol-induced feeding ↓ by NTX and bicuculline, but not by saclofen	No effect on feeding
GABA _B	Baclofen	↑ Feeding; Baclofen-induced feeding ↓ by saclofen, but by NTX or bicuculline	↑ Feeding; Baclofen-induced feeding ↓ by NTX and saclofen, but not bicuculline

Nacc GABA and opioid antagonism of GABA_B agonist feeding: Significant differences in spontaneous food intake were observed among injection conditions after 1 ($F=8.29$, $P<0.0001$), 2 ($F=4.98$, $P<0.003$), and 4 ($F=2.68$, $P<0.049$) h. Baclofen significantly increased food intake in the Nacc shell over the 4 h time course. Baclofen-induced feeding was eliminated by pretreatment in the Nacc shell with the GABA_B antagonist, saclofen after 1 and 2, but not after 4 h (Figure 3, lower panel). In contrast, pairing either bicuculline or naltrexone with baclofen in the Nacc shell resulted in significant increases in food intake after 1 h.

C. Discussion:

Since food intake is stimulated by administration of either opioid or GABA receptor subtype agonists in the Nacc shell, it was hypothesized that GABA antagonists should reduce μ -opioid agonist feeding elicited from the Nacc shell, and further hypothesized that opioid antagonism should reduce feeding elicited by GABA_A and GABA_B agonists in the Nacc shell. The primary and most surprising finding of the present study was that GABA receptor subtype antagonists in the Nacc shell exerted differential actions upon feeding elicited by the μ -selective opioid agonist, DAMGO. Thus, pretreatment with the GABA_A-selective antagonist, bicuculline in the Nacc shell produced significant and dose-dependent *increases* in DAMGO-induced feeding without altering food intake per se. Since DAMGO-induced feeding in the Nacc shell is blocked by both general (naltrexone) and μ -selective (β -funaltrexamine) opioid antagonists (Ragnauth et al., 2000a), the present study thus examined the effects of pretreatment with either naltrexone or β -funaltrexamine in the Nacc shell, and found that both antagonists blocked the enhanced food intake elicited by combinations of bicuculline and DAMGO in the Nacc shell. These data indicate that a local opioid, and particularly, a local μ opioid, synapse is essential for the full expression of this combined ingestive response.

It also appears that alterations in food intake elicited by the different bicuculline conditions were not due to the seizure behaviors noted in approximately 60% of the animals immediately after injection. Thus, such seizure behavior could not explain the increases in food intake following bicuculline-DAMGO pairing, the blocking of this effect by opioid antagonist pretreatment, or the lack of ingestive effects by bicuculline alone.

In contrast, pretreatment with the GABA_B-selective antagonist, saclofen in the Nacc shell produced significant and dose-dependent decreases in DAMGO-induced feeding without altering food intake per se. The differential actions of GABA receptor subtype antagonists upon DAMGO-induced feeding in the Nacc shell occurred at relatively comparable equimolar doses of saclofen (4-6 nmol) and bicuculline (1.6 nmol). In agreement with a previous study (Stratford and Kelley, 1997), the present study confirmed the receptor subtype specificity of GABA agonists and antagonists such that feeding elicited by the GABA_A-selective agonist, muscimol was prevented by pretreatment with the GABA_A (bicuculline), but not GABA_B (saclofen) antagonist in the Nacc shell, and feeding elicited by the GABA_B-selective agonist, baclofen was blocked by pretreatment with the GABA_B (saclofen), but not GABA_A (bicuculline) antagonist in the Nacc shell. Opioid mediation of GABA agonist-induced feeding was not as clear-cut. Naltrexone and muscimol treatment in the Nacc shell prevented the significant increases in feeding elicited by muscimol alone. In contrast, significant baclofen-induced feeding was present in naltrexone-pretreated rats. These data suggest that opioid antagonism in the Nacc shell produces greater effects upon feeding elicited by GABA_A than GABA_B receptor agonists. The lack of clear-cut significant reductions induced by opioid antagonist upon GABA agonist-induced feeding requires a caveat concerning interpretation. Future studies using selective opioid receptor subtype antagonists may clarify these latter findings. Thus, in addition to dependence upon functioning opioid (μ , δ_1 , δ_2 and κ_1), and D₁ dopamine receptors for the full expression of DAMGO-induced feeding in the Nacc shell (Ragnauth et al., 2000a; Ragnauth et al., 2000b), the present study indicates that μ -opioid agonists

rely upon intact GABA receptors in the Nacc shell for a normal ingestive response.

What evidence is there to indicate why and how GABA receptor subtypes in the Nacc shell differentially regulate feeding elicited by μ opioid agonists? A simple explanation for the ability of the GABA_B antagonist, saclofen to reduce DAMGO-induced feeding in the Nacc shell is that this relevant GABA receptor is “downstream” from the μ -opioid receptor activated by DAMGO. Moreover, since the GABA_B agonist, baclofen produces feeding that is not significantly affected by naltrexone pretreatment in the Nacc shell, this suggests that this “downstream” GABA_B receptor in the Nacc shell is not dependent upon an opioid synapse for its full activation. Explanations for the interaction between μ -opioid and GABA_A receptors in mediating feeding in the Nacc shell are more complex. First, since significant feeding elicited by the GABA_A receptor agonist, muscimol, is prevented by either GABA_A (bicuculline) or general opioid (naltrexone) antagonist pretreatment in the Nacc shell, this argues that the relevant opioid synapse is either coincident with or “downstream” from the GABA_A receptor. Second, since either general or selective μ opioid antagonist pretreatment in the Nacc shell blocks the enhanced feeding response elicited by combined bicuculline and DAMGO administration, this also argues that the relevant opioid synapse is either coincident with or “downstream” from neurons with opioid and/or GABA_A receptors. Third, since bicuculline enhances DAMGO-induced feeding without eliciting feeding by itself in the Nacc shell, this suggests that the subpopulation of GABA_A receptors mediating this latter response differs from the subpopulation of GABA_A receptors mediating muscimol-induced feeding in the Nacc shell, and therefore, this former subpopulation should be coincident with μ -opioid receptors

mediating DAMGO-induced feeding. There is no direct anatomical or biochemical evidence testing these hypotheses, although our laboratory has just begun a study examining whether DAMGO in the Nacc shell activates Fos-like immunoreactivity, and whether such potential activation is differentially mediated by GABA_A and GABA_B receptor subtype antagonists.

There is some indirect evidence suggesting the possibility of interrelationships between GABA and opioids in the Nacc. First, reciprocal inhibitory actions exist between GABA and opioid systems in the Nacc such that systemic morphine withdrawal inhibits GABA release in a brain-slice preparation (Chieng and Williams, 1998; Napier and Mitrovic, 1999), and GABA agonists inhibit systemic heroin-induced increases in Nacc dopamine (Gerasimov et al., 1999). While these studies suggest that some form of mutual inhibition occurs, the fact that at least one of the manipulations occurred outside of the Nacc shell does not argue conclusively for a localized interaction. Second, both GABA and enkephalin are found together in spiny cells in the Nacc (Meredith, 1999), and form reciprocal projections between both the Nacc and the ventral pallidum and the Nacc and the VTA (Churchill et al., 1992; Churchill and Kalivas, 1992; Kalivas et al., 1993; Meredith et al., 1993; Van Bockstaele and Pickel, 1995; Zahm et al., 1985). Indeed, electron microscopy studies indicate that μ -opioid receptor-like immunoreactivity can be found on GABA-containing neurons in the Nacc (Svingos et al., 1997). Third, GABA is contained in medium spiny neurons within the Nacc which form both extrinsic projections as well as local dense networks overlapping their own dendrites, suggesting that they regulate signal-to-noise ratios of the modulatory actions of other endogenous

neurotransmitters including the opioids (Shi and Rayport, 1994). Finally, there is evidence for differential actions of GABA receptor subtype antagonists in mediating functional effects within the Nacc with some effects mediated through GABA_A receptors and others mediated through GABA_B receptors. Thus, microdialysis studies indicate that local bicuculline, but not saclofen infusions dose-dependently increased extracellular acetylcholine in the Nacc, while all GABA agonists decreased this signal (Rada et al., 1993). In contrast, baclofen, but not muscimol infusions decreased local levels of serotonin in the Nacc (Tao et al., 1996). Moreover, Nacc pretreatment with baclofen, but not muscimol decreased cocaine-maintained behaviors (Shoaib et al., 1998). Given the role of the Nacc in reinforced behavior (e.g., see review: Salamone, 1994), it is important to clearly elucidate the complex neurochemical systems (Meredith, 1999) mediating the wide variety of rewarding, hedonic and homeostatic responses maintained by the Nacc, including the mediation of ingestive responses.

Some caution is warranted in making conclusions about the site-specific actions of the pharmacological agents employed in the current study given the methodological issues related to drug diffusion. It has been demonstrated that injectate volume of 1.0 μ g will remain within a 1 mm radius sphere of the injection site (Myers, 1996; Lomax, 1966). However, this presents an issue for the current study given that the neighboring Nacc core and ventral striatum are less than 1 mm from the Nacc shell. Furthermore, Schroeder et al. (1991) has demonstrated that when naltrexone is microinjected into the hindbrain, less than 5% of the drug remained in the injected area after 15 min. However, some support for the conclusion that the actions of GABA agonists/antagonists upon opioid-induced

feeding occurred in the Nacc shell are the series of studies that have been conducted by Kelley and co-workers. Bakshi and Kelley (1993a; 1993b) administered morphine into five striatal subregions and demonstrated the most robust feeding response into the anteroventromedial regions, which includes the Nacc shell, and minimal increases in food intake into proximal dorsal, lateral, and posterior striatal sites. In addition, naloxone infused into dorsolateral striatum had no effect on sucrose drinking (Kelley et al., 1996). Furthermore, regional mapping studies of the striatal regions have demonstrated that the GABA_A agonist muscimol elicited the most robust feeding response in the ventromedial Nacc shell (Stratford and Kelley, 1997; Basso and Kelley, 1999). Pecina and Berridge (2000) conducted a microinjection study investigating Fos immunoreactivity with six placements within the accumbens shell region and six placements within the Nacc core. Fos plumes from morphine microinjections that increased eating were compared to plumes from nearby ineffective microinjection sites. It was shown that the accumbens opioid eating site was localized to medial caudal subregion of the Nacc shell. In addition, animals with misplaced cannulae placements in proximity to the Nacc shell in the current study did not show significant feeding responses.

VI. EXPERIMENT 2. EAA Receptor Subtype Agonists Induce Feeding in the Nacc Shell in Rats: Opioid Antagonists Actions and Interactions with the μ opioid agonist, DAMGO

A. Specific Procedures:

Protocol 1: In assessing whether AMPA induced feeding, the first group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n=12), b) AMPA 0.05 μ g (0.2 nmol, 0.025 μ g each side, n=7), c) AMPA 0.25 μ g (1.0 nmol, 0.125 μ g each side, n=7), or d) AMPA 0.5 μ g (2.0 nmol, 0.25 μ g each side, n=8). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 2: In assessing whether opioid antagonism altered AMPA-induced feeding, the second group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n=18), b) AMPA (0.5 μ g, n=8), and naltrexone at doses of c) 0.1 μ g (0.26 nmol, 0.05 μ g each side, n=6), d) 1 μ g (2.6 nmol, 0.5 μ g each side, n=5) or e) 10 μ g (26 nmol, 5 μ g each side, n=6) paired with AMPA (0.5 μ g). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection. To assess whether naltrexone altered food intake per se under these conditions in Protocols 2 and 4, an additional subgroup of six rats received vehicle and naltrexone (10 μ g) with food intake assessed 1, 2 and 4 h thereafter.

Protocol 3: In assessing whether NMDA induced feeding, the third group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle (n=10), b) NMDA 0.03 μ g (0.2 nmol, 0.015 μ g each side, n=7), c) NMDA 0.3 μ g (2 nmol, 0.15 μ g each side, n=8), d) NMDA 1.0 μ g (6.67 nmol, 0.5 μ g each side, n=8) or e)

NMDA 3.0 μg (20 nmol, 1.5 μg each side, $n=7$). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 4: In assessing whether opioid antagonism altered NMDA-induced feeding, the fourth group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle ($n=7$), b) NMDA (1.0 μg , $n=7$), and naltrexone at doses of c) 0.1 μg ($n=5$), d) 1.0 μg ($n=5$), or e) 10 μg ($n=6$) paired with NMDA. Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 5: In assessing whether cotreatment with either AMPA or NMDA altered DAMGO-induced feeding, the fifth group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle ($n=15$), b) DAMGO 2.5 μg (4.8 nmol, 1.25 μg each side, $n=15$), and DAMGO (2.5 μg ,) paired with either c) AMPA (0.5 μg , $n=6$) or NMDA at doses of either d) 0.3 ($n=9$) or e) 1.0 ($n=8$) μg . Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 6: In assessing whether opioid antagonism altered enhanced feeding responses elicited by AMPA and DAMGO cotreatment, the sixth group of rats received the following bilateral microinjection conditions in the Nacc shell: a) vehicle ($n=18$), b) DAMGO (2.5 μg , $n=18$), c) AMPA (0.5 μg) paired with DAMGO (2.5 μg) ($n=6$), d) naltrexone (1.0 μg , $n=6$) paired with AMPA (0.5 μg) and DAMGO (2.5 μg), or β -funaltrexamine at doses of e) 1.3 μg (2.67 nmol, 0.65 μg each side, $n=6$) or f) 4.0 μg (8.2 nmol, 2.0 μg each side, $n=6$) paired with AMPA (0.5 μg) and DAMGO (2.5 μg). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

B. Results:

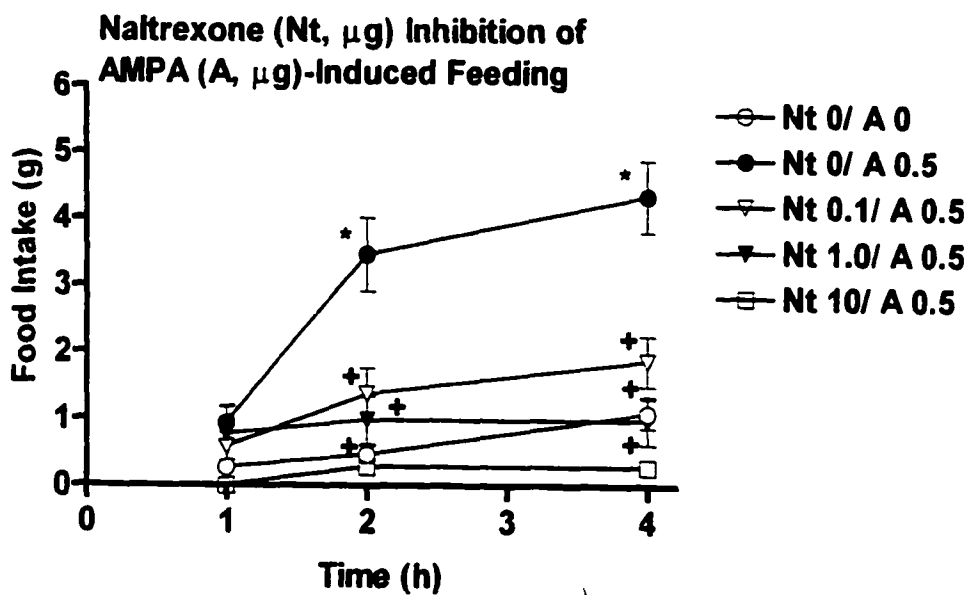
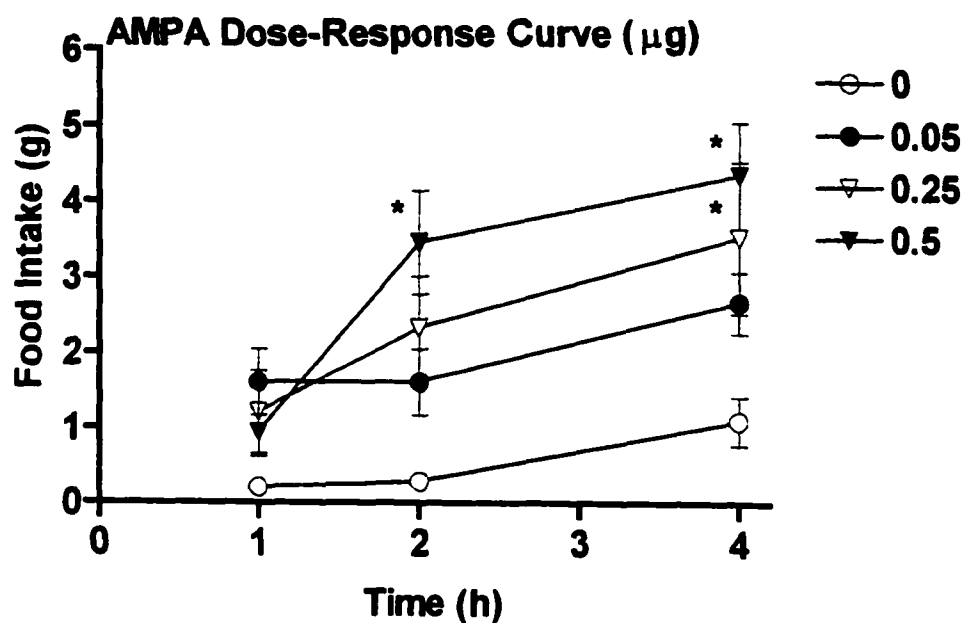
Histological verification: Bilateral cannulae placements were all localized to the shell region of the Nacc which were medial to the core region and lateral to the ventral diagonal band area; these placements were similar to those reported previously by this laboratory (Bodnar et al., 1995; Ragnauth et al., 2000a; Ragnauth et al., 2000b; Znamensky et al., 2001).

AMPA-induced feeding elicited from the Nacc shell: Significant differences in food intake were observed among injection conditions after 1 ($F(3, 36) = 3.75, P < .019$), 2 ($F = 10.09, P < 0.0001$) and 4 ($F = 8.75, P < 0.0002$) h. AMPA produced dose-dependent and time-dependent increases in food intake relative to control treatment following administration into the Nacc shell with the highest (0.5 μg) dose significantly increasing intake after 2 and 4 h, and the intermediate (0.25 μg) dose significantly increasing intake after 4 h (Figure 4, upper panel). In addition, casual observation of the animals found that AMPA in the Nacc shell produced increases in motor activity in 60% of the animals that persisted for approximately 5-15 min after the injection. The highest AMPA dose (0.5 μg) produced seizures in 3 of the 8 tested rats that persisted for less than 2 min immediately following the microinjection.

Opioid Antagonism of AMPA-induced feeding elicited from the Nacc shell: Significant differences in food intake were observed among injection conditions after 1 ($F(4, 72) = 8.41, P < .0001$), 2 ($F = 35.13, P < .0001$) and 4 ($F = 52.23, P < .0001$) h. The significant increases in AMPA-induced feeding elicited from the Nacc shell after 2 and 4 h were completely eliminated by pretreatment with all naltrexone doses ranging from 0.1 to

Figure 4. Upper Panel: Alterations in food intake (g, $\pm$ S.E.M.) following administration of AMPA (0.05-0.5 μ g) in the Nacc shell relative to control treatment. Lower Panel: Alterations in food intake (g, $\pm$ S.E.M.) following administration of AMPA (0.5 μ g) in the Nacc shell relative to control treatment in rats pretreated with the general opioid antagonist, naltrexone (0.1-10 μ g). The asterisks (*) in this and subsequent figures indicate significant increases in food intake following agonist relative to control treatment (Tukey comparisons, $P < 0.05$). The crosses (+) in this and subsequent figures indicate significant decreases in food intake following antagonist pretreatment relative to agonist treatment alone (Dunnett comparisons, $P < 0.05$).

FIGURE 4



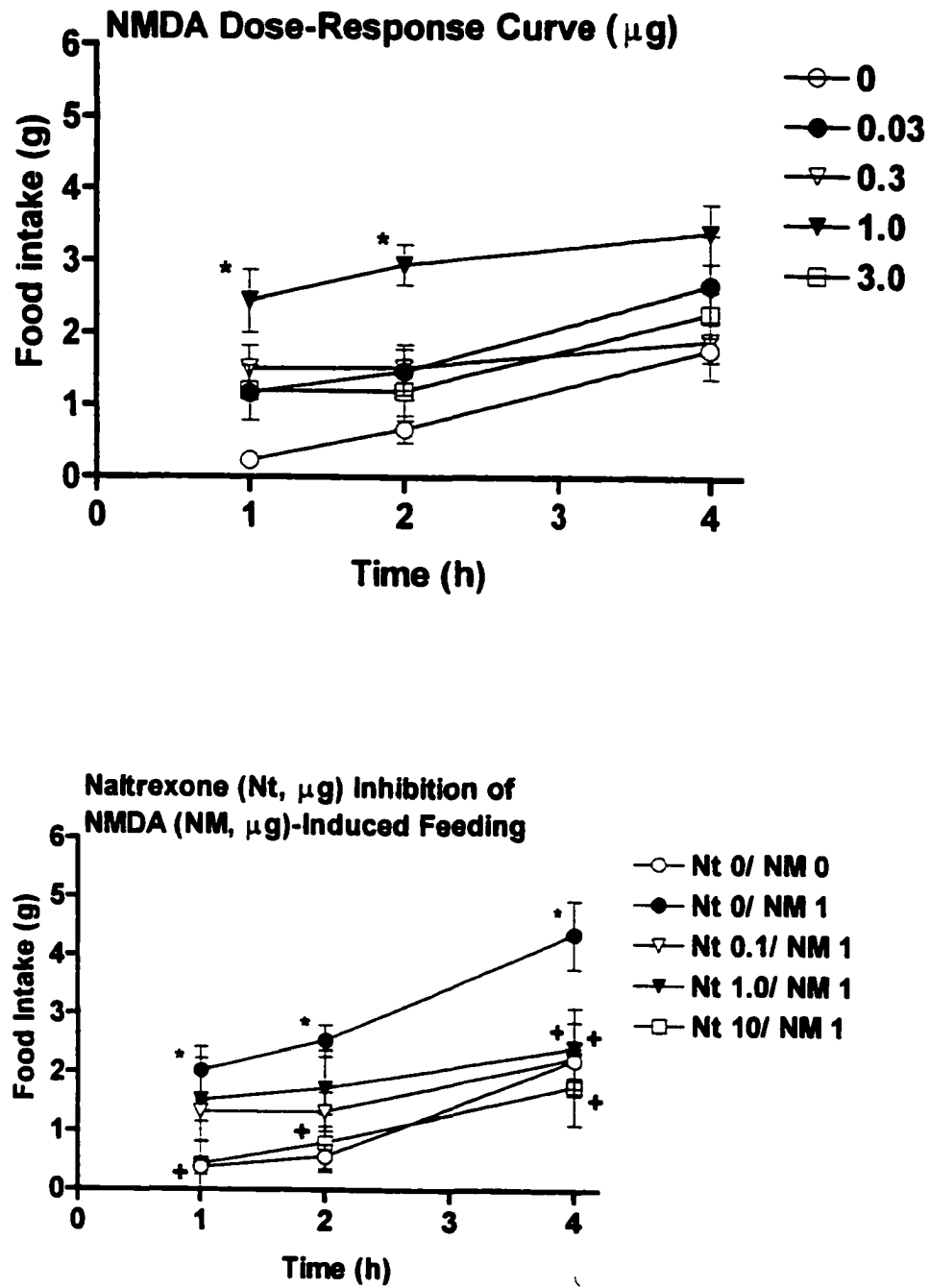
10 μg (Figure 4, lower panel). The elimination of AMPA-induced feeding in the Nacc shell by naltrexone was not due to an inhibitory effect of naltrexone on this form of intake per se since naltrexone itself failed to alter intake relative to vehicle treatment after 1 ($t(5) = 1.56$, n.s.: vehicle ($0.45, \pm 0.21$ g); naltrexone ($1.35, \pm 0.60$ g)), 2 ($t = 2.18$, n.s.: vehicle ($0.65, \pm 0.35$ g); naltrexone ($1.67, \pm 0.60$ g)) or 4 ($t = 2.37$, n.s.: vehicle ($1.10, \pm 0.43$ g); naltrexone ($2.30, \pm 0.64$ g)) h.

NMDA-induced feeding elicited from the Nacc shell: Significant differences in food intake were observed among injection conditions after 1 ($F(4, 36) = 6.64$, $P < 0.0004$) and 2 ($F = 8.68$, $P < 0.0001$) h, but not after 4 h. NMDA in the Nacc shell produced an inverted U-shaped function which dose-dependently (1 μg) and time-dependently (1-2 h) increased food intake (Figure 5, upper panel). A lower (0.03-0.3 μg) NMDA dose range, including a dose equimolar to an effective AMPA dose, was ineffective in stimulating feeding elicited from the Nacc shell. Further, a higher (3.0 μg) NMDA dose in the Nacc shell failed to elicit feeding over the 4 h time course, and was preceded in 5 of the 7 rats by increased motor activity which persisted for up to 5-20 min following injection. The highest NMDA dose (3.0 μg) also produced seizures in 3 of the 7 tested rats that persisted for less than 2 min immediately following the microinjection.

Opioid antagonism of NMDA-induced feeding elicited from the Nacc shell: Significant differences in food intake were observed among injection conditions after 1 ($F(4, 24) = 3.84$, $P < .0001$), 2 ($F = 4.91$, $P < .005$) and 4 ($F = 5.32$, $P < .0033$) h. The significant increases in NMDA-induced feeding elicited from the Nacc shell were completely eliminated across the time course by naltrexone pretreatment at a 10 μg dose

Figure 5. Upper Panel: Alterations in food intake (g, $\pm$ S.E.M.) following administration of NMDA (0.03-3.0 μ g) in the Nacc shell relative to control treatment. Lower Panel: Alterations in food intake (g, $\pm$ S.E.M.) following administration of NMDA (1.0 μ g) in the Nacc shell relative to control treatment in rats pretreated with the general opioid antagonist, naltrexone (0.1-10 μ g).

FIGURE 5



(Figure 5, lower panel). The two lower (0.1 and 1.0 μg) naltrexone doses only eliminated NMDA-induced feeding elicited from the Nacc shell after 4 h.

Feeding elicited by DAMGO and either AMPA or NMDA cotreatment in the

Nacc shell: Significant differences in food intake were observed among injection

conditions after 1 ($F(4, 56) = 19.08, P < .0001$), 2 ($F = 40.86, P < .0001$) and 4 ($F = 69.15, P < .0001$) h. DAMGO in the Nacc shell produced significant increases in food intake across the 4 h time course (Figure 6). Cotreatment of AMPA and DAMGO in the Nacc shell significantly enhanced feeding after 2 and 4 h relative to both vehicle alone and to DAMGO alone. In contrast, cotreatment of an equimolar dose (0.3 μg) of NMDA with DAMGO in the Nacc shell failed to alter the magnitude of DAMGO-induced feeding. Indeed, cotreatment of a higher (1.0 μg) dose of NMDA with DAMGO in the Nacc shell significantly reduced the magnitude of DAMGO-induced feeding after 2 and 4 h.

Opioid antagonism of feeding elicited by AMPA-DAMGO cotreatment in

the Nacc shell: Significant differences in food intake were observed among injections conditions after 1 ($F(4, 68) = 34.74, P < .0001$), 2 ($F = 45.12, P < .0001$) and 4 ($F = 66.26, P < .0001$) h. The significant increases in food intake across the time course following cotreatment of AMPA and DAMGO in the Nacc shell were completely eliminated by pretreatment with either a 1.0 μg dose of naltrexone or either the 1.3 or 4.0 μg doses of the selective μ opioid antagonist, β -FNA (Figure 7). (see Table 11).

Figure 6. Alterations in food intake (g, $\pm$ S.E.M.) following cotreatment of either DAMGO (2.5 μ g) and AMPA (0.5 μ g) or DAMGO (2.5 μ g) and NMDA (0.3-1.0 μ g) in the Nacc shell relative to control or DAMGO treatment alone.

FIGURE 6

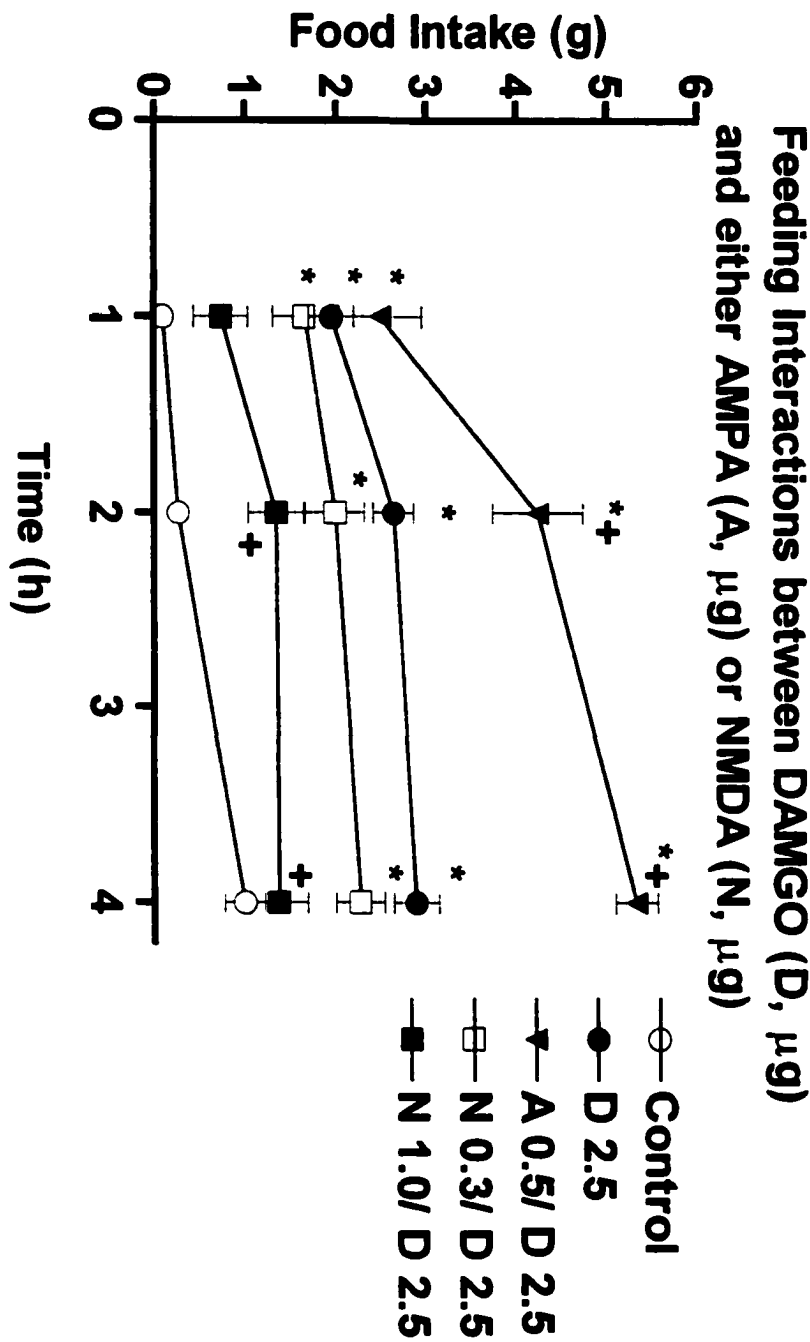


Figure 7. Alterations in food intake (g, $\pm$ S.E.M.) following cotreatment of DAMGO (2.5 μ g) and AMPA (0.5 μ g) in the Nacc shell relative to control treatment in rats pretreated with either the general opioid antagonist, naltrexone (1.0 μ g) or the μ opioid antagonist, β -FNA (1.3-4.0 μ g).

FIGURE 7

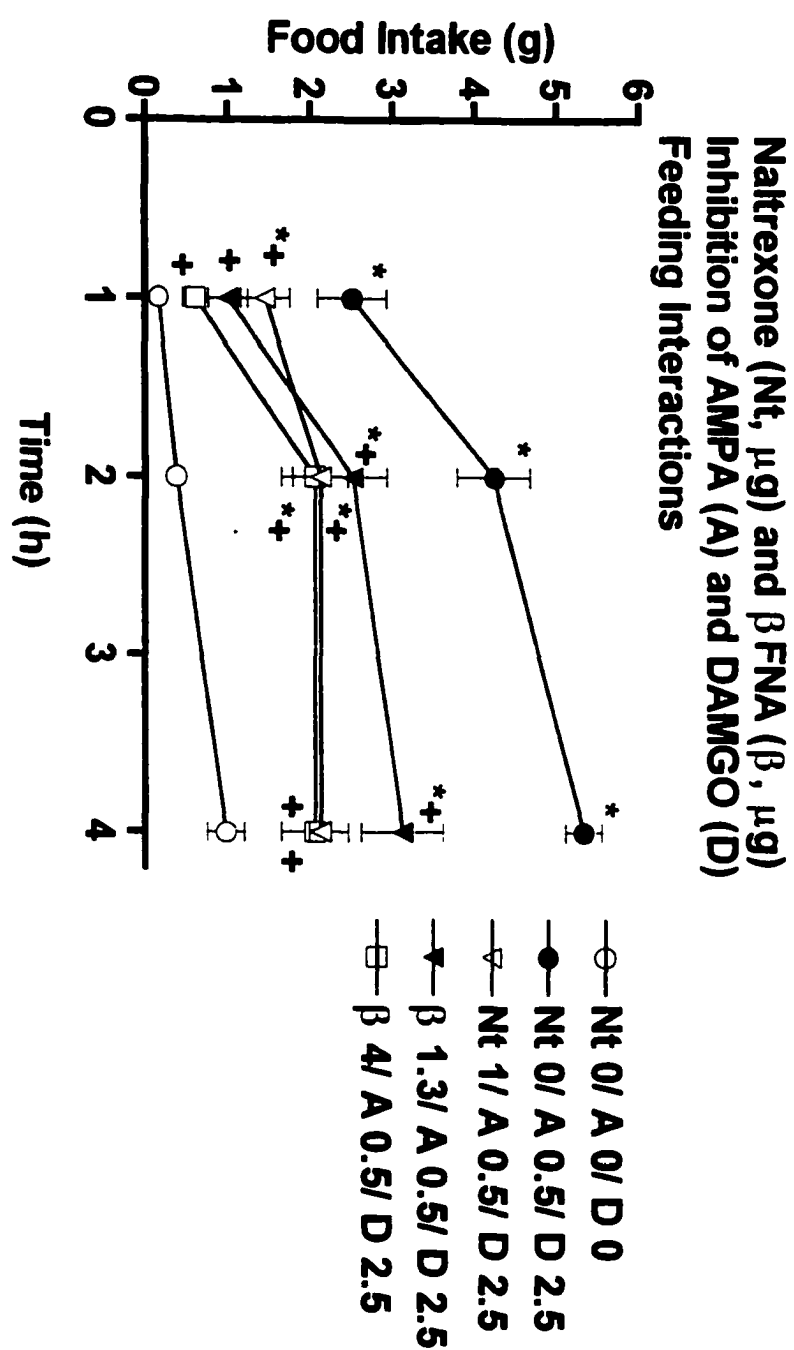


Table 11. EAA agonist-DAMGO Feeding Interactions

Receptor Subtype	EAA agonist-induced feeding	μ + EAA on Feeding Behavior
AMPA	$\uparrow$ feeding in Nacc shell; NTX $\downarrow$ AMPA-induced feeding	DAMGO + AMPA in Nacc shell = $\uparrow$ feeding; NTX $\downarrow$ DAMGO + AMPA feeding
NMDA	$\uparrow$ feeding in Nacc shell; NTX $\downarrow$ NMDA-induced feeding	DAMGO + NMDA in Nacc shell = $\downarrow$ feeding

C. Discussion:

AMPA-induced feeding elicited from the Nacc shell: The findings by Kelley and co-workers (Kelley, 1999a; Kelley and Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998) that the AMPA/kainate antagonist, DNQX produced an intense ingestive response following administration into the Nacc shell supported the concept that removal of tonic excitatory inputs in the Nacc shell with selective AMPA antagonists disinhibits neural circuitry involved in the initiation of ingestive behavior (Kelley, 1999a). Based on this line of reasoning, it would then be hypothesized that AMPA pretreatment in the Nacc shell should fail to alter feeding per se, but should reduce feeding elicited by DAMGO in the Nacc shell by producing local tonic inhibition. Alternatively, since EAA agonists are capable of stimulating food intake when applied to specific sites like the lateral hypothalamus (Khan et al., 1999; Stanley et al., 1993a; Stanley et al., 1996; Stanley et al., 1993b), an alternative hypothesis would suggest that the EAA agonists, AMPA and NMDA, would also increase feeding elicited from the Nacc shell, and potentially enhance feeding elicited by DAMGO. This latter hypothesis was confirmed since administration of AMPA into the Nacc shell elicited significant and dose-dependent increases in feeding behavior, albeit across a different time course from DNQX. Whereas DNQX in the Nacc shell elicited an intense, short-latency and short-duration feeding response in a 30-min ingestive test (Kelley and Swanson, 1997; Maldonado-Irizarry et al., 1995; Stratford et al., 1998), AMPA in the Nacc shell failed to elicit significant feeding at any dose after 1 h, but significantly increased feeding after 2 and 4 h.

Thus, although it appears that the similar stimulatory actions of AMPA agonists

and antagonists in the Nacc shell are time-dependent, why would they produce similar stimulatory actions upon feeding behavior in the Nacc shell? Kelley and co-workers (Kelley, 1999a) proposed that removal of tonic excitatory inputs in the Nacc shell by selective AMPA antagonists disinhibits neural circuitry involved in the initiation of ingestive behavior. However, the similar stimulatory actions of AMPA agonists and antagonists within the Nacc shell strongly suggest that a particular pattern of EAA activity is necessary to maintain a normal level of ingestive behavior, and that either increases or decreases in these patterns leads to increased intake. Support for the concept of opposite changes in neurotransmitter patterns leading to similar alterations in intake can be found in dopaminergic effects upon ingestion. For instance, general and particularly D_1 -selective agonists and antagonists decrease food intake under palatable, deprivation, glucoprivic sham feeding and free feeding conditions (Gilbert and Cooper, 1985; Hobbs et al., 1994; Martin-Iverson and Dourish, 1988; Rusk and Cooper, 1989; Schaefer et al., 1994; Terry, 1996). Indeed, the Nacc shell has been a proposed site of action for many of these dopaminergic ingestive effects (e.g., see review: Salamone, 1994). However, the findings of delayed AMPA feeding (after 2 hours) is unexpected given that EAA transmitters operate through ligand-gated channels and do not use G-protein coupling. A possible explanation for these findings is the modulation of AMPA responses by protein kinases and/or phosphorylation (Bettler and Mülle, 1995; Gellerman et al., 1997). For example, the catalytic subunit of protein kinase A (PKA) increases AMPA-receptor function when added to the intracellular medium. However, PKA does not modulate AMPA receptors through phosphorylation, and therefore it is suggested that there is an indirect effect

through regulatory proteins (Bettler and Mulle, 1995). Demonstration of phosphorylation of AMPA receptors in vitro by Ca^{2+} calmodulin-dependent kinase (CAM-KII), and also by protein kinase C (PKC) has been shown (Bettler and Mulle, 1995). The evidence for modulation of AMPA receptors through phosphorylation, the action of protein kinases, and possibly other regulatory proteins may have functional consequences (Gellerman et al., 1997). Thus, a cascade of biochemical events may be involved in the delayed feeding response with AMPA administration in the Nacc shell.

NMDA-induced feeding in the Nacc shell: Two competing hypotheses could also be proposed for a putative role for NMDA receptors in the Nacc shell in feeding behavior. On the one hand, the non-competitive NMDA receptor antagonist, MK-801 significantly increases food intake under satiated and deprived as well as normal and palatable conditions (Burns et al., 1998; Burns and Ritter, 1997) through increased gastric emptying and vagal afferent activation of the NTS and DVC (Treece et al., 1998; Treece et al., 2000); this would suggest that NMDA itself in the Nacc shell should either fail to affect or reduce intake. On the other hand, NMDA, like AMPA microinjected into the lateral hypothalamus stimulates food intake (Stanley et al., 1993a; Stanley et al., 1993b). Again, the latter hypothesis was confirmed since administration of NMDA in the Nacc shell also elicited significant feeding responses. NMDA displayed a shorter latency of peak effects, a shorter duration of action, and a less potent response than AMPA across an equimolar dose range. Indeed, the increased food intake elicited by NMDA and AMPA in the Nacc shell was quite similar in magnitude and potency to the ability of lateral hypothalamic infusions of either glutamate, kainic acid, AMPA or NMDA to selectively

stimulate feeding (Stanley et al., 1993a; Stanley et al., 1993b). However, the stimulatory actions of EAA agonists upon feeding elicited from the lateral hypothalamus and Nacc appear to activate different circuits since microdialysis studies indicate that feeding increases extracellular glutamate in the lateral hypothalamus, and decreases extracellular glutamate in the Nacc (Rada et al., 1997). Conclusions about the site-specificity of NMDA actions in the Nacc shell are tentative at this point given that NMDA drugs are hydrophilic and may diffuse out of the injected site (Spinella et al., 1996; Naastrom et al., 1993a; Naastrom et al., 1993b). It has been demonstrated that both competitive and non-competitive NMDA receptor antagonists redistribute rostrally towards the brainstem after intrathecal administration (Naastrom et al., 1993b). However, Spinella et al. (1996) has shown that when NMDA antagonists, AP7 or MK-801, were administered into sites lateral and dorsal to the injected site, e.g. RVM, they failed to alter morphine analgesia elicited from the mesencephalic periaqueductal gray. In the current study, given that a low dose of NMDA (1 μ g) was administered into the Nacc shell, there may be less likelihood that the feeding responses occurred even if diffusion to adjacent brain sites occurred.

The feeding responses elicited by both NMDA and AMPA in the Nacc shell were preceded by increased motor activity and seizure activity in subsets of the tested rats, particularly at the higher doses. It should be noted however that the time courses of these behaviors (15-20 min and 2 min respectively) were considerably shorter than the onset of the ingestive responses. Although one might speculate that one or both behaviors might have elicited subsequent feeding behavior, it should be noted that EAA agonist-induced seizure and motor behaviors could not explain subsequent feeding since they occurred

both in the presence (following AMPA and NMDA alone) and absence (following opioid antagonism) of feeding. Further studies investigating EAA-induced alterations in behavior in the Nacc shell should also include formal measures of locomotor and seizure activity.

Opioid Antagonism of AMPA- and NMDA-induced feeding: Feeding elicited by either AMPA or NMDA administered into the Nacc shell was significantly and dose-dependently blocked by pretreatment with the general opioid antagonist, naltrexone, indicating that a local opioid synapse is necessary for the full expression of feeding elicited by these EAA agonists. Importantly, naltrexone's antagonism of AMPA-induced or NMDA-induced feeding was not due to generalized inhibition by naltrexone in the Nacc shell since the antagonist alone failed to alter food intake relative to vehicle under these conditions. The failure of naltrexone in the Nacc shell to reduce feeding in non-deprived animals during the light cycle is in contrast to its marked inhibitory effects in the Nacc shell upon food intake in rats following either food deprivation, 2-deoxy-D-glucose-induced glucoprivation, or acute exposure to a palatable 10% sucrose solution (Bodnar et al., 1995; Kelley et al., 1996). Such a pattern of differential results is consistent with the greater ability of naltrexone to inhibit food intake under deprivation and palatable conditions (Levine et al., 1982; Rudski et al., 1994) relative to intake under normal, non-deprived conditions tested in the present study.

EAA agonist interactions with opioid-induced feeding: Differential feeding interactions were observed following cotreatment of particular EAA agonists with the μ -selective opioid agonist, DAMGO. Thus, significant enhancements in food intake were noted when AMPA and DAMGO were co-administered into the Nacc shell relative to

either DAMGO alone or AMPA alone. This enhanced feeding response appeared to involve opioid and particularly μ receptor synapses since pretreatment in the Nacc shell with either general (naltrexone) or μ -selective (β -FNA) opioid antagonists dose-dependently reduced the increased intake following Nacc shell cotreatment of AMPA and DAMGO. In contrast, cotreatment of NMDA at a dose equimolar to that of the effective AMPA dose with DAMGO in the Nacc shell failed to alter food intake relative to that observed following Nacc shell administration of DAMGO alone. Indeed, cotreatment of a higher dose of NMDA with DAMGO in the Nacc shell significantly reduced the magnitude of DAMGO-induced feeding.

The precise mechanisms by which μ opioid and EAA agonists interact with each other is unknown, but some strong suggestive evidence exists. First, based on initial mapping studies (Bakshi and Kelley, 1993a), it appears that opioid-induced feeding is far more effective when elicited from the Nacc shell, as compared to the Nacc core and surrounding striatal regions. A recent c-fos study by Pecina and Berridge (2000) demonstrates that sites supporting the most potent hedonically-driven feeding responses to morphine are in the Nacc shell, and selectively activate the medial caudal subregion of the Nacc shell and the rostral ventral pallidum, but fail to penetrate the Nacc core. The neurochemical and cytoarchitectural definition of the Nacc core and shell (e.g., Jongen-Relo et al., 1993; Meredith et al., 1989) has been discussed in terms of close appositional relationships between distributions of opioid peptides and opioid receptors in the Nacc core (Jongen-Relo et al., 1993), and the well-described opioid peptide and opioid receptor distribution mismatches in the Nacc shell (Herkenham et al., 1984; Jongen-Relo et al.,

1993; Voorn et al., 1989), suggestive of opioid modulatory actions for the latter structure. Indeed, the opioid clusters in the Nacc shell (Herkenham et al., 1984) are found in an area that is considered a transition zone between the extended amygdala and the rest of the striatum (Alheid and Heimer, 1988; Meredith and Totterdell, 1999), suggesting limbic involvement.

Major excitatory input into the Nacc shell arises from a number of structures including the entorhinal and prefrontal cortex, the hippocampus, particularly the subiculum, and the basal amygdala (Brog et al., 1993; Groenewegen et al., 1987; Meredith and Totterdell, 1999; Sesack and Pickel, 1990; Sesack and Pickel, 1992; Totterdell and Meredith, 1997; Totterdell and Smith, 1989). These cortical inputs most typically use glutamate as the major neurotransmitter (Meredith et al., 1990; Sesack and Pickel, 1992; Totterdell and Meredith, 1997). Glutamate receptors of the AMPA/kainate and NMDA subtypes mediate glutamate transmission in the Nacc shell (Pennartz et al., 1990). Whereas AMPA receptors are localized primarily on post-synaptic sites, NMDA receptors in the Nacc shell are found on both presynaptic and post-synaptic sites, primarily on dendrites, but also on axons and terminals (Gracy and Pickel, 1996; Gracy et al., 1997; Tarazi et al., 1998). Indeed, ultrastructural studies indicate that μ opioid receptors and NMDA receptors display dual localization in the Nacc shell, and act at either similar common dendrites and/or presynaptic NMDA receptor afferents containing μ -opioid receptor dendrites (Gracy et al., 1997b). Further, dynorphin activation of κ opioid receptors also acts upon EAA activity through either presynaptic release or postsynaptic responses (Svingos et al., 1999), although this latter mechanism is unlikely to explain the present

results given the inability of κ -opioid agonists in eliciting feeding following administration into the Nacc shell (Bakshi and Kelley, 1993a). Moreover, locally-identified opioid and particularly enkephalinergic, peptide neurons in the Nacc (Meredith et al., 1993; Van Bockstaele et al., 1994) have been proposed to sharpen responses to the cortical Nacc input through lateral inhibition of axon collaterals (Meredith and Totterdell, 1999; Pennartz et al., 1993; Pennartz et al., 1994). If this cortical input is glutaminergic, this could be a proposed mechanism of interaction between opioid and EAA agonists.

Given these potential interactions between opioid and EAA systems within the Nacc shell, why did AMPA cotreatment act to enhance μ -opioid agonist-induced feeding, while NMDA cotreatment act to reduce μ -opioid agonist-induced feeding? Within the Nacc core, DAMGO decreases both NMDA-mediated and non-NMDA-mediated excitatory postsynaptic potential amplitudes induced by local stimulation which is blocked by general and μ opioid antagonism (Martin et al., 1997). However, when EAA agonists are exogenously administered, DAMGO markedly produced a μ -antagonist-sensitive enhancement of NMDA currents, but markedly reduced non-NMDA (kainate/AMPA) currents which were further enhanced by μ antagonist pretreatment. Whether similar activity occurs in the Nacc shell and whether this mediates the agonist-induced ingestive effects is not known. O'Donnell (see review: O'Donnell, 1999) has proposed that differential NMDA and AMPA receptor activity within the Nacc may result from receptor-specific interactions with dopamine. Within the striatum, D_1 receptor activation potentiates NMDA-receptor-mediated responses, whereas D_2 receptor activation decreases AMPA-receptor-mediated responses (Cepeda et al., 1998; Levine et al., 1996a; Levine et al.,

1996b). Since tyrosine hydroxylase terminals in the Nacc are associated with NMDA receptors in the Nacc (Gracy and Pickel, 1996), O'Donnell (1999) proposes that NMDA-mediated "up-states" in the Nacc are maintained by activation of D₁ receptors, whereas AMPA receptors are maintained by activation of D₂ receptors during "down-state" NMDA-mediated responses. Just as NMDA and AMPA differentially interact with DAMGO-induced feeding elicited from the Nacc shell, D₁ and D₂ antagonists display differential effectiveness upon DAMGO-induced feeding in the Nacc shell (Ragnauth et al., 2000b). Thus, D₁ antagonist pretreatment in the Nacc shell, like NMDA cotreatment, acts to reduce DAMGO-induced feeding. In contrast, DAMGO-induced feeding is refractory to D₂ antagonist pretreatment in the Nacc shell, and potentiated by AMPA cotreatment. These data thereby suggest complex modulatory interactions among opioid, dopaminergic and EAA receptors in the Nacc shell just as has been proposed for EAA and GABA in mediating feeding elicited from the Nacc shell (Kelley, 1999a; Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Stratford and Kelley, 1999; Stratford et al., 1998). These modulatory interactions are along the lines of ensemble coding proposed by O'Donnell (1999), neural integration systems proposed by Kelley (1999a), and/or sensori-motor gating proposed by Meredith and Totterdell (1999). Such modulatory schema have been proposed for the role of the Nacc shell in both appetitive and aversive behaviors in general (Kelley, 1999a; Salamone, 1994), and to responses to stress, fear and drugs of abuse in particular (Beck and Fibiger, 1995; Carlezon and Wise, 1996; Kalivas and Duffy, 1995; King and Finlay, 1997; O'Donnell, 1999; Tidey and Miczek, 1996).

VII. EXPERIMENT 3. Alterations in Food Intake Elicited by GABA and Opioid Agonists and Antagonists Administered into the VTA of Rats.

A. Specific Procedures:

Protocol 1: In assessing GABA_A receptor antagonism upon μ -opioid agonist-induced feeding elicited from the VTA, the first group of rats received the following bilateral microinjection conditions in the VTA: a) vehicle (n= 9), b) DAMGO (5 μ g), 2.5 μ g each side, n= 9), c) bicuculline (75 ng (1.62 nmol), 37.5 ng each side, n=6), and bicuculline at doses of either d) 7.5 ng (0.162 nmol, 3.75 ng each side, n= 6) or e) 75 ng (n=7) paired with DAMGO (5 μ g). GABA antagonist treatments preceded agonist treatments by 20 min in this and all subsequent protocols, and cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 2: In assessing GABA_B receptor antagonism upon μ -opioid agonist-induced feeding elicited from the VTA, the second group of rats received the following bilateral microinjection conditions in the VTA: a) vehicle (n= 7), b) DAMGO (5 μ g, n= 7), c) saclofen (3.0 μ g (12 nmol), 1.5 μ g each side, n=6), and saclofen at doses of either d) 1.0 μ g (4 nmol, 500 ng each side, n= 6), e) 1.5 μ g (6 nmol, 750 ng each side, n=6) or f) 3.0 μ g (12 nmol, 1.5 μ g each side, n=6) paired with DAMGO (5 μ g). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

Protocol 3: In assessing GABA_A agonist-induced feeding elicited from the VTA, the third group of seven rats received the following bilateral microinjection conditions in the VTA: a) vehicle, muscimol at doses of b) 50 ng (25 ng each side, 2.6 nmol), c) 100 ng

(50 ng each side, 5.2 nmol) or d) 200 ng (100 ng each side, 10.4 nmol), and DAMGO (5 μ g). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection. This dose range of muscimol was chosen because of their previously-established ability to elicit feeding following microinjections into the VTA or other intracerebral sites (Arnt and Scheel-Kruger, 1979; Klitenick and Wirtshafter, 1988). DAMGO was administered to this group to demonstrate that feeding could be elicited from these specific cannula placements.

Protocol 4: In assessing GABA and opioid antagonism of GABA_B agonist-induced feeding elicited from the VTA, the fourth group of rats received the following bilateral microinjection conditions in the VTA: a) vehicle (n=11), b) baclofen 200 ng (100 ng each side, 9 nmol, n=11), and either c) saclofen 1500 ng (750 ng each side, 6 nmol, n=7), d) bicuculline 75 ng (37.5 ng each side, 1.62 nmol, n=7), or e) naltrexone 5 μ g (2.5 μ g each side, 13.2 nmol, n=7) paired with baclofen (200 ng). Cumulative intakes were assessed 1, 2 and 4 h following the last microinjection.

B. Results:

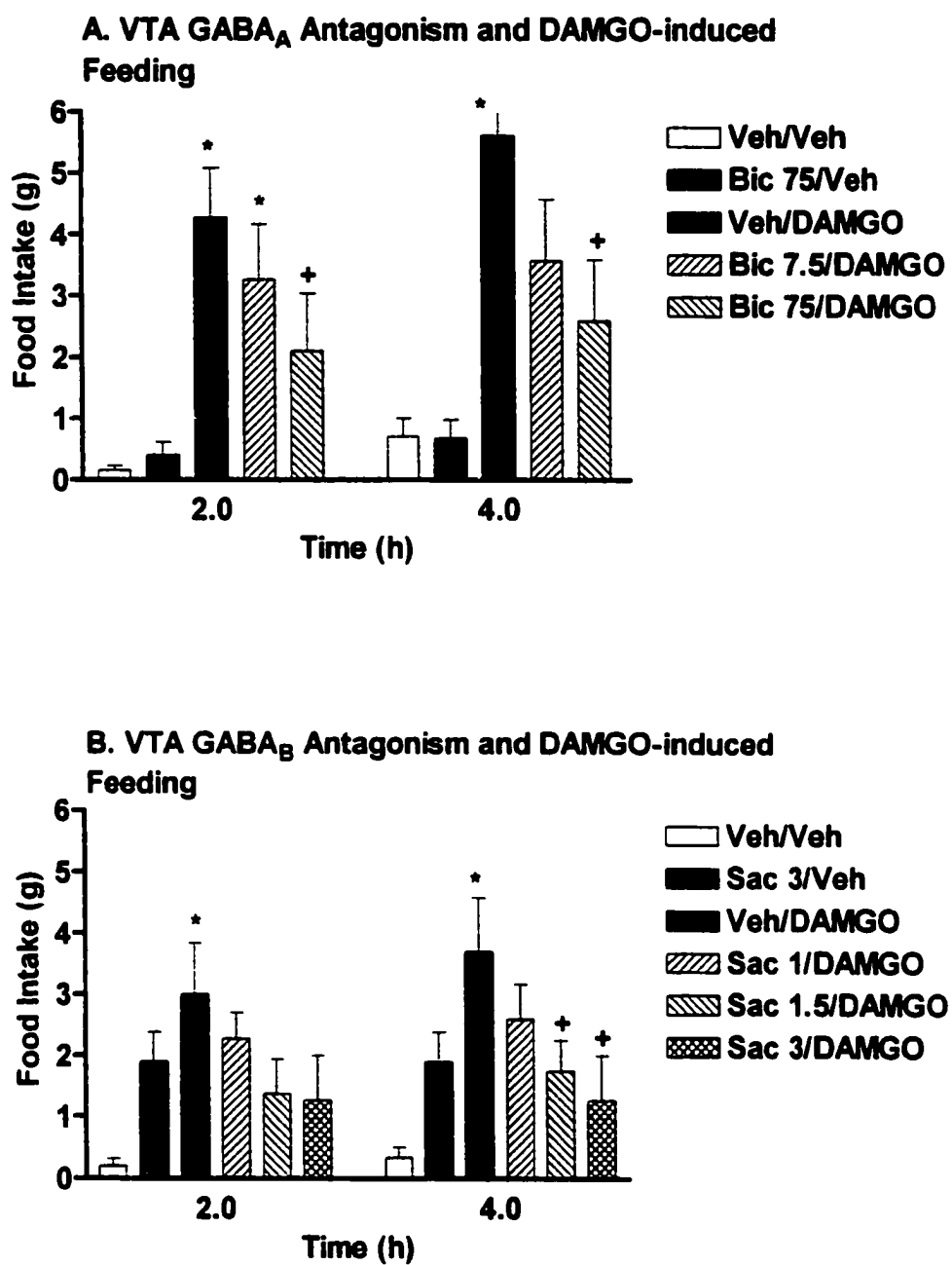
Histological Placements: The cannulae placements of all animals were found to be within the area of the VTA, bordered as far rostrally as the level of the fasciculus retroflexus and commissure of the superior colliculus, bordered as far caudally as the level of the interpeduncular nucleus, and bordered as far dorsally as the caudal linear nucleus of the raphe.

VTA GABA_A receptor antagonism and VTA DAMGO-induced feeding:

Significant differences in food intake were observed among injection conditions after 2 ($F(4,32) = 15.30, P < 0.0001$) and 4 ($F = 10.75, P < 0.0001$) h. As illustrated (Figure 8, upper panel), DAMGO significantly increased food intake relative to control treatment after 2 and 4 h. Pretreatment in the VTA with the higher (75 ng), but not lower (7.5 ng), dose of the selective GABA_A receptor subtype antagonist, bicuculline, significantly decreased DAMGO-induced feeding after 2 and 4 h, suggesting that GABA_A receptors in the VTA are involved in the full expression of DAMGO-induced feeding. Administration of bicuculline alone into the VTA failed to alter food intake over this time course. Behavioral observation of the animals revealed that the higher, but not lower, bicuculline dose produced seizure-like behaviors (e.g., barrel rolls, body rotation, back flips and twitching of forepaws, vibrissae and mouth) in about 50% of the treated rats. These behaviors occurred within 2 min after injection, and persisted for at most 10 min. Animals displayed normal behaviors after 20 min when food was introduced. These behaviors were noted in bicuculline-treated rats in both the presence and absence of DAMGO (see Table 9).

Figure 8. Alterations in food intake (g, $\pm$ S.E.M.) following administration of DAMGO (5.0 μ g) in the VTA relative to control treatment in rats pretreated with either the GABA_A antagonist, bicuculline (Bic: 7.5 -75 ng, upper panel) or the GABA_B antagonist, saclofen (Sac: 1.0-3.0 μ g, lower panel). The asterisks (*) in this and the subsequent figure indicate significant increases in food intake following agonist relative to control treatment (Tukey comparisons, $P < 0.05$). The crosses (+) in this and the subsequent figure indicate significant decreases in food intake following antagonist pretreatment relative to agonist treatment alone (Dunnett comparisons, $P < 0.05$).

FIGURE 8



VTA GABA_B receptor antagonism and VTA DAMGO-induced feeding:

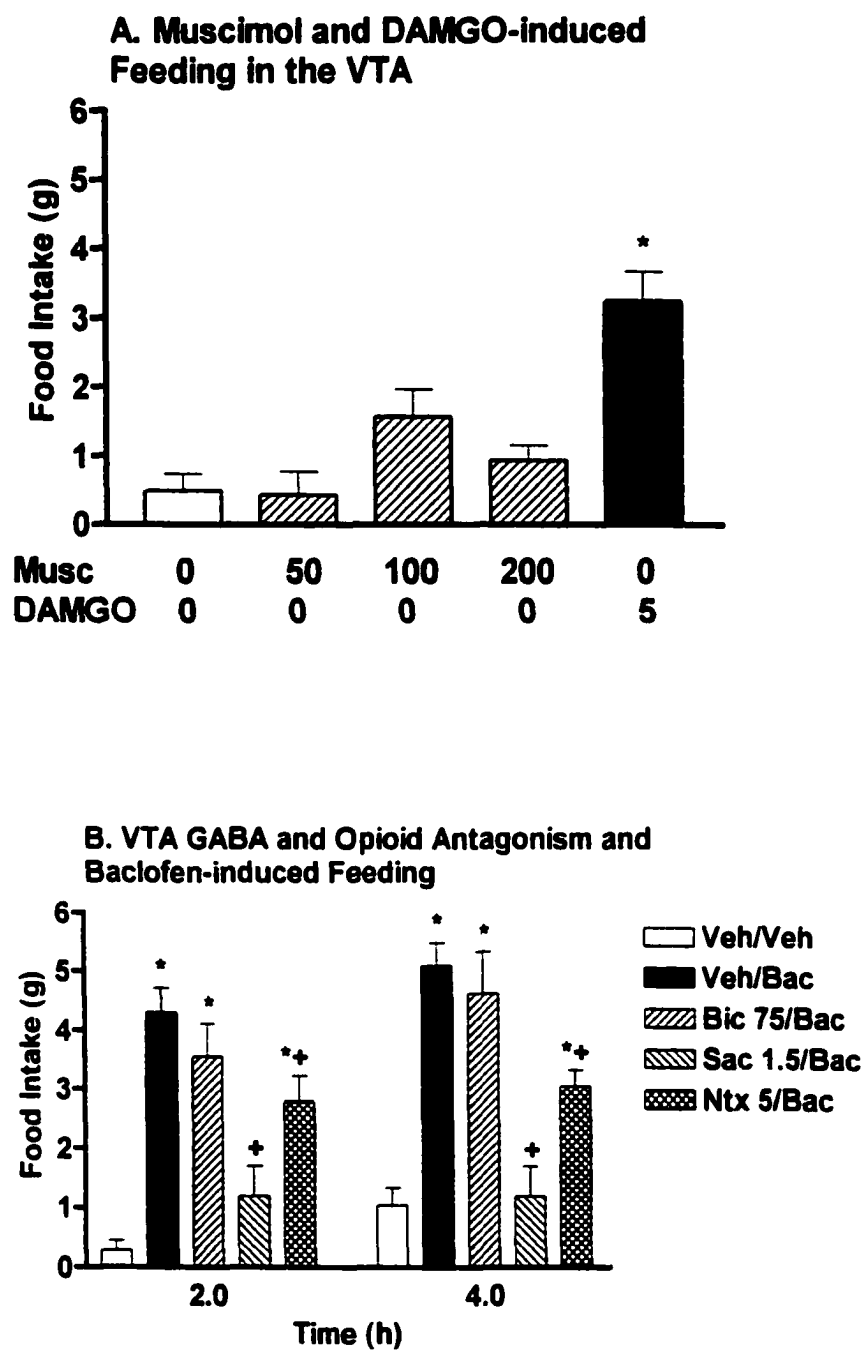
Significant differences in food intake were observed among injection conditions after 2 ($F(5,30)=3.04$, $P<.025$), and 4 ($F=4.65$, $P<0.003$) h. As illustrated (Figure 8, lower panel), DAMGO significantly increased food intake relative to control treatment after 2 and 4 h. Pretreatment in the VTA with the higher (1.5 and 3.0 μg), but not lowest (1.0 μg), doses of the selective GABA_B receptor subtype antagonist, saclofen, significantly and dose-dependently decreased DAMGO-induced feeding after 4 h, suggesting that GABA_B receptors in the VTA are involved in the full expression of DAMGO-induced feeding. Although saclofen alone in the VTA increased intake after 2 and 4 h, this did not reach statistical significance. In contrast to bicuculline, behavioral observation of the animals failed to reveal any overt seizure-like activity.

VTA Muscimol and DAMGO and food intake: Significant differences in food intake were observed among injection conditions after 4 ($F(4,24)=12.78$, $P<0.0001$) h. As illustrated (Figure 9, upper panel), none of the tested muscimol doses in the VTA significantly increased intake at any time point. However, the same group of rats displayed the expected significant increases in food intake after 4 h following DAMGO treatment in the VTA, indicating that these cannula placements were capable of eliciting food intake, and that this GABA_A agonist was ineffective in eliciting feeding over this dose range in the VTA. Observation of the animals failed to reveal any overt alterations in other behaviors (see Table 10).

VTA GABA and opioid antagonism of baclofen-induced feeding: Significant differences in food intake were observed among injection conditions after

Figure 9. Upper Panel: Alterations in food intake (g, $\pm$ S.E.M.) following administration of either the GABA_A agonist, muscimol (Musc: 50-200 ng) or the μ opioid agonist, DAMGO (5.0 μ g) in the VTA relative to control treatment. **Lower Panel:** Alterations in food intake (g, $\pm$ S.E.M.) following administration of the GABA_B agonist, baclofen (200 ng) in the VTA relative to control treatment in rats pretreated with either the GABA_A antagonist, bicuculline (Bic: 75 ng), the GABA_B antagonist, saclofen (Sac: 1.5 μ g), or the general opioid antagonist, naltrexone (Ntx: 5.0 μ g).

FIGURE 9



2 ($F(4, 40)=21.26$, $P<0.0001$) and 4 ($F=28.26$, $P<0.0001$) h. As illustrated (Figure 9, lower panel), baclofen in the VTA significantly increased food intake relative to control treatment after 2 and 4 h. Baclofen-induced feeding was eliminated by VTA pretreatment with the selective GABA_B antagonist, saclofen, after 2 and 4 h, indicating receptor subtype-specific antagonism of this response. In contrast, VTA pretreatment with the selective GABA_A antagonist, bicuculline, failed to alter the pattern or magnitude of baclofen-induced feeding across the time course, further confirming receptor subtype-specific antagonism. VTA pretreatment with the general opioid antagonist, naltrexone, significantly reduced the magnitude of baclofen-induced feeding after 2 and 4 h, although the magnitude of this antagonism was less than that of saclofen. These data suggest that an opioid synapse in the VTA is involved in part in the full expression of baclofen-induced feeding. Behavioral observation of the animals revealed that baclofen administration into the VTA increased motor activity, circling behavior, gnawing and grooming. These behaviors occurred almost immediately following baclofen injection and persisted for up to 15 min thereafter. These increases in VTA baclofen-induced behavioral activity occurred in the presence of bicuculline and naltrexone, but were markedly reduced following saclofen pretreatment.

C. Discussion:

The first major finding of the present study was that VTA pretreatment with either GABA_A or GABA_B antagonists each significantly and dose-dependently reduced feeding elicited by the μ opioid agonist, DAMGO in the VTA. Given the immunoreactive and autoradiographic demonstration of anatomical co-localization of GABA and enkephalinergic neurons in the Nacc which project to the VTA (Kalivas et al., 1993), it has been previously suggested that these neurons produce functional co-regulation (Churchill et al., 1992; Churchill and Kalivas, 1994; Kalivas et al., 1993; Sesack and Pickel, 1995; Van Bockstaele and Pickel, 1995). The significant dose-dependent decreases in DAMGO-induced feeding elicited by bicuculline in the VTA did not appear to be due to any intrinsic ingestive actions of the GABA_A antagonist since VTA bicuculline alone failed to increase chow feeding responses. It should be noted that bicuculline increased sweet milk intake following VTA microinjections (Kelly et al., 1977), and increased chow intake following ventral pallidum microinjections (Stratford et al., 1999) with which the VTA has reciprocal connections. The observation of bicuculline-induced seizure-like behaviors in approximately 50% of the animals within 2-10 min after injections into the VTA does not appear to explain the results since these effects had a far shorter time course than the actions upon DAMGO-induced feeding, and that these behaviors were noted in bicuculline-treated rats in both the presence and absence of DAMGO. Interestingly, in contrast to the reduction of DAMGO-induced feeding elicited from the VTA with bicuculline pretreatment, bicuculline enhanced DAMGO-induced feeding elicited from the Nacc shell (Znamensky et al., 2001), and these data thereby indicate that GABA_A

antagonist actions upon DAMGO-induced feeding appear to be site-specific.

VTA pretreatment with the GABA_B antagonist, saclofen, also significantly and dose-dependently reduced DAMGO-induced feeding albeit over a more limited time course (4 h), and at higher molar doses. VTA GABA_B antagonist-induced reductions of DAMGO-induced feeding were not accompanied by any other behavioral abnormalities, but the effective saclofen doses produced small, but non-significant increases in feeding when administered alone into the VTA. Thus, the small stimulatory ingestive actions of VTA saclofen may explain its longer latency to reduce DAMGO-induced feeding. This pattern of VTA GABA_B antagonist-induced reductions upon DAMGO-induced feeding is similar to that previously observed in the Nacc shell (Znamensky et al., 2001).

The current study also examined whether the GABA_A and GABA_B agonists, muscimol and baclofen respectively, would induce food intake following administration into the VTA since muscimol induced feeding following microinjections into the VTA and related neural sites, including the Nacc, substantia nigra, median raphe and dorsal raphe nuclei (Arnt and Scheel-Kruger, 1979; Basso and Kelley, 1999; Klitenick and Wirtshafter, 1988; Klitenick and Wirtshafter, 1989; Przewlocka et al., 1979; Redgrave et al., 1984; Stratford and Kelley, 1997; Ward et al., 2000; Znamensky et al., 2001). However, the present study found that whereas administration of baclofen into the VTA produced potent feeding responses over the 4 h time course, a wide range of muscimol doses in the VTA failed to elicit feeding. Differential responses have been previously observed for GABA receptor subtype agonists in the VTA and other sites. Microiontophoretically-applied ethanol enhanced baclofen-induced inhibition of VTA GABA firing rates, yet

reduced muscimol-induced inhibition of VTA GABA firing rates (Steffensen et al., 2000). Whereas baclofen, but not muscimol infusions decreased local levels of serotonin in the Nacc (Tao et al., 1996) and decreased cocaine-maintained behaviors in this nucleus (Shoaib et al., 1998), increased acetylcholine levels were observed following muscimol relative to baclofen infusions into the Nacc (Rada et al., 1993). Feeding elicited from the dorsal raphe nucleus was more potent following muscimol relative to baclofen (Przewlocka et al., 1979), whereas muscimol, but not equimolar baclofen administered into the central nucleus of the amygdala (Minano et al., 1992). GABA_A synaptic potentials were never accompanied by GABA_B synaptic potentials within the VTA, and that serotonin reduced GABA_B, but not GABA_A IPSP's in the VTA, suggesting that GABA_A and GABA_B receptors originate from distinct presynaptic fibers (Sugita et al., 1992).

The failure to observe VTA muscimol-induced food intake was not due to misplaced or ineffective cannulae in the particular group of animals since they exhibited potent feeding responses to DAMGO administration into the VTA. Although the lack of muscimol-induced feeding elicited from the VTA appears inconsistent with previous positive studies (Arnt and Scheel-Kruger, 1979; Klitenick and Wirtshafter, 1988), it has been suggested that the magnitude and potency of muscimol-induced feeding is much weaker in the VTA as compared to other related regions such as the substantia nigra and median raphe nucleus (Klitenick and Wirtshafter, 1988; Redgrave et al., 1984). Moreover, the GABA_A antagonist, bicuculline, increased sweet milk intake following VTA microinjections (Kelly et al., 1977). Furthermore, the heterogeneity of the VTA on a rostral-caudal dimension might explain the differential feeding sensitivity to muscimol

since potent feeding responses and increased locomotor activity occurred when muscimol was administered into the caudal, but not rostral VTA (Arnt and Scheel-Kruger, 1979) in the same pattern as was observed for muscimol-induced ingestive effects in the substantia nigra (Redgrave et al., 1984). Animals included in the present study had bilateral cannula placements in the VTA as far rostral as the level of the fascicularis retroflexus and the commissure of the superior colliculus and as far caudal as the level of the interpeduncular nucleus. To assess whether rostral-caudal VTA placement differentiations affected GABA agonist-induced feeding, we performed post-hoc analyses of food intake 2 h following either muscimol (100 ng) or baclofen (200 ng) administered into rostral or caudal VTA placements. Significantly greater baclofen-induced effects relative to muscimol-induced effects were observed ($F(1,12)= 20.00$, $P<0.008$) without significant rostral-caudal effects ($F(1,12)= 2.21$, n.s.) or any interaction between agonist and rostral-caudal dimension ($F(1,12)= 0.63$, n.s.). Therefore, baclofen, but not muscimol, produced potent increases in chow intake along the full rostral-caudal dimensions of the VTA.

As expected, baclofen-induced feeding was selectively eliminated by VTA pretreatment with the selective GABA_B antagonist saclofen, but not with the selective GABA_A antagonist bicuculline, indicating the same pattern of GABA receptor subtype selectivity observed previously in the Nacc shell (Stratford and Kelley, 1997; Znamensky et al., 2001). VTA pretreatment with the general opioid antagonist, naltrexone significantly reduced baclofen-induced feeding, suggesting that an opioid synapse participates in this GABA_B receptor-mediated effect. However, it should be noted that the magnitude of the naltrexone-induced reductions of baclofen-induced feeding elicited from

the VTA was not as pronounced as that observed for saclofen. The opioid antagonist actions upon baclofen-induced feeding appear to be site-specific since naltrexone failed to significantly reduce baclofen-induced feeding when the antagonist and agonist were administered into the Nacc shell (Znamensky et al., 2001).

In conclusion, there is considerable theoretical (e.g., (Kelley, 1999a; Salamone, 1994; Self and Stein, 1992; Wise and Bozarth, 1987)) and empirical evidence linking the VTA and Nacc shell in the mediation of reward and reinforcement. Both μ opioid agonists and antagonists administered in the VTA increase both locomotor activity and dopamine release in the Nacc (Devine et al., 1993a), and neurons containing GABA and enkephalin project from the Nacc, ventral pallidum and diagonal band of Broca to the VTA (Kalivas et al., 1999) with close appositions observed between GABA and enkephalin neurons (Sesack and Pickel, 1995), suggesting that enkephalin and GABA are released from convergent terminals, and therefore may be involved in the co-regulation of common neurons. Further, since muscimol in the Nacc shell activates c-fos-like immunoreactivity in a number of structures including the VTA (Stratford and Kelley, 1997), it was expected that the VTA would support a similar pattern of opioid and GABAergic effects upon ingestive behavior observed in the Nacc shell. Whereas the GABA_B antagonist, saclofen significantly reduced feeding elicited by the μ opioid agonist, DAMGO following microinjections into either the Nacc shell or the VTA, the VTA differed markedly from the Nacc shell in three important respects. First, DAMGO-induced feeding was respectively enhanced and reduced by bicuculline pretreatment into the Nacc shell and VTA. Second, muscimol was capable of eliciting potent increases in food intake following Nacc shell, but

not VTA microinjections. Finally, although baclofen-induced feeding displayed GABA receptor subtype antagonist specificity following administration into both sites, GABA_B agonist-induced feeding was significantly reduced by opioid antagonist pretreatment in the VTA relative to the Nacc shell. Therefore, in examining potential relationships between neuromodulatory systems following intracerebral administration, it is of importance to recognize the possibility of distinct site-specific effects between related nuclei.

VIII. GENERAL DISCUSSION

GABA-Opioid Interactions: Given that intracerebral administration of μ opioid agonists significantly increase food intake when administered into the Nacc shell, along with evidence for co-localization of GABA and μ receptors (Sesack and Pickel, 1995), it was expected that GABA antagonists are involved in the modulation of μ opioid-induced feeding in the Nacc shell. The first experiment of the present study showed that GABA receptor subtype antagonists produced differential actions upon feeding elicited by the μ -selective opioid agonist, DAMGO. Pretreatment with the GABA_A-selective antagonist, bicuculline, in the Nacc shell produced significant increases in DAMGO-induced feeding without altering food intake per se. Furthermore, the ability of general (naltrexone) and μ -selective (β -funaltrexamine) opioid antagonists to block the enhanced food intake elicited by combinations of bicuculline and DAMGO indicates that a μ opioid synapse is essential for the full expression of this combined ingestive response. In contrast, pretreatment with the GABA_B-selective antagonist, saclofen, in the Nacc shell produced significant decreases in DAMGO-induced feeding without altering food intake per se. The finding that the respective GABA_A and GABA_B antagonists, bicuculline and saclofen, exerted differential actions upon DAMGO-induced feeding in the Nacc shell is a demonstration of the receptor selective actions of the GABA receptor subtypes. Furthermore, a confirmation of GABA receptor subtype specificity was demonstrated by the finding, previously shown by Kelley and co-workers (Stratford and Kelley, 1997), that feeding elicited from the Nacc shell by the GABA_A-selective agonist, muscimol was prevented with the GABA_A (bicuculline), but not the GABA_B (saclofen) antagonist, and feeding elicited by the

GABA_B-selective agonist, baclofen, was blocked by pretreatment with the GABA_B (saclofen), but not with the GABA_A (bicuculline) antagonist in the Nacc shell. The opioid mediation of GABA agonist-induced feeding was not as clear-cut. Naltrexone and muscimol treatment in the Nacc shell prevented the significant increases in feeding elicited by muscimol alone. In contrast, naltrexone failed to reduce baclofen-induced feeding. The differential effects of opioid antagonists upon GABA agonist-induced feeding in the Nacc provides further confirmation of the subtype specificity of the GABA_A and GABA_B receptors. The mechanism proposed by which GABA agonists produce feeding is through disinhibition. Thus, the removal of a tonic inhibitory tone in the VTA and Nacc shell results in an increase in food intake.

Intracerebral studies have contributed to the elucidation of site-specific actions of neurotransmitter systems. Since the VTA has reciprocal connections with Nacc shell (Van Bockstaele and Pickel, 1995), it was expected that VTA pretreatment with either the GABA_A antagonist, bicuculline or the GABA_B antagonist, saclofen would alter feeding elicited by VTA DAMGO in the same differential manner as observed in the Nacc shell (Znamesky et al., 2001). However, it was found that VTA pretreatment with either GABA_A or GABA_B antagonist each significantly reduced feeding elicited by DAMGO in the VTA. Therefore, the contrasting findings elucidate the site-specific effects of the GABA_A receptor subtype. Previous studies have demonstrated differential actions of bicuculline in related brain regions upon ingestive behavior. Administration of bicuculline in the ventromedial hypothalamus reduced food intake (Hansen and Ferreira, 1986; Kelly, 1977), whereas intra-lateral hypothalamic bicuculline increased ingestion of sweet milk

(Kelly, 1977). In addition, bicuculline administration in the ventral pallidum, a brain region that has strong reciprocal connections with the Nacc shell and VTA, increased food intake (Stratford et al., 1999). However, administration of bicuculline in the amygdala failed to produce ingestive effects (Hansen and Ferreira, 1986). The differential actions of GABA agonists demonstrated in the Nacc shell and VTA is also suggestive of site-specificity of these receptor subtypes upon ingestive behavior. Thus, whereas administration of baclofen in the VTA produced potent feeding responses, a wide range of muscimol doses in the VTA failed to elicit feeding. Differential feeding responses have been previously observed for the GABA receptor subtype agonists in other neural sites. In addition to muscimol-induced feeding in the Nacc (Znamensky et al., 2001), and other related neural sites, including the dorsal and median raphe nuclei and substantia nigra (Redgrave et al., 1984; Klitenick and Wirtshafter, 1988; Klitenick and Wirtshafter, 1989), muscimol administration into the VMH and PVN increased food intake. However muscimol administration in the LH, as well as in the central nucleus of the amygdala, decreased food intake (Minano et al., 1992; Kelly, 1977). The GABA subtype receptor selective actions were also demonstrated in that intra- VTA baclofen was eliminated by VTA pretreatment with the selective GABA_B antagonist saclofen, but not with the selective GABA_A antagonist bicuculline. Furthermore, the reduction of baclofen-induced feeding with pretreatment with the general opioid antagonist, naltrexone, suggests that an opioid synapse participates in this GABA_B receptor-mediated effect. In addition, the opioid antagonist actions upon baclofen-induced feeding appear to be site-specific since naltrexone failed to significantly reduce baclofen-induced with administration into the

Nacc (Znamensky et al., 2001). Site-specific actions upon ingestive behavior have also been demonstrated with the administration of baclofen into various neural sites. In the present study, Experiments 1 and 3 demonstrated potent feeding responses with intra-Nacc or intra-VTA baclofen administration (Znamensky et al., 2001; Ward et al., 2000), whereas administration of baclofen into the central nucleus of the amygdala did not alter food intake (Minano et al., 1992).

The other neurotransmitter systems that are involved in the GABA mediation of μ opioid induced feeding in the Nacc shell and VTA is of particular interest. Dopamine is a likely candidate in the modulation of GABA-opioid interactions in the Nacc shell and VTA. A previous study (Ragnauth et al., 2000b) demonstrated the role of dopamine receptor antagonism upon feeding elicited from DAMGO in the Nacc shell. The selective D_1 antagonist SCH23390 significantly reduced DAMGO-induced feeding in the Nacc shell whereas the selective D_2 antagonist raclopride produced less consistent effects upon DAMGO-induced feeding elicited from the Nacc shell.

Electrophysiological and autoradiographic studies demonstrate the receptor colocalization of GABA and dopamine receptors in the Nacc and VTA, as well as functional implications for co-regulation (Klitenick et al., 1992; Johnson and North, 1992; Churchill et al., 1992). Furthermore, these studies demonstrate the existence of GABA-dopamine interactions between the Nacc and VTA. Nacc GABAergic neurons project to dopamine cells in the VTA (Brebner et al., 2000) and VTA dopamine neurons project to the Nacc, which are under tonic inhibition mediated by $GABA_A$ receptors (Nowak et al., 1998; Badiani et al., 1995a). The application of $GABA_A$ antagonists into the VTA excites

dopamine neurons and GABA agonists inhibit dopamine neurons (Nowak et al., 1998). Furthermore, dopamine receptor antagonists reduce GABA turnover rates in the Nacc and ventral pallidum (Rada et al., 1993). Differential actions of D₁ and D₂ receptors upon GABA release in the Nacc have been demonstrated (Rada et al., 1993). Microdialysis studies suggest that D₁ receptors stimulate striatal GABA release, and D₂ receptors inhibit it (Rada et al., 1993). Autoradiographic studies demonstrate that D₂ receptors are present on dendrites/spines and axon terminals of GABAergic principal and local circuit neurons in the medial Nacc shell (Meredith and Totterdell, 1999).

Differential actions on dopamine release of the GABA agonists, muscimol and baclofen, in the VTA have been demonstrated. Autoradiographic studies have suggested VTA administration of the GABA_B agonist baclofen inhibits dopamine release in the VTA and Nacc (Olpe et al., 1977; Churchill et al., 1992; Hooks and Kalivas, 1995), whereas VTA treatment with the GABA_A agonist muscimol results in a disinhibition of dopamine neurons in the VTA and Nacc (Churchill et al., 1992; Hooks and Kalivas, 1995). Like GABA_A receptors, stimulation of μ opioid receptors inhibits activity of GABAergic afferents to DA neurons, removing tonic inhibition (Klitenick et al., 1992).

The differential actions of the GABA_A and GABA_B receptors upon dopamine release in the VTA and Nacc may be based in their differential receptor localization (Churchill et al., 1992; Klitenick et al., 1992). A model for GABA_A and GABA_B receptor localization in the VTA has been proposed (Churchill et al., 1992; Klitenick et al., 1992). It is suggested that GABA_A receptors are colocalized with μ receptors on GABA interneurons, whereas dopamine (DA) cells contains GABA_B and D₂ receptors.

Furthermore, GABA afferents from Nacc and ventral pallidum, which also contain μ receptors, innervate the VTA DA cells, and synapse on the GABA_B receptor on DA cells (Klitenick et al., 1992) as well as making synaptic contact with the GABA_A receptors on the GABA interneurons. The differential effects of DA release in the VTA by the respective GABA_A and GABA_B agonists muscimol and baclofen may have implications for the differential findings in food intake between muscimol and baclofen in the VTA. Further, an inter-site study demonstrated that DA antagonist fluphenazine when administered into the Nacc, but not the striatum, reduced the feeding response elicited by muscimol in the dorsal raphe nucleus (Bendotti et al., 1986).

In addition to dopamine, other neurochemical systems may play a lesser role in the modulation of GABA-opioid mediation of food intake in the Nacc shell, including the cholinergic, norepinephrine, and serotonin systems. In the VMH, a brain region involved in ingestive behavior, norepinephrine-induced feeding was reduced by bicuculline (Grandison and Guidotti, 1977). This ingestive response may be parallel in other brain regions involved in ingestive behavior, including the Nacc shell, or this response may be site-specific. A study examining inter-site interactions demonstrated that muscimol-induced feeding elicited from the dorsal raphe nucleus, a midbrain region that has reciprocal connections with the VTA, decreased serotonin metabolism in the Nacc (Bendotti et al., 1986). Acetylcholine appears to be involved in opiate reward mechanisms in general. In the Nacc high levels of Ach are correlated with aversive states and low levels appear to be correlated with opiate reward (Rada et al., 1996). Since cholinergic neurons make up a significant percentage of Nacc local circuit neurons (Meredith et al., 1993), and

cholinergic neurons modulate synaptic responses and membrane properties of principal neurons and attenuate both excitatory (EPSP) and inhibitory (IPSP) post-synaptic potentials (Meredith and Totterdell, 1999), co-modulation of acetylcholine with other transmitter systems, possibly GABA as well as glutamate, may be involved in opioid-mediated reward mechanisms.

There is a differentiation between GABA_A and GABA_B receptors on a neurophysiological level, which may have functional implications. Intracellular recording of dopamine neurons within the VTA has demonstrated that GABA_B receptor stimulation elicits a late onset, long-duration hyperpolarization mediated by increased potassium conductance, whereas stimulation of GABA_A receptors produces a short-lived hyperpolarization mediated by increased chloride flux (Johnson and North, 1992). A differentiation of the GABA_A and GABA_B receptors is also implicated by their respective differential agonists, muscimol and baclofen, and antagonists, bicuculline and saclofen. However, interaction between GABA_A and GABA_B receptors has been shown in the cerebellum. Baclofen inhibited muscimol-stimulated Cl⁻ uptake, suggesting that activation of GABA_B receptors can inhibit the function of GABA_A receptors (Hahner et al., 1991). The colocalization of the GABA receptor subtypes has been demonstrated in the dorsolateral septal nucleus, and in the CA1 region of the hippocampus. However, it is unknown whether interactions between GABA_A and GABA_B receptors occurs in the Nacc shell and the VTA.

The differential synaptic arrangement, and well as differential effects on dopamine may be the basis for the receptor-selective functions of GABA. In addition, further

elucidating the role of dopamine in the GABA modulation of μ opioid agonist food intake in the Nacc and VTA will shed some light on the site-specific effects of these ingestive actions.

EAA-Opioid Interactions: Experiment 2 of the current study examined the role of EAA receptors in the mediation of μ opioid agonist feeding in the Nacc shell. Previous studies have demonstrated a role for the excitatory amino acid (EAA) receptors in the Nacc shell in the mediation of feeding (Stratford et al., 1998 ; Stanley et al., 1993; Stanley et al., 1997; Kelley and Swanson, 1997). Kelley and co-workers demonstrated that microinjections of AMPA/kainate antagonists into the Nacc shell produced short-term and intense increases in feeding, whereas NMDA antagonists were ineffective (Stratford et al., 1998). The lack of ingestive effects of the NMDA receptor in the Nacc shell is in contrast to the potent feeding responses elicited from the Nacc shell by the administration of AMPA/kainate antagonists, which is a demonstration of the subtype selective actions of these EAA receptors. In the current study, administration of AMPA alone into the Nacc shell elicited significant and dose-dependent increases in feeding behavior, albeit across a different time course from DNQX. Whereas DNQX in the Nacc shell elicited an intense short-latency in feeding in a 30-min test, AMPA in the Nacc shell elicited feeding after 2 h. AMPA and DNQX feeding may occur as the result of differential interactions with other neurochemical systems. The ingestive effects of AMPA appear to involve opioid synapses since pretreatment in the Nacc shell with the general (naltrexone) opioid antagonist significantly decreased Nacc shell AMPA feeding. However, systemic pretreatment with naltrexone had no effect on DNQX feeding elicited from the Nacc shell (Maldonado-

Irizarry et al., 1995), which suggests that DNQX-induced food intake elicited from the Nacc shell may not be dependent upon opioid synapses. In addition to AMPA increases in food intake elicited from the Nacc shell, administration of NMDA into the Nacc shell elicited significant food intake with a shorter latency of peak effects and shorter duration than AMPA. Furthermore, feeding elicited from NMDA in the Nacc shell was reduced by Nacc shell pretreatment with naltrexone, also indicating a role for opioids in the full expression of this ingestive response.

Differential feeding interactions were observed following cotreatment of EAA agonists with the μ -selective opioid agonist, DAMGO. Significant enhancements in food intake were noted when AMPA and DAMGO were co-administered into the Nacc shell relative to either DAMGO alone or AMPA alone. This enhanced feeding response appeared to involve opioid and particularly μ receptor synapses since pretreatment in the Nacc shell with either general (naltrexone) or μ -selective (β -FNA) opioid antagonists reduced the increased intake following Nacc shell cotreatment with AMPA and DAMGO. In contrast, cotreatment with NMDA with DAMGO in the Nacc shell reduced the magnitude of DAMGO-induced feeding.

As with the GABA system, the investigation of the involvement of other neurochemical systems is indicated to understand the mechanisms of μ -EAA interactions upon food intake in the Nacc shell. Dopamine is also a likely candidate for the contribution to EAA- μ opioid feeding interactions. First of all, dopamine has been implicated in ingestive behavior (Ragnauth et al., 2000b; Salamone, 1994). A series of ultrastructural and neurophysiological studies (Gracy et al., 1997; Martin et al., 1997) have demonstrated

the co-localization of dopamine and glutamate receptors, and possible co-modulation of these receptors.

Neuroanatomical and neurochemical studies suggest that presynaptic modulation of DA terminals in the Nacc by activity of direct glutamatergic inputs from the hippocampus may increase DA release in the Nacc (Floresco and Phillips, 1999). Electrophysiological evidence suggests that stimulation of DA receptors inhibits glutamate release in the neostriatum, and this attenuation was antagonized by the D₂ antagonist sulpiride but not the D₁ antagonist, SKF-83565 (Hsu et al., 1995). Thus, the D₂ receptors play a greater role in the inhibition of excitatory synaptic transmission in the neostriatum. Studies utilizing retrograde tracing techniques (O'Donnell, 1999) have demonstrated that DA neurons in the Nacc can establish functional glutamatergic synapses. Kelley and co-workers (Maldonado-Irizarry et al., 1994) have indicated dopamine-glutamate interactions in ingestive behavior in the Nacc shell. Systemic administration of D₁ and D₂ antagonists reduced DNQX feeding elicited from the Nacc shell by half, suggesting a modulatory role for dopamine in this ingestive response. Levine and co-workers (Cepeda et al., 1998; Levine et al., 1996) have demonstrated DA-glutamate interactions in striatal neurons, and furthermore there is a differentiation between the actions of D₁ and D₂ receptors. D₁ receptor activation potentiates NMDA-receptor mediated responses, whereas activation of D₂ receptors decreases AMPA-receptor mediated responses. Thus, it appears that dopamine plays a modulatory role upon glutamate mediation of μ agonist-induced feeding in the Nacc shell.

The findings from the current study presented evidence for the roles of inhibitory

and excitatory neurotransmission in the Nacc shell and VTA upon μ opioid agonist feeding in these structures. The current study demonstrated the receptor-selective actions of the EAA receptor subtypes, NMDA and AMPA, as well as the GABA receptor subtypes, GABA_A and GABA_B. In addition, site-specificity of these receptor subtypes have been demonstrated which lends support to the use of intracerebral administration of pharmacological agents to thoroughly understand the basis for ingestive behavior. In addition to elucidating the interactions between opioids and neurochemical systems intrinsic to the Nacc and VTA, future studies could potentially involve examining neurochemical interactions between these neural sites. It is hypothesized that the mechanism in which the VTA exerts modulatory effects upon opioid-induced feeding is the dopaminergic projection from the VTA to the Nacc shell (Swanson, 1982). This indicates that the VTA may be a major component of the afferent circuitry to the Nacc shell in the mediation of opioid-induced feeding. One possible future study that involves testing inter-site interactions between the VTA and the Nacc shell is determining whether feeding elicited by μ agonists in the VTA is reduced by pretreatment of dopaminergic antagonists into Nacc shell. In addition, given the reciprocal GABAergic connections between the Nacc shell and the VTA, an inter-site study could involve determining whether feeding elicited by μ agonists in the VTA is reduced by pretreatment of GABA_A or GABA_B antagonists into the Nacc shell. The VTA is also a candidate for Nacc shell efferents (Zahm et al., 1985; Kalivas et al., 1993). Thus, inter-site evaluations could involve determining whether feeding elicited by μ agonists in the Nacc shell is altered by VTA pretreatment with either GABA_A or GABA_B antagonists.

The lateral hypothalamus is a brain site that has been implicated in ingestive behavior. Stanley and co-workers (Stanley et al., 1996; Stanley et al., 1993) have demonstrated potent feeding responses after administration of EAA agonists in the lateral hypothalamus. Furthermore, the Nacc shell sends projections to the lateral hypothalamus, therefore the lateral hypothalamus may be a potential candidate involved in the mediation of opioid-induced feeding in the Nacc shell. Kelley and co-workers (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1999) provide evidence for a functional link between the Nacc shell and the lateral hypothalamus. The potent feeding response observed after administration of DNQX, the AMPA/kainate antagonist, in the Nacc shell was inhibited by administration of the GABA_A agonist muscimol into the lateral hypothalamus. Furthermore, administration of the NMDA receptor selective antagonist D-AP5 suppressed feeding elicited by injections of muscimol into the Nacc shell. In addition, injections of muscimol into the Nacc shell increased synthesis of the nuclear protein Fos in neurons in the LH (Stratford and Kelley, 1999). The proposed projection from the Nacc shell to the LH is inhibitory. It is suggested that the mechanism of action is a GABAergic projection from the Nacc shell to the LH, and inhibition of the Nacc shell projection neurons would suppress the release of GABA from fibers terminating in the LH, which would disinhibit LH neurons resulting in an increase in food intake (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1999).

Based on the evidence for a functional link between the Nacc shell and LH a potential study might determine whether feeding elicited by μ agonists into the Nacc shell is altered by GABA_A agonists or antagonists or GABA_B agonists or antagonists

administered into the lateral hypothalamus.

The findings from the current study can be extended to the understanding of ingestive behavior in the larger context of motivation and reward mechanisms. The Nacc and the VTA are two brain sites that are proposed to be essential components of anatomical system that is involved in reward perception and motivation (Kalivas and Nakamura, 1999; Kalivas et al., 1999). The Nacc and the VTA have primary roles in this motivational circuitry that includes a convergence of inputs, including ventral pallidal, amygdalar, hippocampal, and prefrontal cortex, which allow for the integration of limbic reward information with motor output (Kalivas et al., 1999; Kalivas and Nakamura, 1999), and integrating input from transmitter systems, including enkephalins, dopamine, glutamate, and GABA into reward motivated behavioral responses. Recording of neurons in Nacc showed neurons displayed alterations in firing rate prior to arrival of reward, so this brain site may be involved in the anticipation of predictable rewards (Martin and Ono, 2000). Lesions placed in the medial shell of the Nacc impaired integration of position and reward value information, without altering the motivation for intake (Albertin et al., 2000). The VTA is a site that is considered to be involved in reinforcement processes and has been consistently demonstrated to support morphine reinforcement. Morphine injected into the VTA significantly increases conditioned place preference (CPP) (Barr and Rossi, 1992; McBride et al., 1999; Glimcher et al., 1984). Furthermore, met-enkephalin injected into the VTA produces CPP, and DAMGO and DPDPE are self-infused into the VTA, suggestive of their rewarding properties (McBride et al., 1999).

μ opioid receptors, in addition to modulating food intake in the Nacc shell and VTA, are involved in the hedonic value of palatable substances (Bodnar, 1996). Furthermore, studies have demonstrated μ opioid receptor involvement in the sensitization of reward in the Nacc (Carr, 1996; Bakshi and Kelley, 1994), which may enhance the response to food taste. A perturbation in opiate sensitization mechanisms may amplify the reward value of food and drugs of abuse (Carr, 1996). Thus, this mechanism of opiate sensitization is most likely a component in eating disorders and has clinical implications. For example, naltrexone, the general opioid antagonist, diminishes binge-eating in bulimic patients (Carr, 1996). In addition, there is demonstration of MOR-like immunoreactivity to astrocytic processes in the Nacc, and this may have implications for addiction in that alterations in astrocytic protein synthesis and release have been observed in morphine-treated brains (Svingos et al., 1996).

The involvement of other neurochemical systems may further enhance the opioid-induced sensitization of food reward. The series of current studies demonstrated the capability of NMDA/AMPA and GABA agonists to elicit feeding when administered into the Nacc shell and VTA, and furthermore demonstrated the alterations of μ opioid agonist feeding produced by NMDA/AMPA agonists in the Nacc shell, and by GABA antagonists in the Nacc shell and VTA. Thus, the interactions of the μ opioid systems with GABA or EAA receptor subtypes has implications for mediating the reward value of palatable substances, as well as implications for eating disorders. However, in the current series of studies administration of GABA agonists and EAA's increased food intake of rat chow, which is not considered particularly palatable. Therefore, this suggests that GABA and

EAA's are probably less involved in the palatability of ingested substances, but are more involved in the regulation of food intake per se. Common neural sites are involved in the rewarding effect of drugs and mediating the incentive value of food (Carr, 1996), and therefore, studies on the effects of certain neurotransmitter systems on drug reward will also be relevant for this discussion.

GABA involvement in reward processes in the Nacc shell and VTA is shown by the finding that GABA_A antagonists are self-administered into the anterior VTA, and injections of GABA_A antagonists into anterior VTA enhance DA release in the Nacc (McBride et al., 1999). Furthermore, blocking GABA_A receptors in the VTA attenuates ethanol intake (Nowak et al., 1998), and elevating synaptic GABA levels by GABA-T inhibitors, (e.g. GVG), reduces heroin reinforcement. In the Nacc, and particularly in the VTA, the GABA_B receptor agonist baclofen reduces cocaine self-administration. It has been suggested that increased GABA levels may play a role in attenuating centrally-mediated reward mechanisms through interactions with the VTA/Nacc DA system (Brebner et al., 2000). There appear to be mutual inhibitory actions between the GABA and opioid system. GABA_B receptors can attenuate the excitatory effects of opiate agonists, and opiate agonists have been shown to reduce GABA release in the VTA with an increase in DA levels and also inhibit Nacc GABAergic projection neurons (Xi and Stein, 2000; Sesack and Pickel, 1995). It has been hypothesized that opiates inhibit the GABAergic cells to disinhibit the mesocorticolimbic systems DA neurons (Xi and Stein, 2000). Thus, it appears that the GABA system, and the GABA_B receptor subtype in particular, modulates the μ receptor-mediated hedonic value and

palatability of reward-related substances, possibly through modulating DA-opioid interactions. The interaction between the GABA system and opioids also have implications for drug addiction. It has been demonstrated that benzodiazepines augment the magnitude of CPP associated with heroin delivery in rats (Walker and Ettenberg, 2001), suggesting the benzodiazepines enhance the rewarding properties of opiates.

GABA-mediated feeding in the Nacc shell does not alter taste or macronutrient selection, but generally increases caloric intake (Basso and Kelley, 1999). Whereas opioids involved in the modulation of palatability, GABA has been suggested to be involved more specifically in the regulation of feeding behavior per se. Thus, GABA does not appear to be involved in palatability per se, but may be involved in modulating opioid mediation of palatability directly or indirectly through the DA system.

With regard to the concept of opioid mediation of palatability, the current series of studies the raised the question whether the mu opioid agonist, DAMGO, altered the hedonic value of the ingesta. This does not appear to be entirely the case, given that rat chow is not typically regarded as a palatable substance. Studies have suggested that in addition to opioid involvement in orosensory reward, opioids may impact feeding associated more with energy needs (Glass et al., 1999).

NMDA and AMPA receptor subtypes are also implicated in reward processes. NMDA receptors are suggested to be involved in learning of the reward value of food and food anticipation (Stanley et al., 1996; Ono et al., 1996). Increasing glutamate levels in the Nacc was correlated with the rewarding efficacy of cocaine (Cornish et al., 1999). Intra-Nacc treatment with the NMDA antagonist, APV, led to a shortening of reaction times by

stimuli predictive of a high reward (Hauber et al., 2000). Furthermore, the AMPA receptor subtype antagonist, DNQX, when administered into the Nacc blocked expression of CPP, which is considered to be memory of primary reward stimulation. (Kaddis et al., 1995; Layer et al., 1993). NBQX blocked expression of conditioned activity, thus it is suggested that conditioned components of behavioral sensitization involve AMPA receptors in the Nacc (Mead et al., 1999).

EAA receptor mechanisms of reward involve the opioid system, as demonstrated by the following findings. NMDA receptor antagonists in the Nacc and VTA inhibit morphine's rewarding properties in CPP tests (Popik and Kolasiewicz, 1999). Furthermore, administration of morphine into the VTA increases expression of GluR1, an AMPA glutamate receptor subunit, and overexpression of GluR1 can enhance the ability of morphine to establish CPP, suggesting that GluR1 is associated with changes in the rewarding efficacy of morphine (Carlezon et al., 2000).

It has been suggested that the development of behavioral sensitization to reward-related substances may depend upon EAA inputs into the Nacc and VTA (Meredith and Totterdell, 1999). Furthermore, dopamine is also implicated in the reward mechanisms of the EAA receptors, particularly NMDA (Meredith and Totterdell, 1999; Kelley, 1999b). There has been the demonstration of long-term enhancement of synaptic strength with the temporal occurrence of cortical striatal excitation, via NMDA receptors and dopaminergic activation, which suggests a dopamine contribution to the NMDA synaptic changes necessary for learning the reward value of food in the Nacc (Kelley, 1999b). Therefore, the EAA receptor subtypes, like the GABA system, are involved in the

μ opioid mediation of reward sensitization.

EAA- μ and GABA- μ mechanisms have implications for eating disorders.

Furthermore, it is likely that these mechanisms are operating through the DA system. The mesolimbic DA system is implicated not only in modulating positive incentives of reward-related stimuli, but also in aversive motivation (Salamone, 1994; Ranaldi and Wise, 2001). Low levels of DA are correlated with an aversive state and cessation of behavior (Mark et al., 1995). Since both DA and opioid systems are involved in the sensitization of reward, hyperactivation of these systems may have clinical implications, which may contribute to drug and food addictions. The GABA_B receptor may have particular involvement, considering that it inhibits dopamine release. Thus, a perturbation of the GABA_B receptors may result in overactivation or underactivation of the DA system, and thus the result may be oversensitization of reward substances, and aversive reactions to reward-related substances, respectively. Hoebel and co-workers (Hoebel et al., 1989; Pothos et al., 1995a; Pothos et al., 1995b) suggest that the idea of a reward-aversion system in ingestive behavior may provide some explanation to the understanding of eating disorders, including hyperphagia and anorexia. Hyperphagia and obesity may be a result of an overactivation of the DA system, whereas low levels of DA are correlated with aversive states, and thus this may be a contribution to the understanding of anorexia.

As mentioned, future studies should examine the interactions between the Nacc shell and the lateral hypothalamus. The LH is another brain site considered to play a critical role in processes to reinforcement and goal-directed behaviors (Clark et al., 1991; Hoebel et al. 1989). It has been demonstrated that the LH is minimally involved in the

direct control of the metabolic functions, but is possibly involved in modulating higher-order cortical processes. Hoebel and co-workers (Hoebel et al., 1989) suggest that perturbations in the excitability of LH neurons have implications for eating disorders. The PFC, a brain area involved in the neural circuitry of reward, is particularly important in integrating information and monitoring behavioral output (Kalivas and Nakamura, 1999). VTA and PFC connections are involved in reward related processes (Duvauchelle et al., 1998). Intra-PFC D₁ antagonist SCH23390 facilitated VTA brain stimulation reward and PFC DA serves a modulatory role over reward elements within the Nacc. Furthermore, a dysfunction in the PFC DA system has been implicated in perseveration of behavior after stimulus termination (Jackson and Moghaddam, 2001). Thus, this may have implications for reward seeking behaviors and addiction in that a perturbation of this system may result in the organisms's inability to discontinue a behavior which may contribute to continual seeking reward and result in addictive behaviors.

The current study demonstrated the involvement of the GABA and EAA receptor subtypes in the full expression of μ opioid food intake. Furthermore, these findings suggest roles for the GABA and the NMDA/AMPA receptor subtypes in modulating the μ opioid mediation of the reward value of palatable substances, and perturbations in these systems have implications for drug and food addictions. In addition, the dopamine system is most likely highly involved in GABA- μ and EAA- μ mediation of the reward value of food and drugs of abuse.

The Nacc shell and VTA are essential brain sites in a motivational circuit involved in integrating reward information for ingestive mechanisms. In terms of motivational

mechanisms, the question arises whether the consequence of feeding behavior through opioid-EAA/GABA interactions in the Nacc shell and VTA is via activation of general or specific drives. First of all, the two component theory of motivation proposes that a generalized drive common to all forms of motivation must first be activated (Pfaff, 1999), which involves connections between ascending reticular activating system and the forebrain. Thus, energization of behavior is initially required to produce an arousal state, and is mediated by a common neuroanatomical system. Therefore, is it the presentation of the stimulus, e.g. food in the case of ingestive behavior, that results in feeding behavior, or are there specific drives related to ingestive behavior? According to the two component process, the specificity of ingestive behavior is a result of the second mechanism that is subsequent to the generalized activation. The generalized motivational state involves limbic-hypothalamic connections. However, the specific drive of ingestive behaviors may involve specific connections within the motivational circuitry, e.g. between the lateral hypothalamus and nucleus accumbens shell.

IX. REFERENCES

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