

INFORMATION TO USERS

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

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

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

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

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

UMI[®]

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

**DIFFERENT BRAIN LOCI MEDIATE NEUROPEPTIDE Y'S EFFECTS ON
FEEDING vs. REWARD**

By

Christina Margaret Brown

**A thesis submitted in conformity with the requirements for the degree of Doctor of
Philosophy, Graduate Department of Psychology, University of Toronto**

© Copyright by Christina Margaret Brown 1999



**National Library
of Canada**

**Acquisitions and
Bibliographic Services**

**395 Wellington Street
Ottawa ON K1A 0N4
Canada**

**Bibliothèque nationale
du Canada**

**Acquisitions et
services bibliographiques**

**395, rue Wellington
Ottawa ON K1A 0N4
Canada**

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-41113-3

Canada

Table of Contents

Abstract	1
Introduction	3
Psychological Rationale: The coexistence of eating disorders and substance abuse	4
Neuropeptide Y (NPY)	9
NPY and feeding	11
History	11
Receptor subtypes	13
NPY and eating disorders	14
NPY and reward	15
Mesolimbic dopamine	16
Mesolimbic dopamine and feeding	17
Mesolimbic dopamine and reward	21
Interaction between NPY and dopamine	22
Anatomy	22
Neurochemistry	23
Behaviour	25
Summary	25
NPY, dopamine, and feeding	26
Two motivations for feeding behaviour: Regulatory vs. Non-regulatory	28
NPY and its effects on feeding and reward: An anatomical and functional model	31
NPY and regulatory feeding behaviour	34
Hypotheses	36

How this will be assessed	36
NPY and non-regulatory feeding behaviour (reward)	36
Hypotheses	38
How this will be assessed	39
Summary of hypotheses	42
General Method	43
Subjects	43
Procedure	43
Surgery	43
Drugs	44
Intracerebral Drug Administration	44
Histology	45
Statistical Analyses	45
Regulatory Feeding Experiments (#1a-1c)	49
Experiment #1a: Effects of NPY on powdered chow intake	50
Procedure	50
Results	51
Experiment #1b: Effects of peripheral FLU on PFH NPY-induced regular chow intake	53
Procedure	53
Results	53
Experiment #1c: Effects of N.Acc FLU on PFH NPY-induced powdered chow	

intake.....	56
Procedure.....	56
Results.....	56
Summary of Regulatory Feeding Experiments.....	57
Non-regulatory Feeding Experiments (#2a-2b).....	58
Experiment #2a: Effects of NPY on sucrose intake.....	61
Procedure.....	61
Results.....	61
Experiment #2b: Effects of N.Acc FLU on PFH NPY-induced sucrose intake...64	
Procedure.....	64
Results.....	64
Summary of Non-Regulatory Feeding Experiments.....	67
Reward Experiments (#3a-3d).....	68
Experiment #3a: Effects of NPY on progressive-ratio operant responding.....	69
Procedure.....	69
Apparatus.....	69
Testing.....	69
Results.....	70
Experiment #3b: Effects of DA antagonism on NPY-induced vs. drug-free PR	
responding for sucrose pellets.....	73
Procedure.....	73
Results.....	74
Experiment #3c: Effects of NPY on conditioned place preference.....	76

Procedure	76
Apparatus	76
Testing	77
Results	78
Experiment #3d: Effects of FLU (I.P.) on NPY-induced CPP	84
Procedure	84
Results	84
Summary of Reward Experiments	88
Methodological Control Experiments (#4a-4c)	90
Experiment #4a: Effects of N.Acc AMPH on locomotor activity	91
Procedure	91
Apparatus	91
Results	92
Experiment #4b: Can N.Acc FLU block other DA-mediated behaviours?	94
Procedure	94
Apparatus	94
Results	95
Experiment #4c: Can N.Acc stimulation with other compounds increase feeding behaviour?	97
Procedure	97
Results	97
General Discussion	100

NPY's involvement in regulatory feeding processes.....	100
NPY's involvement in non-regulatory feeding processes.....	105
NPY's involvement in reward processes.....	109
PR responding for sucrose reward pellets.....	109
NPY and conditioned place preference.....	110
NPY's involvement in eating disorders and substance abuse: A revised hypothesis.....	114
General Conclusions.....	117
References.....	119

List of Figures

Figure 1: The mesolimbic dopamine system.....	17
Figure 2: The two motivators of feeding behaviour.....	29
Figure 3: An anatomical and functional of NPY's effects on regulatory vs. non-regulatory feeding behaviour and reward following its injection into either the PFH or the N.Acc.....	33
Figure 4: Area surrounding the PFH where a cannulae placement was deemed to be accurate.....	46
Figure 5: Area surrounding the N.Acc where a cannulae placement was deemed to be accurate.....	47
Figure 6: Slide sections showing representative bilateral N.Acc and PFH cannulae placements.....	48
Figure 7: Effects of NPY on powdered chow intake after injection into the PFH or the N.acc.....	52
Figure 8: Effects of FLU on PFH NPY-induced chow intake.....	55
Figure 9: Effects of N.Acc FLU on NPY-induced powdered chow intake.....	58
Figure 10: Effects of NPY on sucrose intake after injection into the PFH or the N.Acc.....	63
Figure 11: Effects of N.Acc FLU on PFH NPY-induced sucrose intake.....	66
Figure 12: Effects of NPY injected into the PFH and N.Acc on PR operant responding.....	72
Figure 13: Effects of peripheral FLU on NPY-induced vs. drug-free PR operant responding for sucrose.....	75
Figure 14: Effects of N.Acc and PFH saline administration on place conditioning.....	80

Figure 15: Effects of N.Acc and PFH NPY and AMPH (I.P.) on place conditioning.....	81
Figure 16: Effects of PFH and N.Acc NPY on 1 hr chow intake in rats being tested for NPY place conditioning.....	82
Figure 17: Pearson product-moment correlation between CPP and NPY-stimulated food intake for rats receiving 24 pmol NPY/side in the PFH.....	83
Figure 18: Effects of FLU on PFH NPY-induced chow feeding in rats being tested for NPY + FLU place conditioning.....	86
Figure 19: Effects of FLU on NPY-induced CPP.....	87
Figure 20: Effects of N.Acc AMPH on ambulatory and vertical locomotor activity.....	93
Figure 21: Effects of N.Acc FLU on AMPH-induced locomotor activity.....	96
Figure 22: Effects of N.Acc muscimol and morphine on regular chow feeding.....	99

List of Tables

Table 1: Summary of experimental paradigms and predicted outcomes.....	41
Table 2: Summary of experimental paradigms and observed outcomes.....	104

DIFFERENT BRAIN LOCI MEDIATE NEUROPEPTIDE Y'S EFFECTS ON FEEDING vs. REWARD

Degree of Doctor of Philosophy, Department of Psychology, University of Toronto, 1999

Christina Margaret Brown

Abstract

There is a high co-prevalence of eating disorders and substance abuse in humans. One theory offered to account for this relationship implicates a common biochemical substrate in the pathophysiology of both disorders. Neuropeptide Y (NPY), a 36 amino-acid peptide found throughout neurons of the central and peripheral nervous systems, may be one candidate serving both roles. NPY stimulates voracious feeding in previously satiated rats when injected into the perifornical hypothalamus (PFH). It also has rewarding effects when injected into the nucleus accumbens (N.Acc) as evidenced by its induction of conditioned place preference (CPP) learning.

To clarify the anatomical specificity of NPY's role in mediating feeding and reward, the present dissertation examined the effects of PFH vs. N.Acc injections of NPY on "regulatory feeding" (resulting from homeostatic need) vs. "non-regulatory feeding" (due to rewarding properties of food) as well as other reward-related behaviours. Regulatory feeding was assessed by measuring intake of a non-preferred food, powdered lab chow, while non-regulatory feeding was assessed by measuring intake of a preferred food, sucrose. Other measures of reward were performance of a progressive ratio (PR) operant response for sucrose and CPP. The contribution of dopamine (DA) in mediating these NPY-site effects was also determined.

NPY dose-dependently increased chow (Experiment #1a) and sucrose

(Experiment #2a) intake to the same extent when injected into the PFH but not the N.Acc. Likewise, a dose-dependent increase in PR responding for sucrose occurred following PFH but not N.Acc NPY injections (Experiment #3a). These responses were not blocked by pre-administering the DA receptor blocker, α -flupenthixol, into the N.Acc. A CPP was produced when a low dose (24 pmol/side) of NPY was tested in the N.Acc. (Experiment #3c). A CPP approaching statistical significance that was negatively correlated with food intake occurred with a low dose of NPY in the PFH (Experiment #3d).

These results indicate an anatomical dissociation between certain behavioural effects of NPY in the PFH vs. the N.Acc. The PFH mediates NPY's effects on regulatory feeding but plays little, if any, role in reward-relevant behaviours. Conversely, NPY in the N.Acc supports reward-related but not intake-enhancing effects. Further support for this conclusion comes from findings that DA does not contribute significantly to NPY's regulatory feeding response in the PFH, while its mediation of NPY reward in the N.Acc appears to exist, but is not robust.

Introduction

All organisms must procure, consume, and metabolise nutrients in order to survive. There are times, however, when an organism ingests a substance, not for its energy-producing effects, but for the pleasurable or rewarding state that results from its ingestion. Both human beings and animals are capable of misusing substances, be they foodstuffs or ones having psychoactive properties, such as drugs. When they occur in human beings, these conditions are labelled eating disorders or substance-related disorders. What each of these disorders has in common is the use of a substance by an individual to change mood, to experience pleasure, or “to dull the hardness of unpleasant reality” (Johnston, 1987).

There is a high co-prevalence of eating disorders and substance abuse in human beings. Some authors have proposed that a common diathesis, which predisposes individuals towards developing both disorders, may exist. One way this might be manifested is by an alteration in one or more endogenous neurochemical system(s). Neuropeptide Y (NPY) is a peptide neurotransmitter that exists in neurons of the central and peripheral nervous systems. Endogenous levels of NPY in the hypothalamus increase and decrease in relation to the nutritive state of the organism, and exogenous application of NPY results in voracious feeding in sated animals. As well, NPY appears to have rewarding effects of its own when injected into the nucleus accumbens (N.Acc), a brain area involved in reward-relevant behaviour. These rewarding effects ostensibly depend on the catecholamine neurotransmitter, dopamine (DA), as the pre-administration of a DA antagonist abolishes them.

It is possible that the NPY system is disrupted in individuals suffering from eating

disorders and/or substance abuse. In fact, NPY levels have been found to be altered in both underweight, and weight-restored, anorexic patients. NPY acting on its own receptors in the hypothalamus may underlie behaviour that is activated in response to homeostatic need, such as feeding; while N.Acc NPY may activate non-regulatory feeding (that which results from hedonic, or rewarding, properties of food), and other reward-related behaviour through interactions with the DA system. Thus, a disturbance in the NPY system might result in a predisposition to find a variety of stimuli more rewarding, and might explain the high concordance rate between eating and substance use disorders. To test these assumptions, this thesis assessed the effects of hypothalamic vs. N.Acc NPY on feeding of a palatable vs. a non-palatable food, operant responding for sucrose, and place conditioning in rats. In addition, DA's involvement in the NPY-elicited responses was evaluated.

Psychological rationale: The coexistence of eating disorders and substance abuse

Eating disorders include anorexia nervosa, bulimia, and eating disorder not otherwise specified, a category which includes the newly established binge-eating disorder (BED). The American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders 4th edition (1994) describes the following diagnostic criteria for anorexia nervosa: "a refusal to maintain body weight over a minimal normal weight for age and height, the intense fear of gaining weight or becoming fat (even though underweight), a disturbance in the way in which one's body weight, size or shape is experienced, and in females, the absence of at least three consecutive menstrual cycles" (p. 544-545). The criteria for bulimia are: "recurrent episodes of binge eating, a feeling

of lack of control over eating behaviour during the eating binges, regular use of a purging method in order to prevent weight gain, a minimum average of two binge eating episodes a week for at least three months, a persistent overconcern with body shape and weight, and the disturbance does not occur exclusively during episodes of anorexia nervosa”(p.549-550). The last category, eating disorder not otherwise specified, consists of disorders of eating that do not meet the criteria for the above two disorders. This includes BED, whose criteria are fundamentally the same as bulimia, but does not require the use of compensatory mechanisms to counteract the effects of the binges, which are characteristic of bulimia (p.731).

Some authors have noted similarities in the behaviour patterns that characterise certain eating disorders like bulimia and BED, and substance dependence. The DSM-IV criteria for substance dependence include “a maladaptive pattern of substance use which leads to clinically significant impairment or distress, as manifested by three or more of the following symptoms occurring in the same 12 month period: tolerance to the substance, withdrawal symptoms, the substance is often taken in larger amounts or over a longer time period than intended, there is a persistent desire or unsuccessful efforts to cut down or control the substance use, a great deal of time is spent in activities necessary to obtain the substance, important activities are given up or reduced because of substance use, and/or the substance use is continued despite knowledge of one having problems that are likely to have been caused or exacerbated by the substance”(p.181). According to some authors, most researchers and clinicians agree that eating and substance use disorders have, as a key component, an “unequivocal lack of impulse control” (Garfinkel, Moldofsky, & Garner, 1980).

Although anorexia nervosa is diagnosed in only 0.5-1% (Crisp, Palmer, & Kalucy, 1976) and bulimia in 3% (Drewnowski, Yee, & Krahn, 1988) of young women, higher rates are frequently reported in individuals suffering from substance abuse disorder (Holderness, Brooks-Gunn, & Warren, 1994; Krahn, 1991). Conversely, while the lifetime risk of substance abuse in the normal population ranges from a low of less than 0.5% for heroin use to a high of approximately 13% for alcohol abuse (Jaffe, 1990), the rates in eating disordered individuals are far more inflated (Holderness et al., 1994; Krahn, 1991; Crisp, 1968). In a review of the literature spanning from 1977 to 1992, Holderness et al. found that the reported prevalence rates of alcohol abuse in anorexics averaged 26% across studies, while in bulimics it averaged 22.9%. Drug abuse rates in these populations averaged 25% and 17.05%, respectively. The rate of anorexia nervosa in substance abusing populations was only reported in three studies (2, 5, and 10%), while the rate of bulimia was higher, the average being 20%. Interestingly, familial alcohol and substance abuse rates were reported only for bulimic subjects, and averaged 39.15% and 18.95%, respectively. More recently, Taylor, Peveler, Hibbert, and Fairburn (1993) determined that disturbed eating habits and attitudes, as well as clinical eating disorders, were more prevalent in women receiving alcohol treatment than in a community sample. Selby and Moreno (1995), studying patients presenting at an inpatient treatment unit for eating disorders, divided subjects into subtypes of eating disorders or conditions (anorexic, bulimic, obese) and compared each group's rate of substance abuse, as well as their familial rates, to that of depressed patients. The authors found that bulimic patients reported significantly greater rates of substance abuse (47.6%) than either anorexic (20%), obese (28.1%) or depressed (22.5%) patients. Similarly,

71.1% of bulimics reported familial substance abuse, while anorexics reported 42.9%, obese patients 44.4%, and depressed patients only 27.5%.

The pattern of co-morbidity between eating disorders and substance abuse also exists in males, although it is far less reported. Katzman and Marcus (1991), studying 46 men and 34 women presenting for outpatient substance abuse treatment, found that 20% of men and 20% of women reported eating disorders. Differences between the genders emerged, however, when the method of purging was examined. While women were more likely to abuse laxatives and engage in self-induced vomiting, men were more inclined to abuse exercise, defined as exercising more than two hours each day, seven days a week. Carlat, Camargo, and Herzog (1997) found prevalence rates of substance abuse in eating disordered men to be 37%. This number rose to 61% when only bulimics were considered. Family alcoholism in this sample of 135 patients was reported by 37% of the subjects.

Several theories exist to explain the co-existence of eating disorders and substance abuse. One view sees eating disorders and substance abuse as the expression of a common personality disorder (Lacey & Evans, 1986), which is characterised by a lack of impulse control. While this theory seems to have good face validity, empirical validation remains inconclusive. Some authors (Yeary & Heck, 1989) assert that eating disorders are forms of psychoactive substance abuse, and anecdotal reports exist of a reciprocal relationship between substance abuse and eating disorders, where an improvement in one leads to a deterioration in the other (Taylor et al., 1993). On the other hand, studies which have attempted to characterise the psychological profiles that are common to both substance abusing and eating disordered women have met with little

success (Grilo et al., 1995; Butterfield & LeClair, 1988).

Another explanation for the co-existence of the two disorders evolved from the observation that families of anorexics and bulimics interact more dysfunctionally than do families of non-eating disordered individuals (Sargent, Liebman, & Silver, 1985; Humphrey, 1986). This theory remains unproven, however, due to the inability of most studies to tease apart the temporal associations between eating disorders, substance abuse, and affective illness (Holderness et al., 1994). In addition, the fact that genetic factors appear to be involved in the phenotypic expression of eating disorders (Strober, 1991) as well as some forms of substance abuse, particularly alcoholism (Cotton, 1979), further confuses the matter.

A third theory, the self-medication hypothesis, suggests that eating disordered individuals begin abusing psychoactive substances in order to cope with the social isolation, worry, and dysphoria commonly associated with eating disorders (Holderness et al., 1994). An offshoot of this theory is that both eating disordered and substance abusing groups are attempting to self-medicate their underlying symptoms of depression (Krahn, 1991). Indeed, some authors have found lifetime prevalence rates for major depression in 70% of bulimic subjects (Walsh, Roose, Glassman, Gladis, & Sadik, 1985), a number that rose to 88% when all affective disturbances were considered. Similarly, associations have been documented between depression and substance abuse (Deykin, Levy, & Wells, 1986), including alcoholism (Weissman & Myers, 1980), and antidepressant medications have been reported to be effective in treating some eating disorders, especially bulimia (Kennedy & Goldbloom, 1991). Some authors (e.g., Krahn, 1991) have proposed that one disorder might lead to an increased susceptibility to the

other. For example, the food deprived state that increases preferences for sweet, high-fat foods (the foods most often included in binges) might also increase one's preferences for certain drugs. In a classic study, Franklin, Schiele, Brozek, & Keys (1948) found that normal men who received only half of their normal caloric intake increased their consumption of caffeine and tobacco, the only drugs available to them. Likewise, food deprivation in animals leads to preferences for drugs, and has become a standard method for establishing drug self-administration (Stewart & Grupp, 1984).

The proposition that depression or another underlying genetic mechanism might be involved in the pathophysiology of eating and substance abuse disorders can be investigated using techniques designed to elucidate common biochemical substrates or physiological elements of both disorders. Using these techniques, associations can be established between behaviour that is characteristic of the disorders and a variety of neuromodulators, including monoamines, endogenous opioids, and neuropeptides. One such neuropeptide that has been implicated in disruptions of eating and other reward-relevant behaviour is NPY that, along with its functional interactions with the monoamine DA, serves as the focus of this thesis.

Neuropeptide Y (NPY)

Since its discovery in 1982 by Tatemoto, Carlquist, and Mutt, NPY, a member of the 36 amino acid family of pancreatic polypeptides, has been found to have many biochemical, physiological, and behavioural functions related to feeding, in addition to other effects (Wahlestedt & Reis, 1993). This class of peptides, which also includes peptide YY (PYY), pancreatic peptide (PP), and the non-mammalian pancreatic peptide Y (PY), was so named after avian PP was discovered during the process of isolating insulin

from pancreatic islet cells (Heilig & Widerlöv, 1990). The term NPY comes from the finding that it is abundant in the brain, and that its amino acid sequence ends with a C-terminal tyrosine (the Y being the abbreviation for tyrosine in the single letter amino acid code). NPY is widely distributed throughout neurons of both the central and peripheral nervous systems, and is found in particularly high concentrations in the hypothalamus, a structure critically involved in energy homeostasis, and the control of neuroendocrine/autonomic systems (Leibowitz, 1990). Specifically, the greatest amount of NPY is found in the paraventricular nucleus of the hypothalamus (PVN) (Chronwall et al., 1985). NPY is known to coexist with classical neurotransmitters such as norepinephrine (NE), epinephrine (EPI), and serotonin (5-HT) in certain hypothalamic neurons, but not with DA (Hökfelt et al., 1987; Kyrkouli, Stanley, & Leibowitz, 1990).

There are two basic types of neurons in the brain that have been determined to be NPY immunoreactive (Hendry, 1993): short-axon cells or interneurons, which are predominant in the forebrain and large subcortical regions, such as the striatum and amygdala; and long-projection neurons, which are found principally in the medulla and A1 regions of the brainstem and project to the PVN. Another projection area originates in the arcuate nucleus and sends ipsilateral projections to the PVN and the dorsomedial hypothalamus (Hendry, 1993; Heilig & Widerlöv, 1990). The arcuate nucleus also has connections with the pituitary, other hypothalamic areas, the limbic system, the midbrain periaqueductal gray, and autonomic nuclei of the brain stem. It appears, therefore, that centrally-administered NPY's endocrine and vegetative effects result from its action at this nucleus, as well as through its connections with the PVN (Heilig & Widerlöv, 1990).

Apart from the hypothalamus, the N.Acc is an area that has been shown to contain

some of the highest levels of NPY-like immunoreactivity in the mammalian brain, and the greatest density of neurons containing NPY message in the human brain (Hendry, 1993; Heilig & Widerlöv, 1990; Allen et al., 1983). In fact, the concentration of NPY immunoreactivity in the N.Acc is believed to be regulated by the presence of NPY within a system of afferent axons that selectively innervates it, possibly the projection from the ventral tegmental area (VTA)(Hendry, 1993).

NPY and feeding

History:

One of the many behavioural effects of NPY is induction of feeding in previously satiated animals (Clark, Kalra, Crowley, & Kalra, 1984; Stanley & Leibowitz, 1984; Kalra, Dube, & Kalra, 1988; Paez, Nyce, & Myers, 1991; Paez & Myers, 1991; Pich et al., 1992), with the perifornical hypothalamus (PFH) being the most sensitive injection site (Stanley, 1993; Stanley, Magdalin, Seirafi, Thomas, & Leibowitz, 1993; Currie & Coscina, 1995, 1996; Brown & Coscina, 1995). The orexigenic effect of NPY has been observed in many species (Steinman, Fujikawa, Wasterlain, Cherkin, & Morley, 1987; Morris & Crews, 1990; Nakajima et al., 1990; Okita et al., 1990; Paez & Myers, 1991; Miner, 1992), and is due to an increased motivation to eat rather than pathological or stimulus-bound eating. For example, NPY-injected mice will ingest more milk when required to work for it in a lever press apparatus, will tolerate more shock to the tongue for drinking milk than saline-treated controls, and will overcome a taste aversion for quinine-adulterated milk (Flood & Morley, 1991).

Due to its large molecular size, it is difficult, if not impossible, for NPY (and other peptides) to cross the blood-brain barrier. A study by Levine and Morley (1984) found that

NPY has no feeding-stimulatory effects when injected intraperitoneally (I.P.), suggesting a central mechanism of action of this peptide. Therefore, studies looking at the feeding-stimulatory effects of NPY have employed the central injection technique.

The first study to examine whether NPY causes changes in feeding behaviour was conducted by Clark et al. (1984). These researchers found that injecting NPY into the third ventricle of ovariectomised female rats induced a significant increase in feeding behaviour, thus implying that NPY, or a closely related pancreatic polypeptide-like neuropeptide, plays an important role in neural regulation of food intake. Following this observation, Levine and Morley (1984) and Clark, Kalra, and Kalra (1985) replicated the finding by injecting NPY into the third ventricle of male rats. Both studies found that NPY increased feeding in a dose-related manner during the light phase of the light/dark cycle, the period when rats typically ingest small amounts of food. Stanley, Chin, and Leibowitz (1985) decided to explore more specifically NPY's site of action using a mapping technique that considered seven different brain regions. Results of this study indicated that NPY acts at the hypothalamus. Food intake resulting from injections into hypothalamic regions (i.e., PVN, ventromedial hypothalamus, lateral hypothalamus (LH), medial preoptic area) increased by over 300% as compared to extra-hypothalamic regions (i.e., amygdala, periaqueductal grey, thalamus) and saline-treated controls. Further investigations by Stanley and Leibowitz (1984; 1985) pinpointed NPY's site of action as the PVN. Since then, the PVN was considered the most sensitive site of action of exogenous NPY. More recently, however, Stanley et al. (1993), observing that effects produced by PVN injection of NPY were no greater than those injections at any other hypothalamic site (Stanley et al., 1985), carried out an extensive mapping study to find the exact location of NPY's effect.

Using a microinjection technique that allowed the researchers to inject solutions in a very small volume (10 nl as opposed to the usual 300-500 nl), and thereby reduce the spread of drug from the injection site, Stanley et al. found that the PFH, at the level of the caudal PVN, is the most sensitive hypothalamic site for NPY-induced eating. Furthermore, injections bracketing the PFH in all directions were substantially less effective. Similar results are found when the feeding response resulting from NPY injections in the PVN and the PFH are compared (Brown & Coscina, 1995; Currie & Coscina, 1995, 1996). The PFH has relatively dense concentrations of NPY-terminal immunoreactivity, particularly overlapping hypothalamic neurons that project to brain stem autonomic nuclei (Gray et al., 1986). The axons from NPY brain stem neurons traverse the PFH en route to the PVN (Stanley, 1993), suggesting that it may be the PFH, and not the PVN, which is responsible for the eating-stimulatory effects of NPY. It is currently unknown whether the axons traversing the PFH provide synaptic NPY input to this region. It is interesting to note that, although some NPY neurons have been shown to make classical synaptic connections, many immunoreactive terminals exist as free nerve endings (Heilig & Widerlöv, 1990), making it possible for NPY to exert post-junctional effects at some distance from its site of release. In fact, in their precise mapping study, Stanley et al., (1993) determined the latency to eat in the PFH to be between 18.6 and 23.4 min., while all other sites (including the PVN) had mean latencies of over 33 min. These results suggest that diffusion into the PFH from PVN-aimed cannulae may be responsible for the observed eating effects.

Receptor subtypes:

Although hampered by the lack of selective antagonists, the search for NPY

receptor subtypes has taken place by comparing the effects of NPY with various peptidergic agonist analogues. These compounds include various fragments of the parent peptide, as well as several chemically related peptides such as PYY and PP. Using this technique, researchers have discovered at least five distinct receptor subtypes for NPY. The term Y1 was introduced to refer to the receptor that required the whole NPY or PYY molecule for activation, while Y2 refers to the receptor that is activated only by the C-terminal NPY fragments, like NPY₂₋₃₆ and NPY₂₂₋₃₆ (Larhammar, 1996; Wahlestedt & Reis, 1993; Wahlestedt & Heilig, 1995). Y3 receptors were later distinguished on the basis that some actions of NPY could not be mimicked by PYY (Michel, 1991). This was followed by the discovery of the Y4 receptor (Bond, Walker, Branchek, & Weinshank, 1995) which, based on its high affinity for rat and human PP, was suggested not to be classified as a pure NPY receptor. The search for NPY's "feeding receptor" continued to be fuelled by reports of feeding effects of NPY and some of its fragments, such as NPY₂₋₃₆, which differed from their effects at the Y1 receptor. Recently, such a receptor, termed the Y5 receptor, was cloned from rat hypothalamus (Gerald et al., 1996). This receptor is down-regulated in animal models of obesity such as the obese Zucker rat (Widdowson, 1997), and its gene transcript is expressed in the PVN and lateral hypothalamus, two brain areas implicated in feeding (Gerald et al., 1996). Additionally, NPY Y5 receptor antisense oligodeoxynucleotides decrease fasting-induced meal size and duration, as well as prevent the increases in hypothalamic NPY levels found during food deprivation (Schaffhauser et al., 1997).

NPY and eating disorders:

Cerebrospinal fluid (CSF) levels of NPY are elevated in underweight amenorrheic

anorexic patients. In many of these amenorrheic patients, this elevation persists for up to six weeks of weight restoration (Kaye, Berretini, Gwirtsman, & George, 1990). As anorexics typically display paradoxical attitudes towards diet, resisting food intake while being obsessively preoccupied with food, increased NPY levels are posited to reflect a homeostatic signal that attempts to stimulate feeding behaviour. Alternatively, increased NPY levels may reflect a secondary, compensatory response to food restriction. Indeed, in the same study, a significant inverse relationship between caloric intake and CSF NPY levels was found in normal female controls. A third explanation for these results is that NPY is involved in the accompanying menstrual dysregulation, as normalisation of menstrual function was associated with normalisation of CSF NPY levels.

NPY and reward

The capacity of a stimulus to be rewarding has been defined as its ability to elicit approach behaviour, as well as its ability to increase the probability that the responses preceding it will be repeated (Carr, Fibiger, & Phillips, 1989). NPY has reinforcing, or rewarding, effects of its own. Josselyn and Beninger (1993) found that NPY produced a conditioned place preference (CPP) in rats when injected in a low dose into the N.Acc. In this paradigm, one distinct environment is paired with a specific drug injection while a second environment is paired with injection of saline. During the testing phase, the non-drugged animal is allowed access to both environments. If an animal spends significantly more time in the environment that was previously paired with the drug, a place preference is said to occur, indicating rewarding, or reinforcing, effects of the drug. The CPP effect of NPY was blocked by pretreatment with α -flupenthixol (FLU), a DA receptor blocker. The authors conclude that NPY applied to the N.Acc is rewarding, and that these rewarding

properties may be mediated by increased DA neurotransmission.

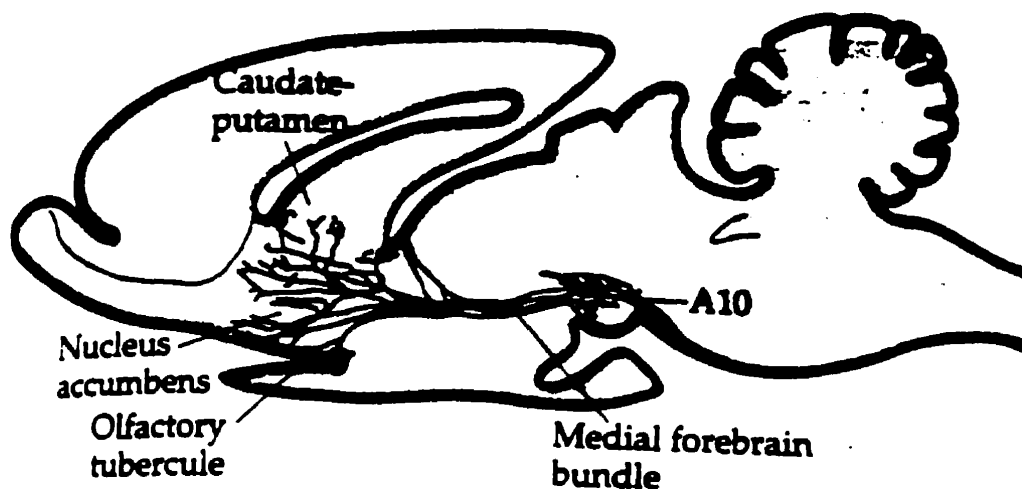
NPY, when injected intracerebroventricularly (icv) in satiated rats, has been found to increase food-reinforced operant responding on both fixed ratio (FR) (Jewett, Cleary, Levine, Schaal, & Thompson, 1992) and progressive ratio (PR) schedules of reinforcement (Jewett, Cleary, Levine, Schaal, & Thompson, 1995; Jewett, Schaal, Cleary, Thompson, & Levine, 1991). The PR schedule has been used to determine an organism's motivation to respond for rewarding stimuli (Roberts & Richardson, 1992), and demands that response requirements escalate during each experimental session, allowing bar-pressing behaviour to extinguish in each animal on each day (Roberts, 1992). The final ratio of responses emitted by an animal is defined as the "breaking point" (Roberts & Richardson, 1992). Typically, drugs that increase the sensitivity of the mesolimbic DA system increase breaking point as the dose of drug is increased (Richardson & Roberts, 1996; Roberts & Bennett, 1993). As well, high (i.e., 95%) sucrose content in reward pellets results in increased breaking point when it is compared to low (i.e., 1%) content, and these effects are blocked by DA receptor antagonism (Cheeta, Brooks, & Willner, 1995). Following NPY administration, breaking points increase, and are comparable to those obtained from animals food-deprived for 36-48 hrs. However, insulin and 2-deoxyglucose, which also increase food intake, have no effects on breaking points (Jewett et al., 1995), indicating that NPY may change the reinforcing value of food in ways unrelated to homeostatic mechanisms regulating nutrient intake.

Mesolimbic dopamine

DA, one of the catecholamine neurotransmitters, is present in neurons of the central nervous system. DA neurons which project to forebrain areas originate in three cell groups

that are labelled A₈, A₉, and A₁₀, and are classified on the basis of their topographic location (Di Chiara, 1995). The two DA systems that are most commonly referred to are: the nigrostriatal DA system, which sends axons from the substantia nigra (A₉) to the caudate-putamen, and is important in the control of movement; and the mesolimbic DA system (see Figure 1), whose neurons originate in the VTA (A₉, A₁₀) of the midbrain and project to various forebrain areas, including the N.Acc (Ungerstedt, 1971). This group of cells is best known for its involvement in motivational and reward processes (Roberts, 1992). Because of this, the mesolimbic DA system, and its possible interactions with NPY, is of considerable interest.

Figure 1. The mesolimbic dopamine system.



Mesolimbic dopamine and feeding

DA neurotransmission appears to play a central role in food reward. One way in which this hypothesis has been tested is by making knife cuts or lesions that deplete DA in specific brain areas and observing resultant feeding behaviour. Alheid, McDermott, Kelley, Halaris, and Grossman (1977) looked at deficits in food and water intake after knife

cuts positioned either ventral or medial to the striatum, a brain area that includes the N.Acc. Rats that received cuts on the parasagittal plane at the lateral edge of the lateral hypothalamus exhibited profound weight loss post-surgery, and never returned to baseline control levels. Furthermore, these rats demonstrated prolonged periods of aphagia and adipsia, in addition to deficits in responding to different types of glucoprivic and hydrational challenges. All cuts that interfered with striatal connections depleted DA from the striatum, with the parasagittal cuts resulting in the most severe effects (13% of control values).

In vivo microdialysis studies have shown that extracellular levels of DA and its metabolites in the N.Acc increase following food presentation and electrical stimulation of the LH which stimulates feeding (Hernandez & Hoebel, 1988). More recently, Martel and Fantino (1996) found that levels of DA and its metabolites in the N.Acc rose during ingestion of a highly palatable diet. The amount of DA released was positively correlated with the amount of food ingested, which, the authors propose, suggests a role for mesolimbic DA in food reward. Other authors have found increased N.Acc and striatal DA metabolism following consumption of a nutritive meal, but no effects following ingestion of a non-nutritive saccharin solution (Blackburn, Phillips, Jakubovic, & Fibiger, 1986). These results suggest that post-ingestional factors, and not simply taste hedonics, are important for DA-dependent feeding.

Other evidence for DA's involvement in feeding behaviour comes from the finding that low doses (i.e., those which do not produce non-specific behavioural activating effects) of amphetamine (AMPH), an indirect DA agonist that acts by increasing the release and blocking the reuptake of DA, reliably increase food consumption in rats when injected

either peripherally (Evans & Vaccarino, 1990) or centrally into the N.Acc (Evans & Vaccarino, 1986; Sills, Baird, & Vaccarino, 1993). When given a choice between different food types, rats receiving AMPH will typically increase their consumption of nutritive, palatable foods, such as carbohydrates (Evans & Vaccarino, 1990). It is clear that the orexigenic effects of AMPH depend on DA transmission: pretreatment with FLU attenuates AMPH-induced increases in sugar consumption (Evans & Vaccarino, 1990), and feeding is observed following administration of *d*-amphetamine, but not *l*-amphetamine, which is two to five times less potent at releasing DA (Evans & Vaccarino, 1987).

The effects that DA has on feeding behaviour appear to be confined to specific aspects of that behaviour. For example, DA receptor blockade with haloperidol has been found to decrease electrically stimulated feeding behaviour, while having no effect on deprivation-induced feeding (Phillips & Nikaido, 1975). These authors postulate that brain stimulation-induced feeding may be subserved by the activation of one or more specific subsystems that are normally involved in the regulation of food intake. Similarly, tail pinch-induced eating, gnawing, and licking behaviour (which parallels stimulus-bound eating) is blocked by haloperidol, pimozide, and spiroperidol pretreatment (Antelman, Szechtman, Chin, & Fisher, 1975). It is possible that the subsystem in which DA is involved is one related to the rewarding aspects of food. One study that provides support for this hypothesis utilised microdialysis methods to study DA release and metabolism in the N.Acc of behaving rats (Salamone, Cousins, McCullough, Carriero, & Berkowitz, 1994). Rats that pressed a lever on a FR5 schedule showed significant increases in extracellular DA and DA metabolites compared to food-deprived controls. Furthermore, the increase in DA levels was not simply related to the action of ingesting food, as rats

receiving massed presentation of food pellets consumed large quantities of food, but showed no significant increase in DA release. Thus, increases in N.Acc DA that accompany operant responding may facilitate the ability of an organism to overcome obstacles, or response costs (Salamone, Cousins, & Snyder, 1997), that separate it from significant stimuli (such as food, or drugs).

Other authors have proposed that DA systems may be involved in the preparatory behaviour associated with feeding. Metoclopramide (a DA antagonist) significantly attenuated conditioned preparatory responses to a conditional stimulus signalling delivery of a meal, while affecting consummatory behaviours only at the highest dose (Blackburn, Phillips, and Fibiger, 1989). Similar effects have been found using voltammetry to monitor N.Acc DA transmission during lever pressing for milk reward (Richardson & Gratton, 1996). These authors concluded that N.Acc DA neurons are activated primarily in response to the incentive, rather than the reinforcing, properties of rewards due to the finding that greater increases in DA activity were observed in the period preceding each lever-press than during presentation of the reward itself. Likewise, presentation of a conditional stimulus predictive of food is associated with an increase in the chronoamperometric response to DA; however, this response remains elevated during and following meal consumption (Phillips, Atkinson, Blackburn, & Blaha, 1993). Finally, rats that have been conditioned to associate a taste with intragastric administration of a nutritive substance show elevated DA levels in the N.Acc following presentation of the conditioned stimulus as compared to unconditioned controls receiving the same treatment (Mark, Smith, Rada, & Hoebel, 1994).

Mesolimbic dopamine and reward

A variety of drugs that are self-administered by animals and human beings have been demonstrated to elicit an increase in N.Acc DA, as measured by microdialysis. This includes, most notably, cocaine (Weiss, Markou, Lorang, & Koob, 1992), and AMPH (Di Chiara & Imperato, 1988), but also encompasses other drugs such as ethanol (Di Chiara & Imperato, 1985), nicotine (Brazell, Mitchell, Joseph, & Gray, 1990), and opiates (Di Chiara & Imperato, 1988). N.Acc DA activity following the self-administration of heroin, however, as opposed to acute, experimenter-given injections, has been found to remain unchanged (Hemby, Martin, Co, Dworkin, & Smith, 1995), indicating contradictory results for this class of drugs.

Another paradigm used to assess reward processes is the electrical stimulation of certain brain structures, or brain stimulation-reward (BSR). This procedure has been used to reinforce behaviour in many species, from goldfish, pigeons, rabbits, dolphins, rats, and chimpanzees to human beings (Rolls, 1975). The systemic administration of DA antagonists blocks the effects of BSR, while apparently leaving motor systems intact. Fouriezos and Wise (1976) injected rats with pimozide, a DA receptor blocker, and showed that subjects would cease responding for BSR in a manner analogous to that which occurs when the reinforcement is terminated or withheld. The rate of responding would initially be high, then gradually decrease until it eventually extinguished, indicating that subjects were capable of responding, but chose not to. Using rate of responding as the dependent measure was initially criticised by Valenstein (1964), who said that rate has a behavioural ceiling; that is, a maximal rate at which the organism can lever-press. Using this method, any further increase in the rewarding effect of the stimulation, therefore, will not be

noticed. Using the frequency threshold method, one that circumvents the problems associated with rate-limiting aspects of BSR, Stellar, Corbett, and Hamilton (1985) found that N.Acc injection of FLU, in doses that left motor functioning intact, produced more than a 30% reduction of medial forebrain bundle stimulation reward, as measured by an increase in the frequency threshold required to elicit self-stimulation.

Just as DA antagonists reduce rewarding brain stimulation, the administration of DA agonists enhances it, providing further evidence of DA's role in reward processes. Gallistel and Karras (1984) found that AMPH administration dose-dependently decreased the frequency at which rats would lever-press for BSR. Cocaine also facilitates BSR by lowering frequency thresholds when administered in moderate doses (Maldonado-Irizarry, Stellar, & Kelley, 1994; McGregor, Atrens, & Jackson, 1992), an effect that was reversed by DA antagonists such as (+)-UH232 (Kling-Petersen, Ljung, & Svensson, 1994). Other DA agonists, such as GBR-12909, a reuptake blocker like cocaine, also decrease thresholds (increase reward) at medium doses (Maldonado-Irizarry et al., 1994).

Interaction between NPY and dopamine

Anatomy:

Support for an anatomical link between NPY and DA comes from labelling studies. Using double immunocytochemistry, Kubota et al. (1988) found that NPY-immunoreactive neurons receive synaptic inputs from DA-ergic axon terminals in the rat neostriatum. These authors suggest that nigrostriatal DA neurons may monosynaptically influence NPY neurons in the striatum. Furthermore, the expression of NPY immunoreactivity in the N.Acc has been determined to be under the influence of the DA-ergic mesencephalic pathway. Unilateral 6-OHDA lesions of the nigral DA-ergic neurons induced a bilateral

decrease in the NPY density which was more marked in the contralateral, rather than the ipsilateral, N.Acc (Salin, Kerkerian, & Nieoullon, 1990). These results, along with the discovery of synaptic associations between tyrosine hydroxylase (a synthetic enzyme of DA and NE) immunoreactive terminals and NPY immunoreactive neurons within the N.Acc (Aoki & Pickel, 1988), provide support for the notion that functional catecholamine-NPY interactions occur within this structure. Finally, cocaine reliably increases NPY-like immunoreactivity in the rat hippocampal dentate gyrus (an area that does not normally express NPY) and decreases it in the adjacent dentate hilar interneurons (that normally do express it) when it is given at doses that produce seizures (Goodman & Sloviter, 1993). The increases in dentate gyrus NPY-like immunoreactivity appeared to be dependant on whether the dose of cocaine produced seizures, as electrical stimulation of the perforant path resulted in similar increases. No decreases in NPY-like immunoreactivity in dentate hilar interneurons were found following this treatment, however, indicating either that cocaine may be having direct effects on NPY levels, or that electrical stimulation may not completely mimic the seizures induced by cocaine.

Neurochemistry:

When NPY is injected icv, it significantly reduces striatal and brain stem DA turnover in α -methylparatyrosine (α -MPT) pretreated rats. α -MPT is a compound which, when injected I.P., inhibits the synthesis of catecholamines, thereby reducing their levels in most brain areas. The icv administration of NPY lessened the reduction of DA in the brain stem and striatum, indicating that NPY modulates the synthesis of DA turnover in the CNS (Vallejo, Carter, Biswas, & Lightman, 1987). Correspondingly, α -MPT administration, as well as treatment with haloperidol, results in significant decreases in both the number, and

staining intensity, of NPY-containing cells in the striatum (Kerkerian, Salin, & Nieoullon, 1988). NPY administration icv also results in increases in striatal DA in freely moving rats when a voltammetric method is used to measure catecholamines (Kerkerian-Le Goff et al., 1992), reaching maximal levels 1 hr after the peptide injection. DA release in surrounding brain tissue is also enhanced significantly in unrestrained animals when microdialysis procedures are used following NPY infusion into the lateral ventricle (Matos, Guss, & Korpinen, 1996) or the hypothalamus (Myers, Lankford, & Roscoe, 1996; Myers, Lankford, & Paez, 1992). The same occurs when NPY is injected into the striatum (Beal, Frank, Ellison, & Martin, 1986) or the lateral ventricle (Heilig, Vécsei, Wahlestedt, Alling, & Widerlöv, 1990) and brain tissue DA levels are measured *ex vivo*, by high performance liquid chromatography (HPLC) analysis. These results support the notion that DA function is modified by NPY, and raises the possibility that DA may be involved in the expression of NPY's behavioural effects.

Peripheral metamphetamine administration stimulates the release of NPY from the PVN of rats within 30 to 60 min post-injection (Yoshihara, Honma, Mitome, & Honma, 1996). On the other hand, NPY levels are significantly reduced in the N.Acc and the cerebral cortex of rats given repeated administrations of cocaine over a two week period (Wahlestedt et al., 1991). Interestingly, these reductions parallel those of DA in the same brain areas after the same treatment, indicating that whatever process is affecting one system might be affecting the other. These authors speculate that the analogous reductions in DA and NPY may relate to the anxiety and depression associated with cocaine withdrawal in humans.

Behaviour:

NPY has been shown to have anxiolytic effects in animal models of anxiety (Broqua, Wettstein, Rocher, Gauthier-Martin & Junien, 1995; Heilig, Söderpalm, Engel & Widerlöv, 1989) and depression (Song, Earley, & Leonard, 1996). Decreased CSF NPY levels have also been found in patients suffering from depression (Nilsson, Karlsson, Blennow, Heilig, & Ekman, 1996; Widerlöv, Lindström, Wahlestedt, & Ekman, 1988) and are associated with increased levels of anxiety (Widerlöv, Heilig, Ekman, & Wahlestedt, 1989).

Other behavioural studies have examined the interaction between NPY and DA and found similar patterns of results. The reinforcing effects of NPY appear to rely on DA activity. As stated above, when NPY is injected into the N.Acc, a CPP is produced, and this effect is blocked by co-administration of FLU (Josselyn & Beninger, 1993). Moore, Merali, and Beninger (1990), studying circling behaviour in rats, found that unilateral striatal NPY injections dose-dependently increased contralateral circling behaviour similar to the turning behaviour that occurs following unilateral striatal injections of DA agonists such as AMPH (Moore et al., 1990). Furthermore, Moore et al., using *in vivo* microdialysis, found similar levels of intrastriatal DA metabolites as did Beal et al. (1986) after NPY injection.

Summary:

Based on the above evidence, it is clear that NPY and DA interact, with NPY administration in some brain areas resulting in increased DA levels, and NPY inhibition having the opposite effect. Similarly, inhibition of the DA system results in significant decreases in NPY expression, while increases in DA stimulate NPY release. One way in

which NPY may be increasing CNS DA levels is by inhibiting the activity of dopamine- β -hydroxylase (DBH), the synthetic enzyme of NE. Cheng, Chang, and Tsai (1992), using a chromatographic analysis of NE formation from DA *in vitro*, found that NPY application resulted in dose-dependent decreases in NE formation. They concluded that NPY might be acting as an endogenous inhibitor of DBH in vesicles where it is co-stored with NE. There are also reports that NPY applied to rat N.Acc *in vitro* produces an increase in the basal activity of tyrosine hydroxylase activity (Westfall & Vickery, 1994). Alternatively, NPY may modulate DA release by means of a σ_1 -like receptor, as NPY-enhanced DA release from rat striatal slices (Ault & Werling, 1997), and NPY enhanced N-methyl-D-aspartate (NMDA)-stimulated DA release from N.Acc slices (Ault, Radeff, & Werling, 1998) are reversed by known σ_1 antagonists.

NPY, dopamine, and feeding

The orexigenic effects of NPY may be due to a direct action on Y5 receptors (Gerald et al., 1996) or to an indirect action of NPY on other neurotransmitters such as DA or NE, either presynaptically through modulation of the release of monoamines, postsynaptically through modulation of the monoaminergic effector response, or through a direct receptor-receptor interaction (Leibowitz, 1989).

A role for DA in the feeding-stimulatory action of NPY was suggested by Myers et al. (1992). Using HPLC analyses, these authors found that, during PFH NPY-induced feeding, the release of both NE and DA from the PFH was enhanced significantly. They concluded that the functional role of NPY in neurons involved in feeding revolves about its action on afferent synapses of either NE-ergic and/or DA-ergic neurons in the hypothalamus. This finding suggests a presynaptic action of NPY on DA release, rather

than a post-synaptic effect on receptors; although the latter cannot be ruled out completely due to possible interneuronal feedback loops that may be activated by NPY. Recently, similar results were obtained when NPY was infused through push-pull cannulae in the preoptic area of the hypothalamus (Myers et al., 1996). In this study, DA levels were increased following NPY injections that resulted in increased feeding behaviour but not after injections that elicited hypothermia alone or hypothermia and feeding. This indicates that the rise in DA levels following NPY administration is specific to its effects on feeding. This idea received further support from a study in which DA levels in the hypothalamus increased during icv NPY-induced feeding while other neurotransmitter levels, such as 5-HT, remained unchanged (Matos et al., 1996).

More evidence for DA's role in NPY-induced feeding comes from studies using DA antagonists. In a study examining the effects of monoamine antagonists on NPY-induced food intake, Levine and Morley (1984) found that NPY induction of food intake was markedly suppressed by peripheral administration of the DA antagonist haloperidol, as well as the opiate antagonist naloxone. The α -adrenergic antagonist phentolamine failed to suppress NPY-induced feeding. At a dose of 1.0 mg/kg, haloperidol significantly suppressed NPY-induced food intake at 1, 2, and 4 hrs post-injection, while the 0.1 mg/kg dose attenuated feeding only at 1 hr post-injection. In addition, NPY-induced water intake was suppressed by both the 0.1 and 1.0 mg/kg doses. The authors caution, therefore, that the doses used may have resulted in non-specific suppression of food intake.

Some studies have found that the inhibition of DA synthesis in the hypothalamus actually potentiates the NPY feeding response (Kyrkouli, Stanley, Hutchinson, Seirafi, & Leibowitz, 1990). As well, when AMPH is injected into the PFH, the feeding effects of

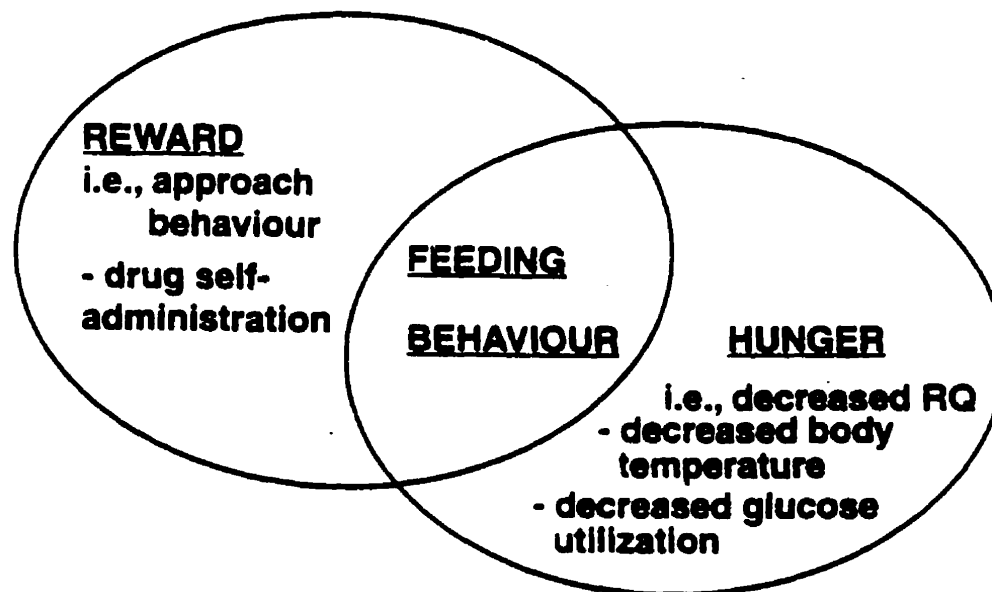
NPY are reduced, an effect that is blocked by a DA, but not NE, antagonist (Gillard, Dang, & Stanley, 1993). These authors also found that a maximally effective dose of DA alone reduced the NPY feeding response by 40%; however, it did not abolish it completely. It was concluded that NPY and DA interact in an antagonistic manner in the PFH, an area where DA acts primarily to inhibit feeding (Leibowitz, 1975; Hoebel, 1985).

Two motivations for feeding behaviour: Regulatory vs. Non-regulatory

There is no doubt that ingestive behaviour is a complex and multi-faceted phenomenon. The notion that it results due to myriad heterogeneous intrinsic and extrinsic factors is difficult, if not impossible, to deny. A person need only think of their own feeding behaviour to acknowledge that there are times when one eats to alleviate the unpleasant sensations of hunger such as a growling stomach, shakiness, dizziness, or weakness. At other times, they indulge in a pleasant-tasting food, such as a dessert, even though there is no nutritive need, and no accompanying unpleasant sensations. The intrinsic state of hunger is accompanied by a number of tangible physiological sequelae including: decreased oxygen utilisation; reduced respiratory quotient (RQ), the proportion of carbon dioxide produced to oxygen consumed (Kleiber, 1975); decreased glucose utilisation; a reduction in body temperature; and a lower overall metabolic rate (Le Magnen, 1985). Some authors have labelled the feeding behaviour that arises for homeostatic reasons “regulatory” feeding, as opposed to “non-regulatory”, which occurs in non-deprived animals in response to such manipulations as tail-pinch (Mittleman, Rushing, & Winders, 1993) and electrical stimulation of the LH (Mittleman, Castañeda, Robinson, & Valenstein, 1986). Other non-regulatory feeding behaviour includes that which occurs in an organism that is sated with one specific food type but will still ingest a different food

type as well as rate its taste more positively than the initial food, a phenomenon called “sensory-specific satiety” (Johnson & Vickers, 1992; Berridge, 1991). For the purposes of this thesis, two motivations for feeding behaviour will be considered and tested: the one that occurs as a result of hunger is termed “regulatory” feeding, while that which occurs due to hedonic, or rewarding, properties is called “non-regulatory”. As displayed in Figure 2, these motivators need not be mutually exclusive: it is possible for feeding behaviour to arise due to one, or both, factors. Furthermore, while hunger is accompanied by certain homeostatic, physiological correlates, reward is a construct hypothesised to be reflected in paradigms other than feeding, such as self-administration and approach behaviour directed towards the stimulus.

Figure 2. The two motivators of feeding behaviour.



These definitions correspond to the two motivators described by Kissileff (1991): the internally-driven, motivating behavioural effects of food deprivation, and the

rewarding, or externally propelled, motivators of feeding. These, Kissileff asserts, are representative of two basic “paradigms” that are intimately involved in ingestive behaviour: regulation and reward. These factors also parallel the interaction between internal states and both unconditional and conditional incentive stimuli put forward by Toates (1994), that underlies the incentive motivation model of feeding behaviour. Booth, Gibson, Toase, and Freeman (1994) acknowledge that an appetite for food is a desire to ingest “small objects or pieces of materials that have acquired personal significance from familiarity, nutritional benefit, and social and emotional value” (p.106), indicating that a number of motivating variables can direct feeding behaviour.

Some classes of drugs, such as the benzodiazepines, increase food intake not by inhibiting satiety or increasing hunger, but by increasing the “positive hedonic evaluation” of ingested food (Cooper & Higgs, 1994). As stated earlier, apart from its involvement in most reward-related behaviours, DA appears to be fundamentally involved in reward-driven feeding behaviour. DA antagonists have been determined to decrease the “reward quality” or incentive value of food (Wise, Spindler, de Wit, & Gerber, 1978), and some authors have speculated that endogenous DA may be involved in eating which occurs over and above normal preferences and intake (Evans & Vaccarino, 1990). Others have posited that separate neurobiological reward systems underlie the motivated behaviour that results when an organism is in a deprived vs. a non-deprived state (Nader, Bechara, & van der Kooy, 1997). Interestingly, these authors found that DA antagonism affects reward processes that occur in deprived states, while lesions of the tegmental pedunculopontine nucleus (TPP), but not DA antagonism, affect those that occur in non-deprived states. Support for DA involvement in non-regulatory feeding behaviour also comes from studies

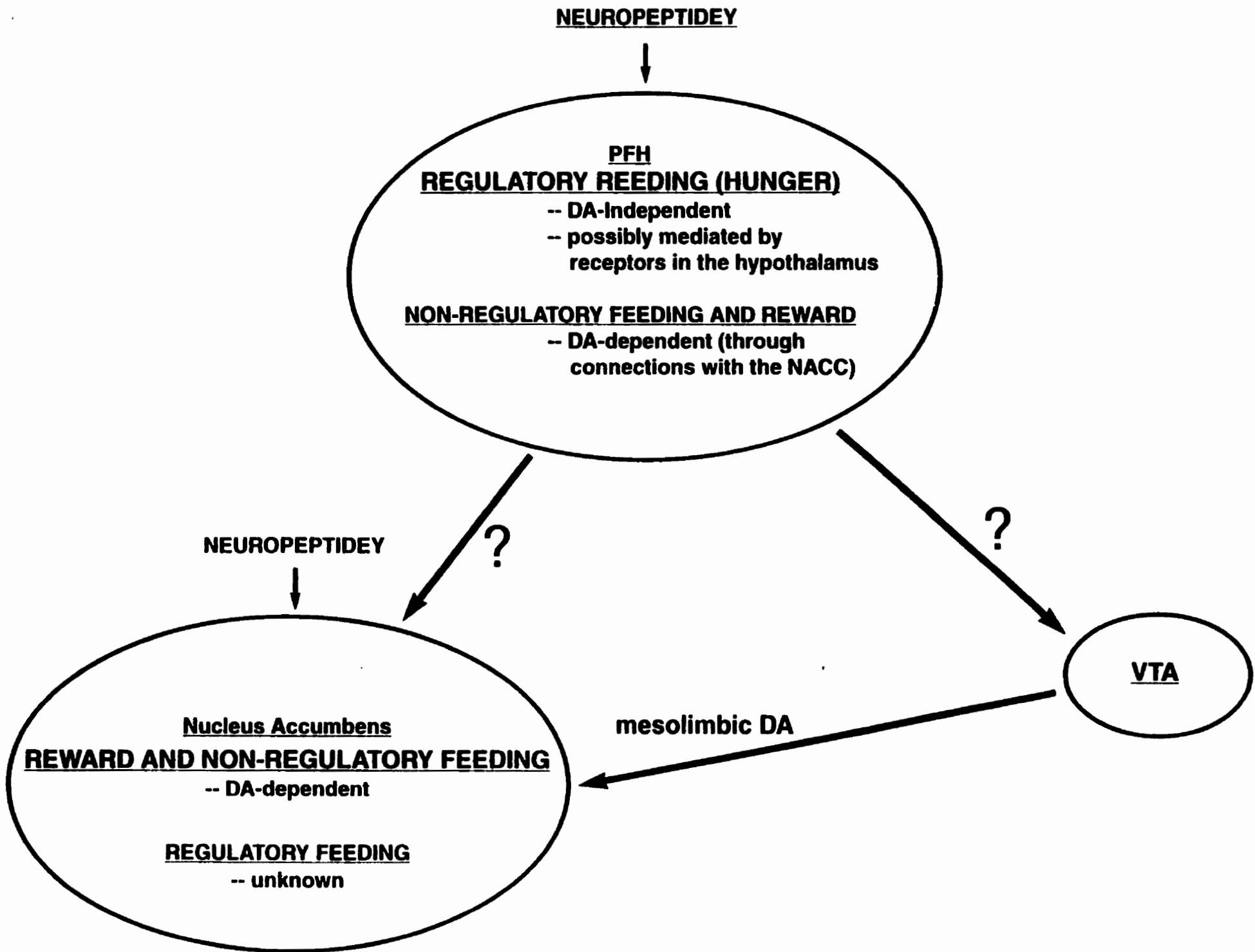
in which rats that eat in response to electrical stimulation of the LH also show increased behavioural sensitisation to repeated AMPH administrations compared to rats that do not display stimulation-induced feeding (Mittleman et al., 1986). In addition, footshock stress, which is known to produce increases in forebrain DA utilisation, did so more in rats exhibiting stimulation-induced feeding than in those not exhibiting feeding behaviour.

NPY and its effects on feeding and reward: An anatomical and functional model

Pursuant to the above definitions, an anatomical and functional model is now proposed, which will be used to generate and test certain hypotheses related to the effects of NPY on regulatory vs. non-regulatory feeding and other measures of reward (see Figure 3). As outlined above, the PFH is a site where NPY has been shown to have its strongest effects on feeding behaviour (Stanley et al., 1993), while the N.Acc seems to underlie its rewarding effects, as measured by CPP (Josselyn & Beninger, 1993). Anatomically, the PFH has direct connections with the N.Acc (Zahm & Brog, 1992) as well as the midbrain VTA (Hoebel, 1984), which contains cell bodies of ascending DA-ergic neurons projecting to the N.Acc. It is possible, therefore, that NPY has effects in the PFH which either directly or indirectly affect mesolimbic DA function. Such an alteration might be expected to be associated with changes in reward-relevant behaviours (Di Chiara, 1995; Wise & Bozarth, 1987; Glickman & Schiff, 1967). Therefore, NPY in the PFH is hypothesised to be involved primarily in regulatory feeding while possibly having some concomitant effects on non-regulatory feeding due to its general effects on hunger. DA levels, if affected by NPY in the PFH, are not expected to contribute to this response, but would likely be involved in accompanying, non-ingestive behaviours, such as increased locomotion. On the other hand, NPY in the N.Acc is hypothesised to be

involved primarily in non-regulatory feeding and reward, an effect likely mediated by its direct actions on DA-containing neurons. What follows is a review of the evidence for the role NPY plays in each, the hypotheses to be tested, and the manner in which these hypotheses will be tested.

Figure 3. An anatomical and functional model of NPY's effects on regulatory vs. non-regulatory feeding behaviour and reward following its injection into either the PFH or the N.Acc.



NPY and regulatory feeding behaviour.

NPY injected into the hypothalamus is believed to increase food intake by acting to inhibit satiety mechanisms or, likewise, to increase hunger. Meal size and duration following hypothalamic NPY administration increase while both the number of meals eaten and the rate of feeding remain unchanged (Leibowitz & Alexander, 1991). Recurrent perfusion of NPY in the hypothalamus, unlike the stimulating effects of other neuroactive factors, produces eating behaviour that is not satiable as long as the level of NPY is chronically elevated (Paez & Myers, 1991). The inference that NPY inhibits satiety and increases hunger is also supported by the finding that hypothalamic NPY administration results in increased intake of nutritive foods but not non-nutritive ones. Stanley (1993) found that rats injected with NPY in the PVN increased their intake of glucose, which is sweet and nutritive, and carbohydrate-MD, a non-sweet nutritive solution, while leaving consumption of saccharin (sweet and non-nutritive) unaffected. Parenthetically, intravenous injections of glucose decrease hypothalamic NPY-elicited food intake to an extent that is equivalent to the caloric value of the infusion (Rowland, 1988). Fructose, which does not cross the blood-brain barrier, is not effective at decreasing the NPY-elicited food intake, indicating that the satiating effect of food on NPY-induced feeding might be mediated by the actions of nutrients directly on CNS neurons.

NPY appears to have effects on metabolic and endocrine factors in addition to being a physiological signal that stimulates feeding. Brain levels of NPY increase when an organism is food-deprived and return to normal once satiated (Sahu, Kalra, & Kalra, 1988). Central NPY administration results in increased RQ (Currie & Coscina, 1996; Brown, 1993; Menendez, McGregor, Healey, Atrens, & Leibowitz, 1990), increased plasma insulin

levels (Moltz & McDonald, 1985), and reduced brown fat thermogenesis (Egawa, Yoshimatsu, & Bray, 1991). These data indicate that NPY affects the entire process of energy utilisation, and not only feeding behaviour. As well, when recurrent injections of NPY are given over a 7 day period, but intake is restricted, rats still display significant weight gain, indicating that NPY has additional weight-increasing metabolic and endocrine effects (Zarjevski, Cusin, Vettor, Rohner-Jeanrenaud, Jeanrenaud, 1993). Conversely, continuous infusion of NPY antibodies icv result in a dose-dependent decrease in 24 hr cumulative food intake (Dube, Xu, Crowley, Kalra, & Kalra, 1994).

Endogenous NPY levels are sensitive to manipulations that alter metabolic fuels and their regulatory hormones. For example, the removal of endogenous glucocorticoids decreases (White, Dean, & Martin, 1990), while the loss of insulin increases (White, Olchovsky, Kershaw, & Berelowitz, 1990), hypothalamic NPY levels. This suggests that NPY may be directly related to levels of some hormone(s) or signal(s) that regulate the utilisation of metabolic fuels.

Recently, a peripheral satiety signal that operates reciprocally with NPY was discovered in mice (Zhang et al., 1994). Called "leptin", this hormone acts as a feedback signal from adipose tissue. Circulating levels have been found to increase exponentially with body mass index (BMI) or percentage body fat (Blum, 1997), and icv-injected leptin decreases deprivation-induced feeding and lowers hypothalamic NPY concentrations (Wang et al., 1997). Using autoradiographic and genetic mapping techniques, leptin receptors have been found to exist in many brain tissues such as the hypothalamus and the choroid plexus (Gehlert & Heiman, 1997). Furthermore, the injection of leptin into the bloodstream lowers the expression of NPY in *ob/ob* mice, which are deficient in circulating

leptin, but has no effect on *db/db* mice, which lack functional leptin receptors (Remesar, Rafecas, Fernández-López, & Alemany, 1997).

Hypotheses:

PFH. NPY injected into the PFH will increase regulatory feeding behaviour through a mesolimbic DA-independent system.

N.Acc. There is currently no evidence to suggest that NPY injected into the N.Acc will have any effects on regulatory feeding; however, due to its known involvement in reward processes, NPY injection into the N.Acc may result in increased chow intake due to its effects on reward value. Therefore, it is hypothesised that there will be a slight increase in chow intake following N.Acc NPY administration.

How this will be assessed. To test these hypotheses, consumption of a nutritive yet non-preferred food type (regular powdered chow) following NPY injection into the PFH or the N.Acc will be measured. As any increases in intake following injection into the PFH will likely be mediated by local feeding receptors and be independent of DA, this effect will be unaffected by N.Acc antagonism of the mesolimbic DA system. However, any effects of N.Acc NPY on chow intake will be blocked by N.Acc DA antagonism. The DA receptor blocking agent, FLU, will be used, based on its ability to non-selectively block both D₁ and D₂ receptors (Arnt, 1985), as well as its ability to block other DA-mediated reward-relevant behaviours (Josselyn & Beninger, 1993; Carr, Fibiger, & Phillips, 1989).

NPY and non-regulatory feeding behaviour (reward)

Although NPY appears to play a primary role in the control of regulatory feeding, a number of studies have revealed that the feeding cues produced by NPY do not fully parallel those associated with deprivation-induced feeding. Seeley, Benoit, and Davidson

(1997) found that 24 hr food-deprived rats that had previously received foot shock paired with icv saline displayed more behavioural immobility (a response to a cue that reliably predicts shock) in a drug-free generalisation test than did non-deprived rats that had received foot shock paired with icv NPY administration. This paradigm measures the interoceptive cues produced by NPY administration and compares them to the cues produced by food deprivation. The results were interpreted as demonstrating that food deprivation activates processes or mechanisms different from those that underlie the orexigenic effects of NPY.

The patterns of behaviour exhibited following NPY administration also do not mimic those present in food-deprived animals. Levine, Kuskowski, Grace, and Billington (1991) found that icv NPY-treated rats ate the same amount, spent a similar amount of time eating, and demonstrated similar latencies to eat when compared to 24 hr deprived animals. Rats injected with NPY were more active, however, than those deprived of food, either when food was present (22% vs. 13% of total time), when there was a chewable object present (47% vs. 14%), or in the absence of either (37% vs. 4%). Detailed analysis of the patterns of ingestion following icv NPY administration show that orosensory information, which is affected by food-deprivation, is not affected by NPY, as feeding patterns are quite different following NPY administration as compared to food deprivation-induced feeding (Lench, Hart, & Babcock, 1994). As well, food hoarding behaviour in rats, which is proportional to body weight loss, is not raised by icv NPY administration (Cabanac, Dagnault, & Richard, 1997), providing further support for the notion that NPY does not completely mimic the physiological state of hunger.

The possibility that NPY operates by mechanisms other than regulatory ones is also

supported by recent findings demonstrating that NPY does not affect the consummatory phase of ingestion. Seeley, Payne, and Woods (1995), using the intraoral intake test (which focuses on the highly stereotyped consummatory phase by introducing a 0.1 M sucrose solution directly into the oral cavity of rats via an indwelling catheter), found that icv NPY failed to increase intake. However, 24 hr food deprivation nearly doubled intraoral intake. These results indicate that NPY administration does not completely mimic the stimulus state associated with food deprivation. These authors also suggest that NPY may have its primary effect on the appetitive (or preparatory), and not the consummatory, phase of food intake. Indeed, it may be that the appetitive phase of NPY-induced food intake is controlled by its effect on DA release, which makes intuitive sense when the reward-relevant effects of DA are considered.

Finally, the finding that rats injected with NPY in the hypothalamus will consume far more of a palatable diet (Stanley & Leibowitz, 1985) than regular chow (Brown & Coscina, 1995) provides support for the notion that feeding stimulated by NPY is, at least in part, non-regulatory in nature. If it stimulated only regulatory processes, then a given dose of NPY would be expected to result in equivalent increases in the consumption of either type of diet.

Hypotheses:

PFH. NPY injected into the PFH will increase feeding of a non-regulatory nature as well as other indices of reward. These effects will likely be mediated by connections with the mesolimbic DA system.

N.Acc. NPY injected into the N.Acc will result in an increase in non-regulatory feeding and other indices of reward, and this effect will be DA-dependent.

How this will be assessed. One way in which these hypotheses will be tested is by measuring the intake of a nutritive, preferred food type (sucrose) following injection of NPY into the PFH or the N.Acc. If PFH NPY is involved in non-regulatory feeding, then the increase in free-feeding sucrose intake following NPY administration should be greater than the increase in chow intake, over and above the amount due to normal preferences. N.Acc DA antagonism with FLU should at least partially block this effect, as a component of this feeding may be regulatory and, thus, not mediated by the DA system. If N.Acc NPY is involved in non-regulatory feeding, then a robust increase in sucrose intake will occur following injection into this site, and this effect will be blocked by N.Acc DA antagonism.

Another way to test these hypotheses is to examine the effects of operant responding for reward pellets. It is predicted that, in non-deprived rats, PFH NPY will produce an increase in PR operant responding for sucrose reward pellets, an index of an organism's motivation to respond for rewarding stimuli. This effect should be blocked by antagonism of the DA system. Likewise, N.Acc NPY should produce an increase in PR responding, an effect that will also be blocked by the antagonism of the DA system.

A third way of testing these hypotheses involves the use of the CPP paradigm. If NPY has rewarding effects when it is injected into the PFH, then its pairing with a distinct environment should result in a CPP for that environment. This effect, if DA-mediated, should be blocked by antagonism of the mesolimbic DA system. If PFH NPY is involved only in regulatory feeding behaviour, then its administration should result in either no effect on CPP, or possibly a conditioned place aversion (CPA), as food-deprivation results in a CPA (Bechara & van der Kooy, 1992; Harrington & van der

Kooy, 1992). Finally, if N.Acc NPY is involved in reward processes, then its administration should result in a CPP that is blocked by DA antagonism. A summary of the experimental paradigms and the predicted outcome of NPY administration are shown in Table 1.

Table 1. Summary of experimental paradigms and predicted outcomes.

<u>PROCESS</u>	<u>EXPERIMENTAL PARADIGM</u>	<u>PREDICTED OUTCOME OF N.ACC-APPLIED NPY:</u>	<u>PREDICTED OUTCOME OF PFH-APPLIED NPY:</u>	
			IF REGULATORY ONLY:	IF BOTH REGULATORY AND NON-REGULATORY:
REGULATORY FEEDING	Free-feeding (chow)	Slight increase in feeding behaviour—blocked by N.Acc FLU	A robust increase—not blocked by N.Acc FLU	A robust increase—partially blocked by N.Acc -FLU
NON-REGULATORY FEEDING (REWARD)	Free-feeding (sucrose)	A robust increase in feeding when compared to effects on chow feeding—blocked by N.Acc FLU	A robust increase (no different from chow feeding)—not blocked by N.Acc FLU	A robust increase (larger than chow feeding)—partially blocked by N.Acc FLU
REWARD	Progressive ratio operant responding for sucrose	An increase in breaking point—blocked by FLU	A slight increase in breaking point—not blocked by FLU	A robust increase in breaking point—blocked by FLU
REWARD	Conditioned place preference	Produce a CPP—blocked by FLU	No CPP produced. Possibly place aversion	Produce a CPP—blocked by FLU

Summary of Hypotheses

In summary, the primary hypothesis is that NPY injected into two distinct brain sites, the PFH and the N.Acc, will have distinct effects on behaviour. NPY injection into the PFH will produce an intrinsic state that parallels the sensation of hunger, and will therefore increase regulatory food intake. Measuring the consumption of a non-preferred food type, powdered chow, will assess this. NPY injection into the N.Acc will increase the rewarding aspects of various stimuli, including non-regulatory food intake, while having no effect on homeostatic mechanisms such as hunger. This will be assessed by measuring: intake of sucrose, a preferred food type; responding for sucrose reward pellets on a PR operant schedule; and the amount of time spent in an environment previously paired with NPY administration vs. that spent in an environment previously paired with saline, as measured by the CPP paradigm.

The secondary hypothesis of this thesis is that mesolimbic DA activity is involved in the rewarding effects of NPY, while playing a minor role, if any, in NPY's effects on regulatory feeding. Therefore, injection of the DA receptor antagonist, FLU, into the N.Acc will result in an inhibition of the NPY-elicited increase in non-regulatory feeding and other rewarding behaviours, while having no effect on NPY-elicited increases in regulatory feeding.

Experiments #1a-c were designed to assess the effects of NPY and FLU on regulatory, or hunger-driven, feeding, while experiments #2a and b were designed to evaluate their effects on non-regulatory feeding. Experiments #3a-d looked at other indices of reward, such as PR operant responding for sucrose and CPP. The last group of experiments, #4a-c, were designed to validate methodological procedures.

General Method

Subjects

All subjects were adult male Sprague-Dawley rats purchased from the Charles River Company (St. Constant, PQ), housed individually in hanging wire-mesh cages and given free access to water and food (standard Purina Rat Chow) unless otherwise stated. Upon arrival, subjects weighed between 225 and 275 g. Animals were subsequently housed in a temperature- and humidity-controlled room (22 degrees Celsius) with lights on from 0900 to 2100 hr and were handled for approximately 5 min per day prior to surgery. Body weights were measured daily throughout testing.

Procedure

Surgery. Bilateral implantation of cranial guide cannulae was done once subjects had attained a body weight of at least 290 g. Animals were anaesthetised with sodium pentobarbital (50 mg/kg, I.P.) and positioned in a stereotaxic apparatus. Two 15 mm, stainless steel, 22 gauge (.39 mm diameter) guide cannulae (Plastic Products, Roanoke, VA) were aimed to terminate above the right and left PFH and/or N.Acc. The coordinates for the PFH were: with the incisor bar set at 3.3 mm below the interaural line, anterior-posterior (AP) -1.9 mm from bregma, midline (ML) ± 1.1 mm from the midsagittal sinus, and dorsal-ventral (DV) -5.6 mm from bregma's DV coordinate. For the N.Acc (when animals were also being implanted in the PFH): with the incisor bar set at -3.3 mm below the interaural line, AP +1.7 mm from bregma, ML ± 1.5 mm from the midsagittal sinus, and DV -5.6 mm from bregma (Paxinos and Watson, 1986). When the N.Acc alone was the target site, the incisor bar was set at 5.0 mm above the interaural line and the co-ordinates were: AP +3.4 mm from bregma, ML ± 1.5 mm from the

midsagittal sinus, and DV – 4.7 mm from bregma (Pellegrino, Pellegrino, & Cushman, 1979). These co-ordinates had been chosen at the beginning of all experiments; the change in height of the incisor bar when doing the quadruple implants necessitated a change in co-ordinates for the N.Acc. In addition, four stainless steel screws were implanted in the skull, and the entire assembly was covered with dental acrylic, anchoring the guide cannulae to the skull. Twenty-eight gauge (.36 mm diameter) stainless steel wire stylets were kept flush inside the guide cannulae in order to prevent them from blocking.

A cannulae placement was determined to be inaccurate if it lay > 0.5 mm in any direction away from the target site. The coronal sections shown in Figure 4 and 5 (taken from Paxinos & Watson, 1986) represent the location of injection sites surrounding the PFH and the N.Acc, respectively, that were considered to be accurate. Any tracts lying outside of this area, either on the rostral-caudal or medial-lateral plane, were considered to be inaccurate, and data from those animals was not included in the analyses. Figure 6 shows representative injection sites for the N.Acc and the PFH.

Drugs. NPY was purchased from Peninsula Laboratories (Belmont, CA). FLU was generously provided by H. Lundbeck and Co. (Denmark), as well as purchased from Research Biochemicals International (RBI; Natick, MA). AMPH was also purchased from RBI, through the Bureau of Drug Research.

Intracerebral Drug Administration. All rats were acclimatised to the central injection procedure by restraining them with a towel and performing a sham injection at least twice on separate days before testing began. NPY (0, 24, 78, 156, and 235 pmol per injection side), AMPH (0 or 10 μ g per injection side) and FLU (0, 1.25, and 5 μ g per

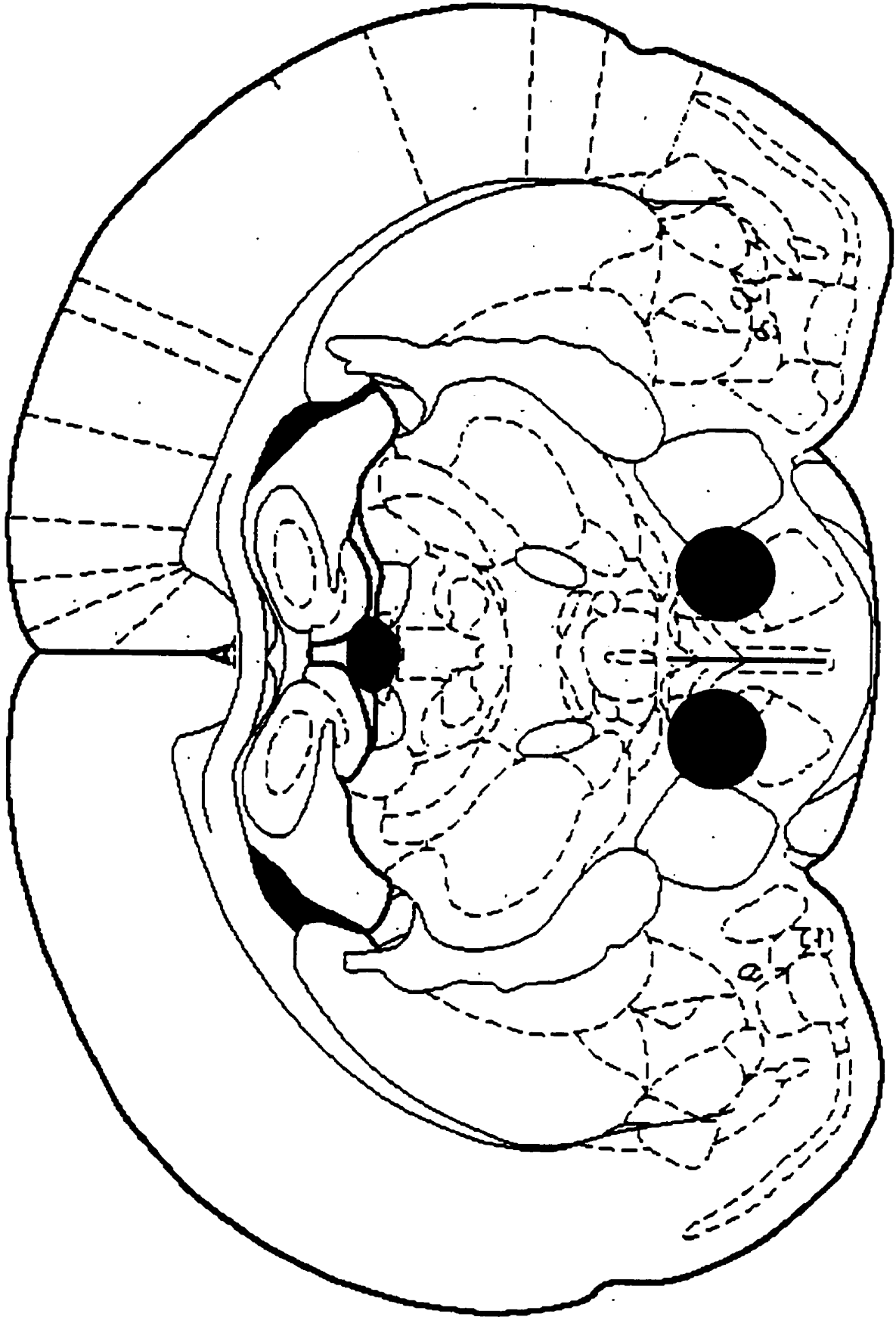
injection side) were dissolved in sterile physiological saline (0.9%) and injected in a **terminating 3 mm below the guide cannula. The injector was attached, by a length of plastic tubing, to a 5 µl Hamilton microsyringe. All solutions were infused manually over a period of 1 min. The injector was left inside the guide cannula for an additional 30 sec after the infusion, to allow for diffusion of the solution away from the tip.**

Behavioural testing began immediately following the last injection. All tests were done between 1000 and 1700 hrs.

Histology. At the conclusion of testing, subjects which had undergone cannulae implantation were sacrificed with an overdose of sodium pentobarbital and then perfused through the heart using isotonic saline followed by a 10% formalin solution. After soaking in formalin solution for at least 24 hrs, the brains were sectioned at 40 µm intervals in a cryostat. These sections were then stained with cresyl violet, and examined under a microscope to determine the exact location of the cannula tips. Rats that had not undergone surgery were sacrificed with carbon dioxide gas.

Statistical Analysis. For the feeding experiments, results were analysed using one-, two-, or three-way analyses of variance (ANOVAs). Post-hoc comparisons, using Fisher's least significant difference (LSD) or Dunnett's test, were made to investigate any treatment differences. For the CPP studies, one-way ANOVAs or Student's *t*-tests were used, depending on the comparisons being made. Post-hoc comparisons using Fisher's LSD were made to elucidate treatment differences. A two-tailed alpha level of .05 was used for all statistical tests, except in Experiment #3c, when one-tailed alpha levels for the *t*-tests were used when *a priori* predictions could be made.

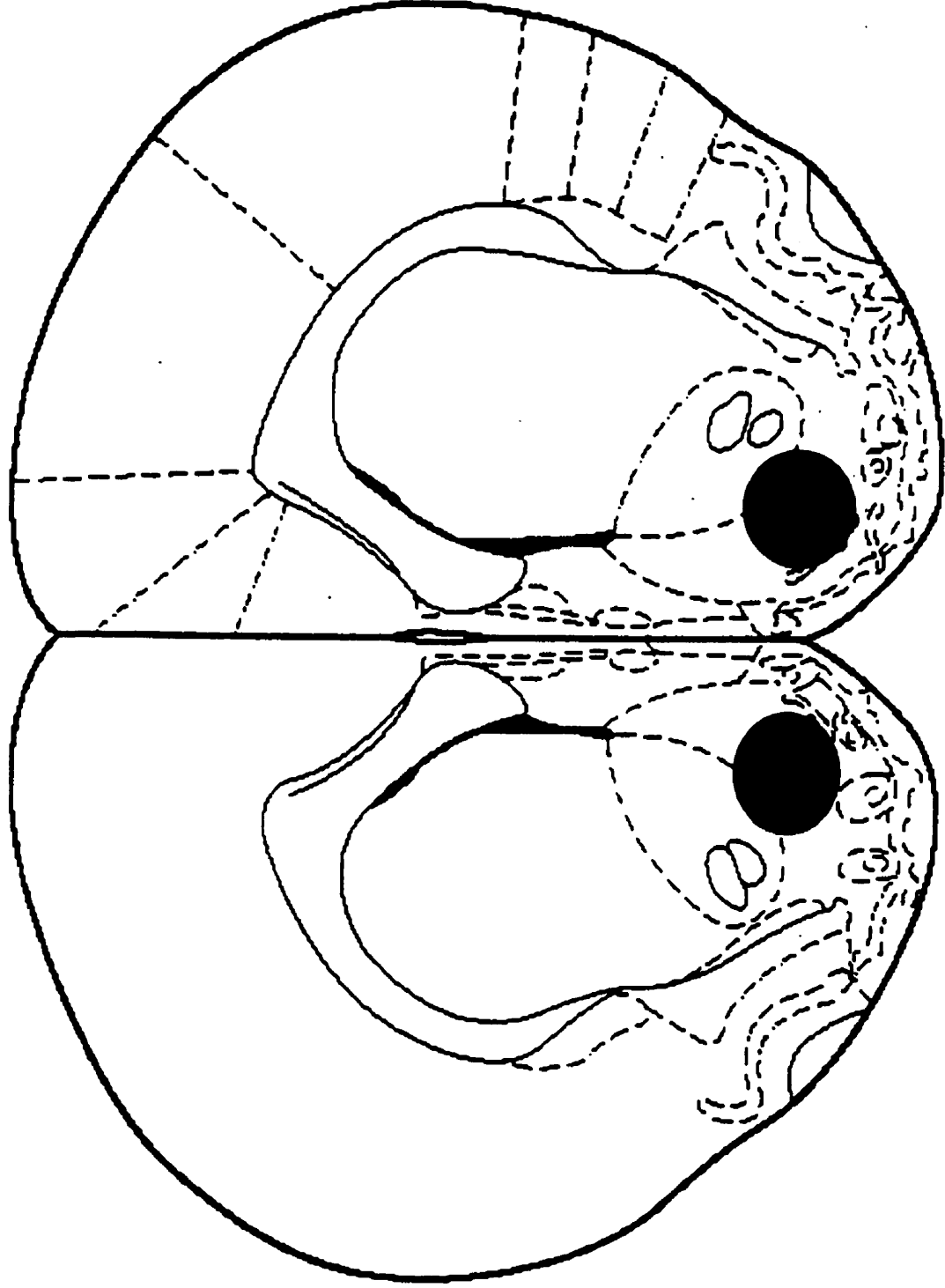
Figure 4. Area surrounding the PFH where a cannulae placement was deemed to be accurate (Paxinos & Watson, 1986).



Interaural 7.20 mm

Bregma -1.80 mm

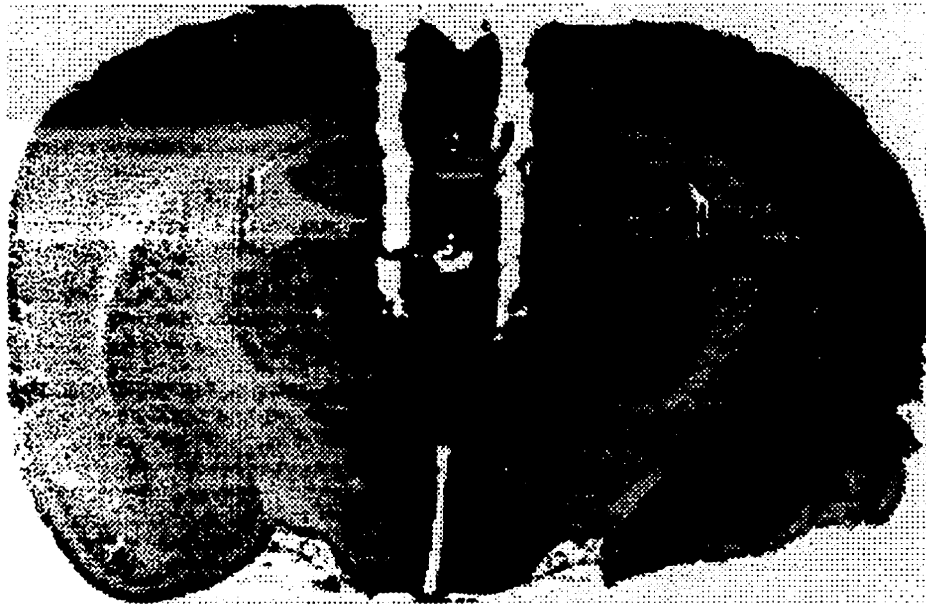
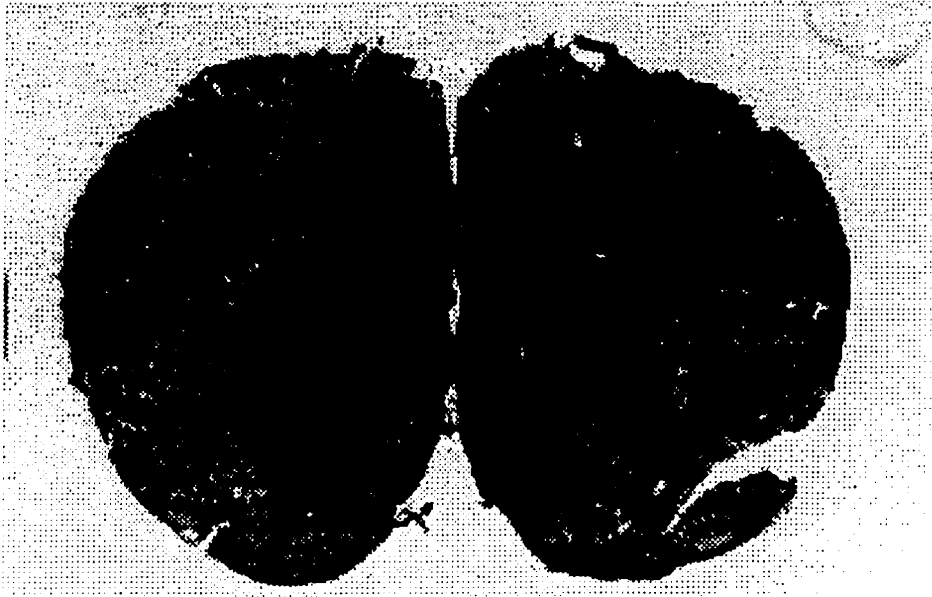
Figure 5. Area surrounding the N.Acc where a cannulae placement was deemed to be accurate (Paxinos & Watson, 1986).



Interaural 10.70 mm

Bregma 1.70 mm

Figure 6. Slide sections showing representative bilateral N.Acc (top panel) and PFH (bottom panel) cannulae placements.



Regulatory Feeding Experiments**(Experiments #1a-1c)**

Experiment #1a: Effects of NPY on powdered chow intake

To ascertain whether PFH- and/or N.Acc-applied NPY has effects on regulatory feeding, and to secure a dose-response curve for these potential effects, this first experiment examined the effects of NPY (0, 24, 78, 156, and 235 pmol per injection side) on powdered chow feeding. Based on previous research (i.e., Flood & Morley, 1991; Lynch, Hart, & Babcock, 1994), any increases in chow intake above baseline can be interpreted as the inhibition of a satiety mechanism or, alternatively, an increase in an animal's motivation to eat.

Procedure

Twenty rats were used in this study. Eight had bilateral cannulae implanted that were aimed to terminate in the N.Acc and 12 had bilateral cannulae aimed at the PFH. All testing took place in the animals' home cages. A few days prior to testing, rats were acclimated to the powdered chow by giving them free access to this food for 24 hrs. On each test day, subjects were pre-satiated by removing all of the pellets in the food hopper and placing four or five chow pellets on the floor of their cages. One hour later, testing with NPY began. All rats received 0, 24, 78, 156, and 235 pmol/side of NPY in a counterbalanced order, and were returned to their home cages along with a stainless steel bowl filled with a pre-weighed amount of powdered chow. Intake (g) minus spillage was measured 1 and 2 hr post-injection. There were at least 2 drug-free days between each test session.

Results

The data from five rats, all from the PFH group, were not included in the analysis

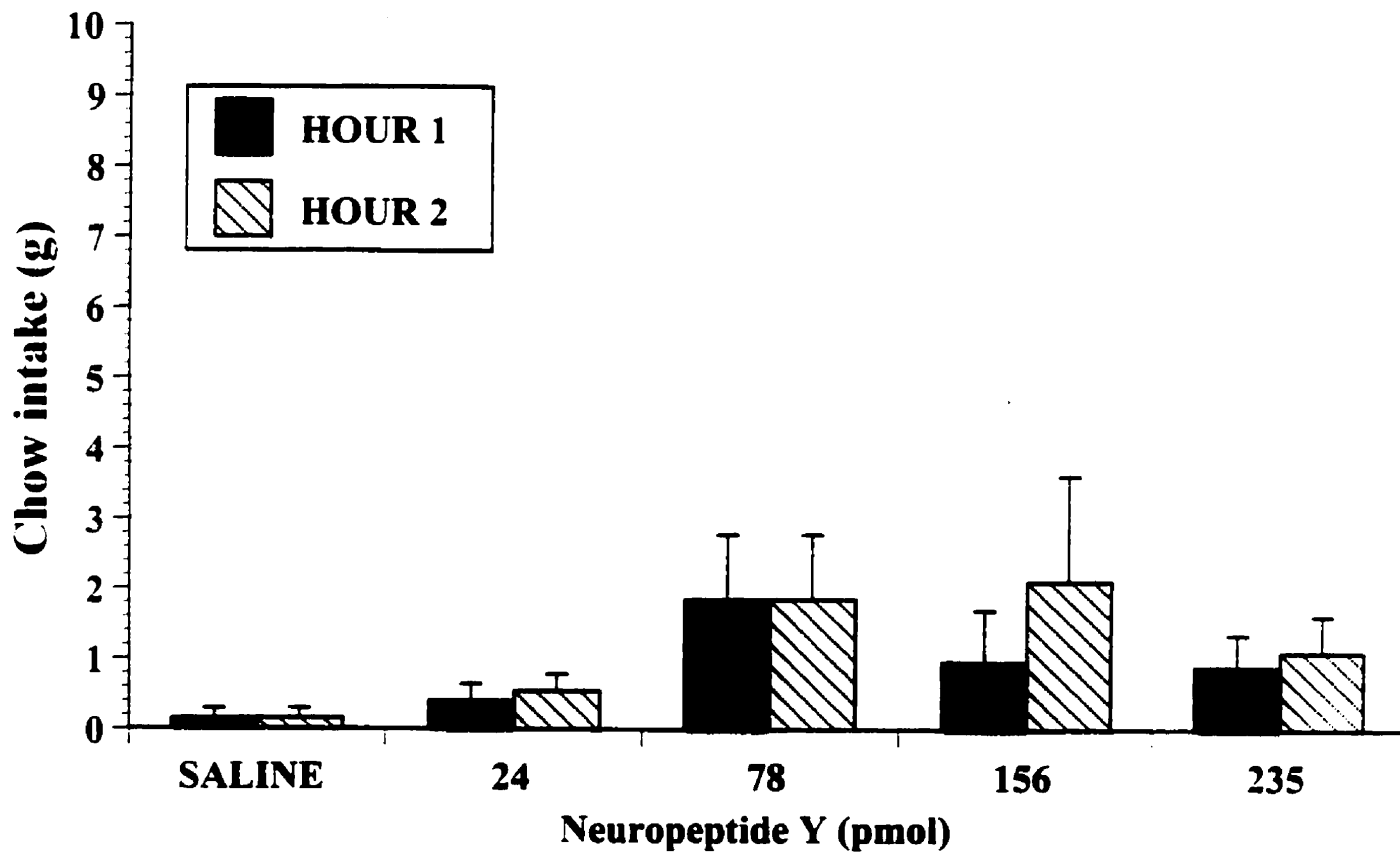
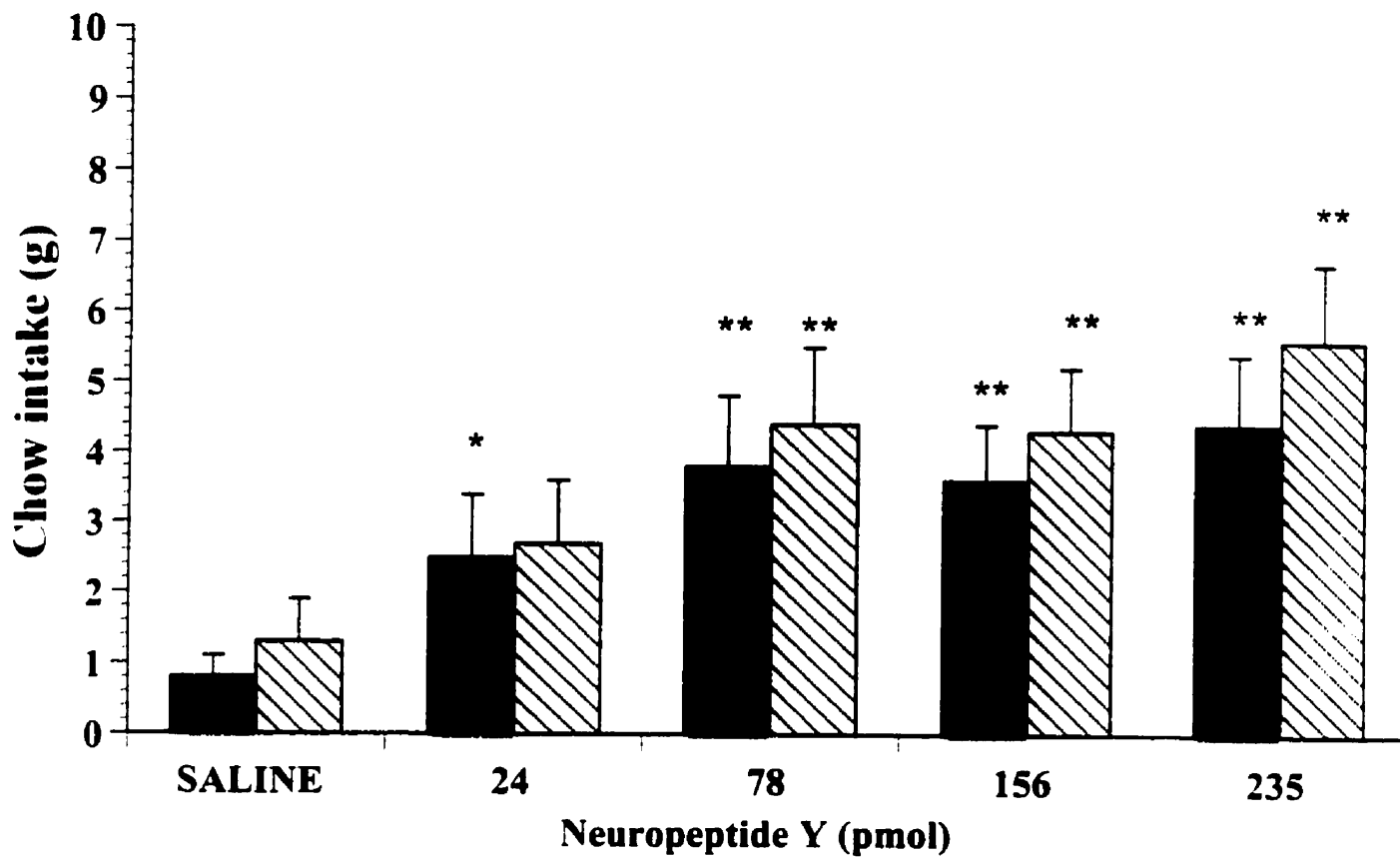
due to inaccurate cannulae placements.

The results from this experiment can be seen in Figure 7. A two-way ANOVA, with area and dose as factors, revealed a significant main effect of area for both 1 hr, $F(1, 13) = 7.06$, $p = .02$, and 2 hr, $F(1, 13) = 7.15$, $p = .02$, intake, with rats receiving NPY in the PFH (top panel) eating substantially more than those receiving NPY in the N.Acc (bottom panel). There was also a main effect of dose, $F(4, 52) = 9.47$, $p < .001$ (1 hr), $F(4, 52) = 7.16$, $p < .001$ (2 hr), with the 78, 156, and 235 pmol doses of NPY all eliciting near-maximal food intake. The area x dose interaction was significant only for 1 hr intake, $F(4, 52) = 2.82$, $p = .03$, with PFH-applied NPY increasing powdered chow intake (relative to baseline) at all doses (top panel), and N.Acc-applied NPY only increasing intake at the 78 pmol dose (relative to baseline)(bottom panel). It is noteworthy that none of the doses of NPY in the N.Acc significantly increased 1 hr intake compared to the PFH rats' saline score ($p > .10$; Fisher's LSD). The apparent increase in powdered chow feeding seen after the 78 pmol dose of NPY in the N.Acc appears to be due to the very low baseline feeding exhibited by this group of rats. Two separate one-way ANOVAs revealed a significant main effect of dose for rats injected with NPY in the PFH, $F(4, 24) = 10.83$, $p < .001$ (1 hr), $F(4, 24) = 10.5$, $p < .001$ (2 hr), while revealing no significant effects for rats receiving NPY in the N.Acc, $F(4, 28) = 2.06$, $p > .10$ (1 hr), $F(4, 28) = 1.47$, $p > .10$ (2 hr).

Figure 7. Effects of NPY on powdered chow intake after injection into the PFH (top panel) or the N.Acc (bottom panel).

* = $p < .05$ (compared to baseline; Dunnett's test)

** = $p < .01$ (compared to baseline; Dunnett's test)



Experiment #1b: Effects of peripheral FLU on PFH NPY-induced regular chow intake

To ascertain whether the DA system plays any role in the feeding-stimulatory actions of NPY on chow intake, the effects of the DA receptor blocker, FLU, on feeding induced by PFH administration of NPY were assessed. It was hypothesised that DA is not involved in the hunger-driven, or regulatory, aspects of NPY-induced feeding, therefore, FLU should not disrupt NPY-elicited powdered chow consumption. The following experiment describes the effects of I.P. FLU on PFH NPY-induced chow feeding.

Procedure

Seventeen rats, all with cannulae implanted bilaterally aimed at the PFH, were used in this study. Nine were tested with 156 pmol/side of NPY and 9 with 24 pmol/side. FLU (0, .05, .1, and .2 mg/kg, I.P.) was dissolved in saline and injected 2.5 hrs prior to testing with NPY. This time course was chosen based on previous reports showing that FLU's peak neuroleptic effects occur at this time point (Ettenberg, Koob, & Bloom, 1981). All rats received each dose of FLU in a counterbalanced order and were pre-satiated as in Experiment #1a. One hr later, NPY was administered and rats returned to their home cages. 1 and 2 hr intake (g) of standard, Purina rat chow (minus spillage) were the dependent measures.

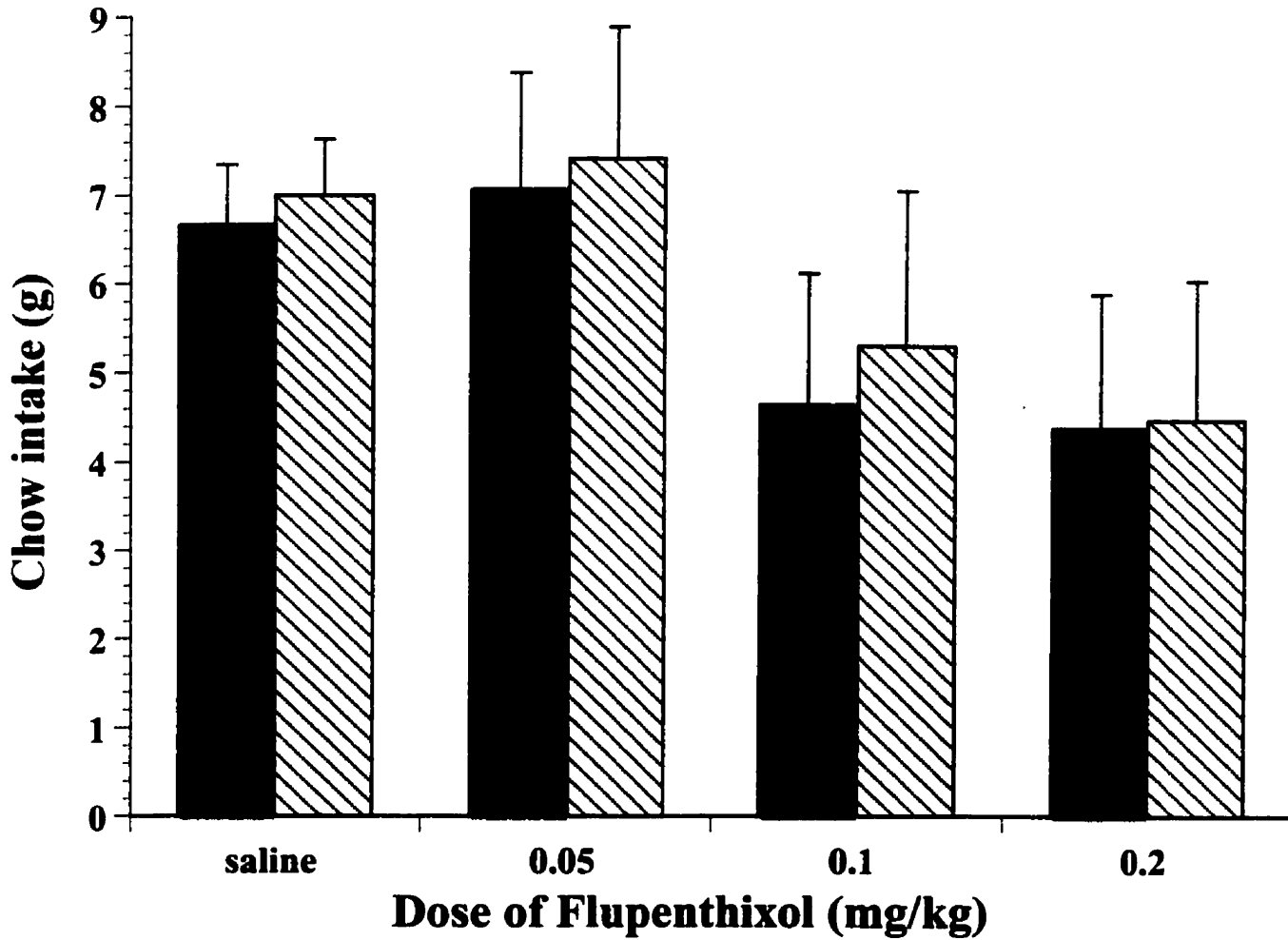
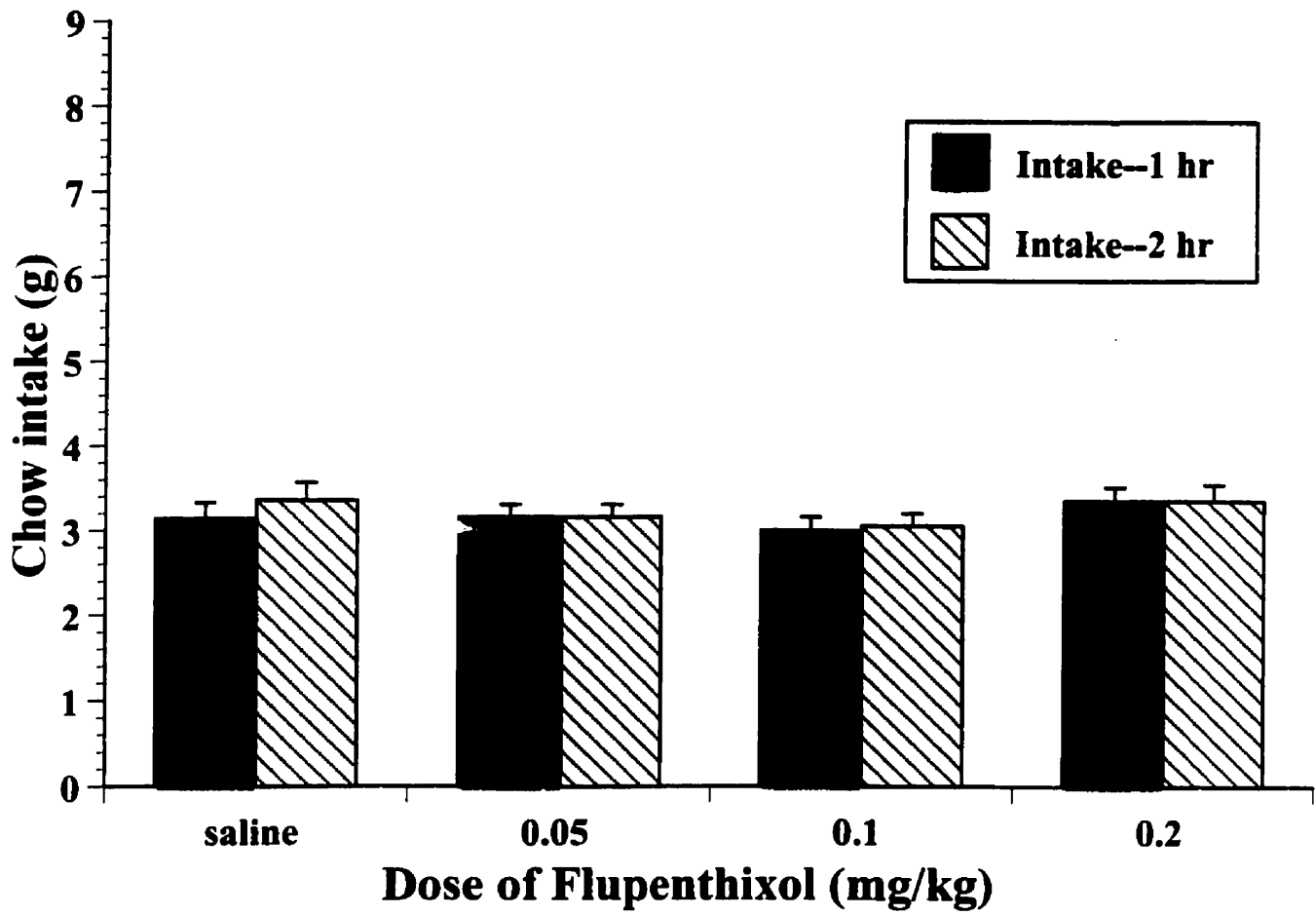
Results

Two rats receiving the 156 pmol dose, and four rats receiving the 24 pmol dose of NPY were determined to have inaccurate cannulae placements, so their data were

excluded from the analysis.

The data from Experiment #1b are shown in Figure 8. Two separate one-way ANOVAs revealed that there was no significant effect of any dose of FLU on either 24 pmol, $F(3, 12) = .039$, $p > .10$ (1 hr), $F(3, 12) = .062$, $p > .10$ (2 hr)(top panel), or 156 pmol, $F(3, 18) = 1.38$, $p > .10$ (1hr), $F(3, 18) = 1.09$, $p > .10$ (2 hr)(bottom panel), NPY-induced chow feeding.

Figure 8. Effects of FLU (I.P.) on PFH NPY-induced chow intake. Top panel: 24 pmol/side NPY. Bottom panel: 156 pmol/side NPY.



Experiment #1c: Effects of N.Acc FLU on PFH NPY-induced powdered chow intake

Although the results of Experiment #1b indicated that peripheral blockade of DA does not play a significant role in PFH NPY-induced feeding, it remained to be determined if a more precise blockade of the mesolimbic pathway might reduce chow intake. It was hypothesised that blockade of the mesolimbic DA system, which is known to be involved in reward processes, would not affect NPY-induced chow feeding, as NPY-induced feeding of this food type is proposed to result from more homeostatically-driven, regulatory signals. Experiment #1c tested this hypothesis by examining the effects of N.Acc-applied FLU on PFH NPY-induced powdered chow intake.

Procedure

Fifteen rats, all with two sets of bilateral cannulae aimed to terminate in the N.Acc and the PFH, were used in this experiment. Rats were acclimatised to the powdered chow as in Experiment #1a. On each test day, they were pre-satiated for one hr then injected with 0, 1.25, or 5 $\mu\text{g}/\text{side}$ of FLU in the N.Acc 30 min prior to an injection of 0 or 156 pmol/side of NPY in the PFH. All rats received all combinations of FLU and NPY in a semi-randomised order. Immediately following the second injection, rats were replaced in their home cages and given free access to powdered chow. 1 and 2 hr intake (g) minus spillage were the dependent measures.

Results

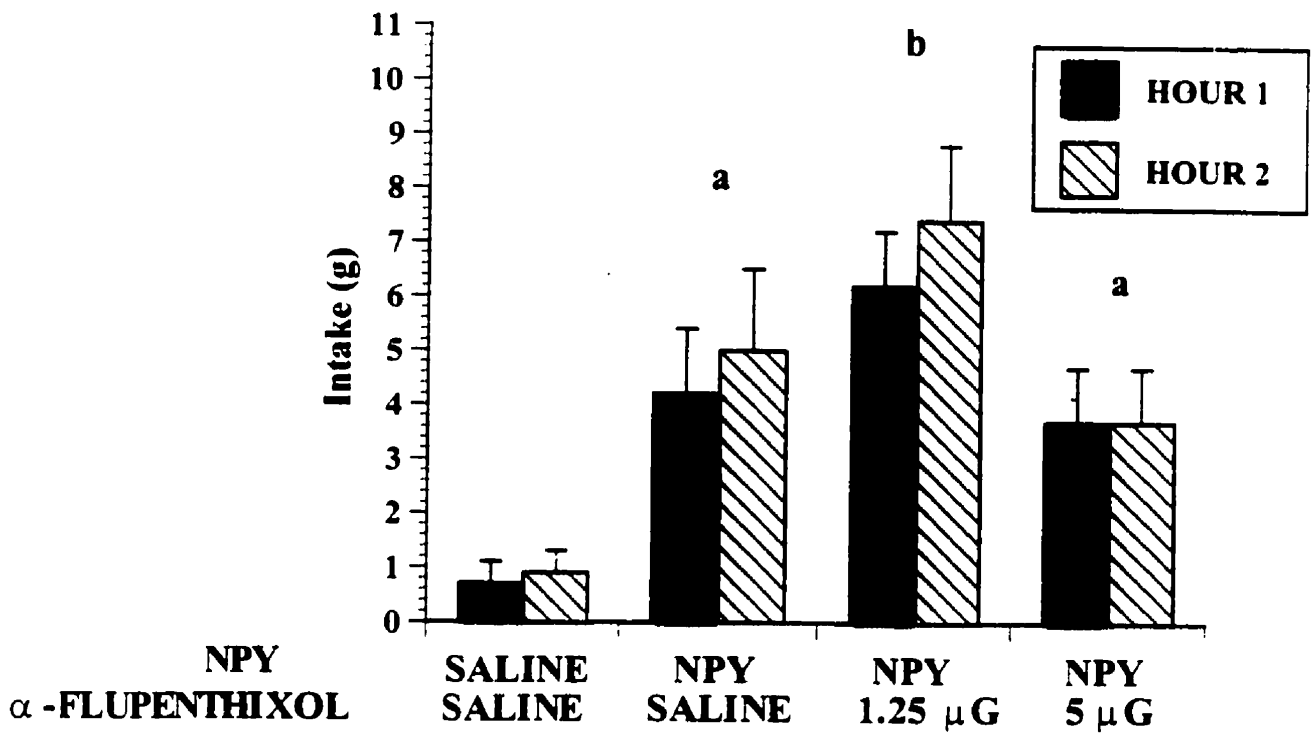
Six of the rats were found to have inaccurate cannulae placements so their data were excluded from the analysis.

The data from this study are displayed in Figure 9. A one-way ANOVA revealed a significant main effect of dose for both 1 hr, $F(3, 24) = 15.02, p < .001$, and 2 hr, $F(3, 24) = 14.34, p < .001$, intake. Post-hoc comparisons showed that NPY stimulated food intake as compared to saline, regardless of the dose of FLU co-administered. Furthermore, the 1.25 μg dose of FLU plus NPY elicited significantly more feeding than did either NPY + SALINE or NPY + 5 μg of FLU when compared to baseline (SALINE + SALINE).

Figure 9. Effects of N.Acc FLU on NPY (156 pmol/side)-induced powdered chow intake.

a = significantly different from SALINE + SALINE ($p < .01$)(Fisher's LSD test)

b = significantly different from SALINE + SALINE ($p < .01$) and NPY + SALINE ($p < .05$) and NPY + 5 μ g FLU ($p < .01$)(Fisher's LSD test)



Summary of Regulatory Feeding Experiments

Experiment #1a demonstrated that NPY injected bilaterally into the PFH produces a robust dose-dependent increase in chow intake, with most of this response occurring within one hr of the NPY injection. On the other hand, NPY injected bilaterally into the N.Acc had little effect on chow intake. FLU, injected either peripherally (Experiment #1b) or directly in the N.Acc (Experiment #1c), had no attenuating effect on PFH NPY-induced chow intake, indicating that mesolimbic DA does not contribute to this response.

These results indicate a primary role of PFH NPY in mechanisms underlying regulatory feeding behaviour that are not mediated by mesolimbic DA activity.

Non-Regulatory Feeding Experiments**(Experiments #2a-2b)**

Experiment #2a: Effects of NPY on sucrose intake

In order to assess NPY's effects on feeding of a more non-regulatory, reward-driven nature, and to generate a dose-response curve for these effects, this experiment examined the effects of NPY on the intake of sucrose, a preferred food type. It was proposed that any increases in sucrose intake, above and beyond that seen with powdered chow, might reflect NPY's stimulation of reward pathways (Sills, Baird, & Vaccarino, 1993).

Procedure

Twenty rats were used in this study. Ten had bilateral cannulae aimed to terminate in the N.Acc, and 10 had bilateral cannulae aimed to terminate in the PFH. All testing took place in the animals' home cages. For one week prior to the beginning of testing, rats were habituated to sucrose (Redpath brand table sugar) by receiving seven daily 1 hr presentations. On each test day, animals were pre-satiated with regular chow for 1 hr (as in Experiment #1a) and injected, in a counterbalanced order, with NPY (0, 24, 78, 156, and 235 pmol/side). Subjects were returned to their home cages, along with a stainless steel bowl filled with a pre-weighed amount of sucrose, and intake (g) minus spillage was measured 1 and 2 hr post-injection. At least two days separated test sessions wherein rats had access only to regular chow.

Results

One rat from the PFH group lost its cannulae assembly during testing, so its data were removed from the analysis.

Figure 10 shows the results from the present experiment. A two-way ANOVA

revealed a significant main effect of area, $F(1, 17) = 15.30, p = .001$ (1 hr), $F(1, 17) = 18.67, p < .001$ (2 hr), with the PFH- implanted rats eating more sucrose than the N.Acc- implanted rats. There was also a main effect of dose, $F(4, 68) = 13.46, p < .001$ (1 hr), $F(4, 68) = 14.14, p < .001$ (2 hr), with all doses of NPY stimulating sucrose intake as compared to baseline. Fisher's LSD test indicated that, at both 1 and 2 hrs post-injection, the 156 and 235 pmol doses of NPY resulted in maximal responding. The area x dose interaction was also significant for both 1 hr, $F(4, 68) = 7.31, p < .001$, and 2 hr, $F(4, 68) = 8.06, p < .001$, intake, with NPY stimulating sucrose intake at all doses in the PFH while having no effect in the N.Acc.

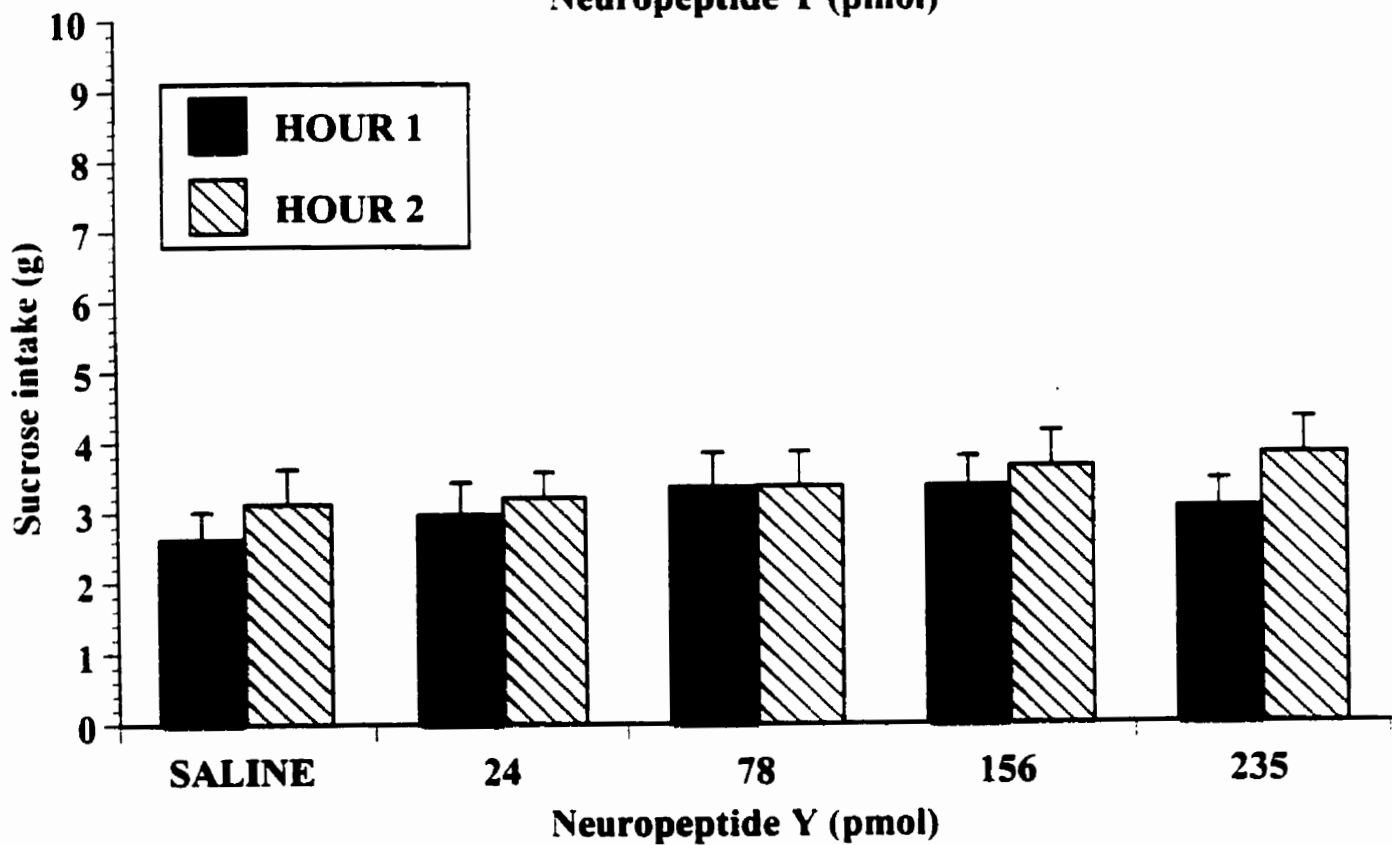
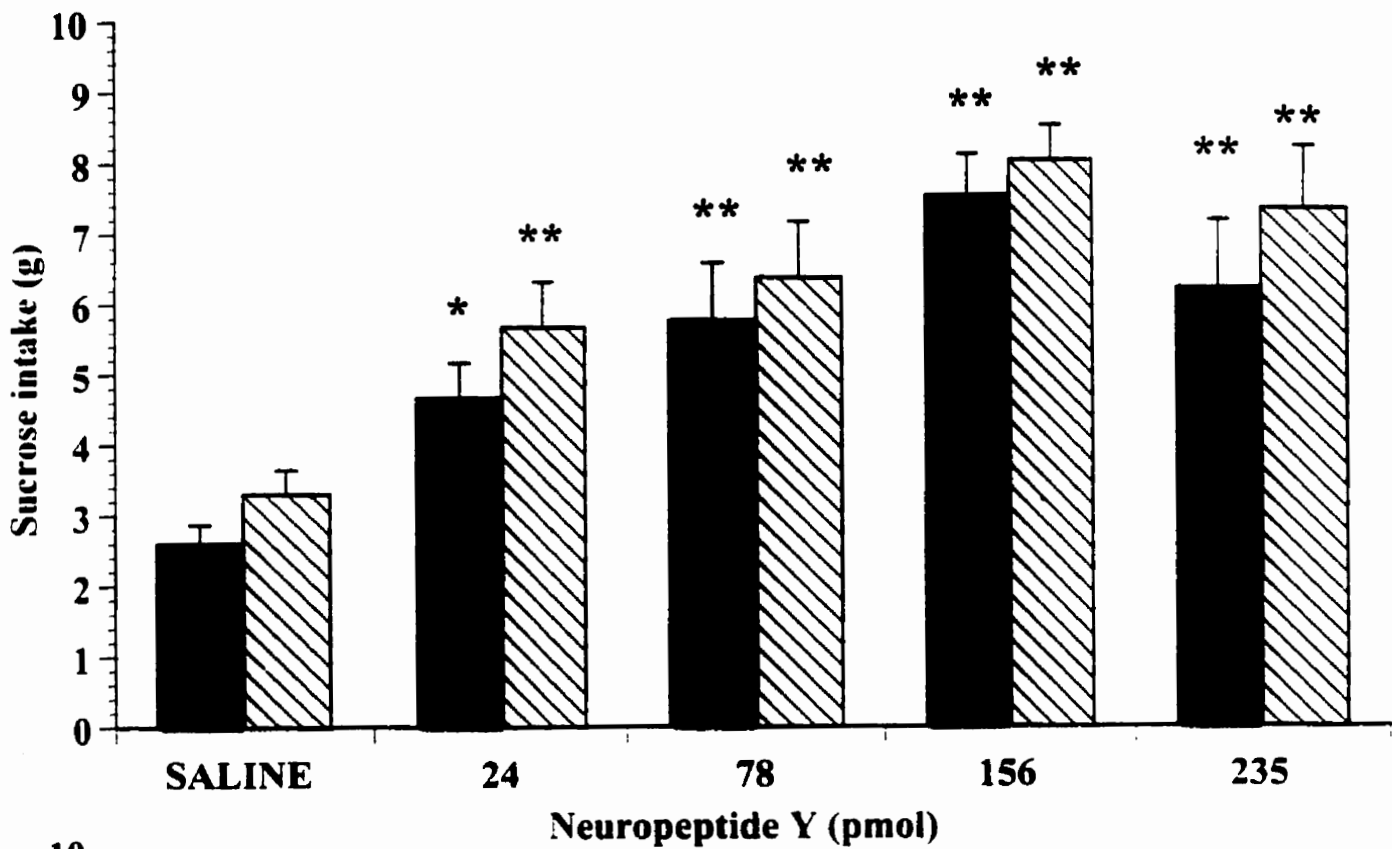
A three-way ANOVA on the data from Experiments #1a and #2a revealed a significant main effect of food type both for 1 hr, $F(1, 30) = 21.45, p < .001$, and 2 hr, $F(1, 30) = 19.14, p < .001$, intake, confirming that rats ate significantly more sucrose than powdered chow regardless of the dose of NPY.

It is important to note that there was no significant food type x dose interaction when data from Experiments #1a and #2a were collapsed and a three-way ANOVA performed, $F(4, 120) = 1.57, p > .10$ (1 hr), $F(4, 120) = .70, p > .10$ (2 hr). This indicates that NPY did not preferentially stimulate intake of sucrose.

Figure 10. Effects of NPY on sucrose intake after injection into the PFH (top panel) or the N.Acc (bottom panel).

* = $p < .05$ (compared to baseline)(Dunnett's test)

** = $p < .01$ (compared to baseline)(Dunnett's test)



Experiment #2b: Effects of N.Acc FLU on PFH NPY-induced sucrose intake

Experiment #2a determined that PFH-injected NPY results in increased sucrose intake. The present experiment was designed to assess whether central blockade of the mesolimbic DA system would have any effects on PFH NPY-induced intake of a preferred food type. As in Experiments #1b and #1c, the hypothesis being tested was that, if mesolimbic DA was involved in the non-regulatory, reward-driven aspects of PFH NPY-induced feeding, such a blockade would preferentially affect intake of a palatable food type, such as sucrose.

Procedure

Ten rats, all with cannulae aimed to terminate both in the N.Acc and the PFH, were used in this experiment. Once acclimatised to the sucrose for seven days (as in Experiment #2a), testing began. On each test day, rats were pre-satiated with regular chow for 1 hr then injected with 0, 1.25, or 5 µg/side of FLU in the N.Acc 30 min prior to an injection of 0 or 156 pmol/side of NPY in the PFH. All rats received all combinations of FLU and NPY in a semi-randomised order. Immediately following the second injection, rats were replaced in their home cages and given free access to sucrose. 1 and 2 hr intake (g) minus spillage were the dependent measures.

Results

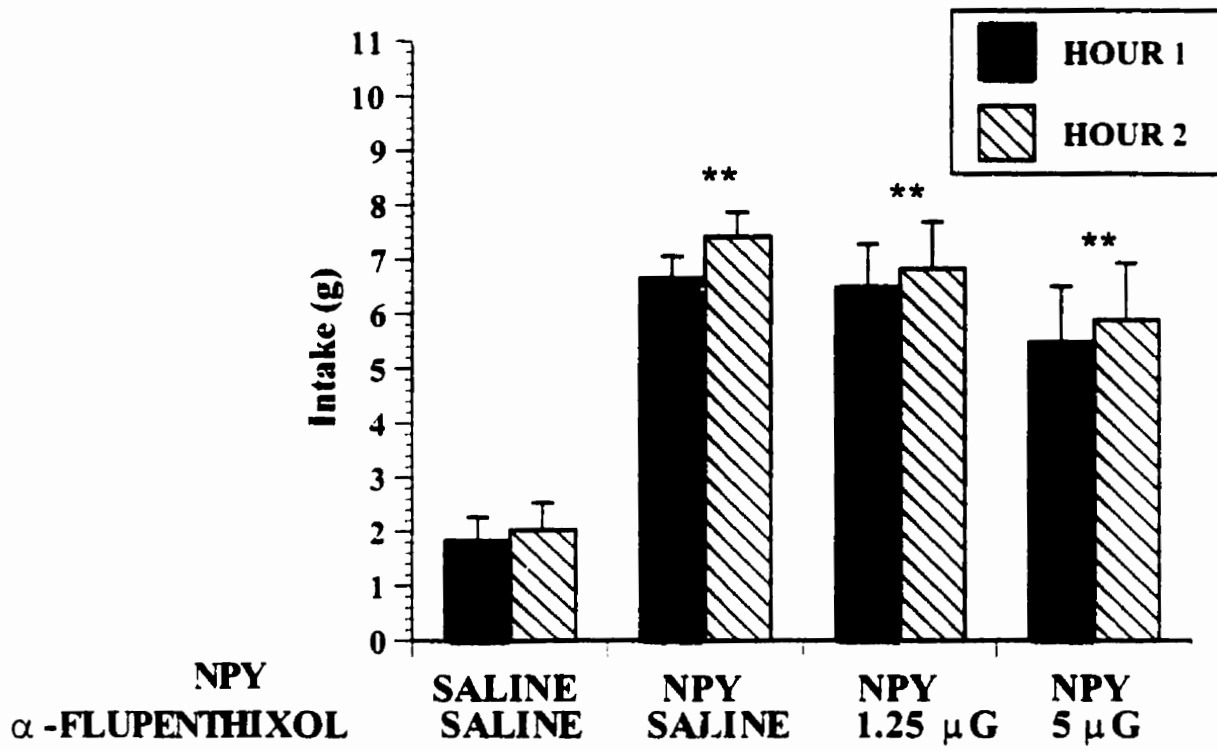
Three rats were found to have inaccurate cannulae placements so their data were excluded from the analysis.

The results from this experiment are displayed in Figure 11. A one-way ANOVA revealed a main effect of dose for both 1 hr, $F(3, 18) = 10.10$, $p < .001$, and 2 hr, $F(3, 18)$

= 10.45, $p < .001$, intake, with NPY stimulating sucrose intake as compared to baseline regardless of the dose of FLU.

Figure 11. Effects of N.Acc FLU on PFH NPY-induced sucrose intake

**** = $p > .001$ (Fisher's LSD test)**



Summary of Non-Regulatory Feeding Experiments

Experiment #2a demonstrated that PFH injection of NPY results in robust increases in sucrose intake; however, these increases are no greater than NPY's effects on chow intake. Furthermore, Experiment #2b showed that N.Acc FLU has no effect on PFH NPY-stimulated sucrose intake, indicating that mesolimbic DA does not contribute to this response. N.Acc NPY had no effect on sucrose intake.

These results suggest that PFH NPY does not selectively influence the rewarding aspects of food but, more likely, acts to stimulate homeostatic mechanisms thereby increasing the intake of a variety of food types.

Reward Experiments**(Experiments #3a-3d)**

Experiment #3a: Effects of NPY on progressive-ratio operant responding

The present experiment was designed to test the hypothesis that NPY injected into the N.Acc or the PFH would increase responding for sucrose reward pellets on a PR schedule. Sucrose, rather than chow, pellets were chosen for this experiment in order to minimise the contribution of NPY's regulatory effects on this measure. That is, if purely nutritional need was the motivating drive behind the animals' responding, they would be expected to respond more vigorously for chow reward than for sucrose reward, as chow represents a more complete dietary source. It was predicted that, if NPY exerts any of its feeding effects on reward mechanisms, PR responding for sucrose would increase following NPY injection into either brain region.

Procedure

Thirty-two rats were used in this experiment. Twenty-four had bilateral guide cannulae implanted in the PFH and eight in the N.Acc.

Apparatus. All operant testing was carried out in eight chambers measuring 28 cm long, 21 cm wide, and 21 cm high (Med Associates Inc., Georgia, VT). Each chamber contained a food pellet dispenser and a retractable response lever that was 4.5 cm wide and 7 cm above the chamber's floor. The centre of the lever was 6.5 cm to the left of a central food hopper positioned 3 cm above the chamber's floor. Each chamber was illuminated by a light and was contained within a sound-attenuating box equipped with a ventilating fan. Apparatus control, and data collection, was accomplished with a 386-SX IBM-type computer.

Testing. One week after their arrival in the laboratory, rats were food-deprived

for 24 hrs and trained to bar-press for 45 mg sucrose reward pellets (Formula F; P.J. Noyes Co. Inc., Lancaster, NH). Once stable responding on a fixed-ratio 1 (FR1) schedule had been attained, home cage feeding was reinstated and animals were switched to a FR3, then a FR5, and finally a PR schedule. Response requirements for the PR schedule were based on the following equation:

$$\text{ROUND } [5 * \text{EXP}(0.2 * \text{reward number}) - 5]$$

and, therefore, increased exponentially through the following series: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737, 901 (Roberts & Richardson, 1992). This PR schedule had previously been demonstrated to generate dose-dependent breaking points for cocaine self-administration in rats (Roberts & Richardson, 1992). The number of reinforcers earned prior to a 20 min period of non-reinforcement represented the breaking point. Once an animal had completed three daily PR sessions, surgery was performed. Two days after surgery, the PR schedule was re-introduced. Once stable responding was re-established ($\pm 10\%$ over a period of three days), testing with NPY (0, 78, 156, and 235 pmol/side) began (at least 5 days of post-operative recovery). Five min after subjects received one of the four doses of NPY in a semi-randomised, counterbalanced order, they were placed in the operant chambers. Numbers of responses made and reinforcers earned were the dependent measures.

Results

Eight rats from the PFH group had inaccurate cannulae placements, so their data were excluded from the analysis.

Results are displayed in Figure 12. For the number of responses made (top panel), a two-way ANOVA, with area and dose as factors, revealed a significant main

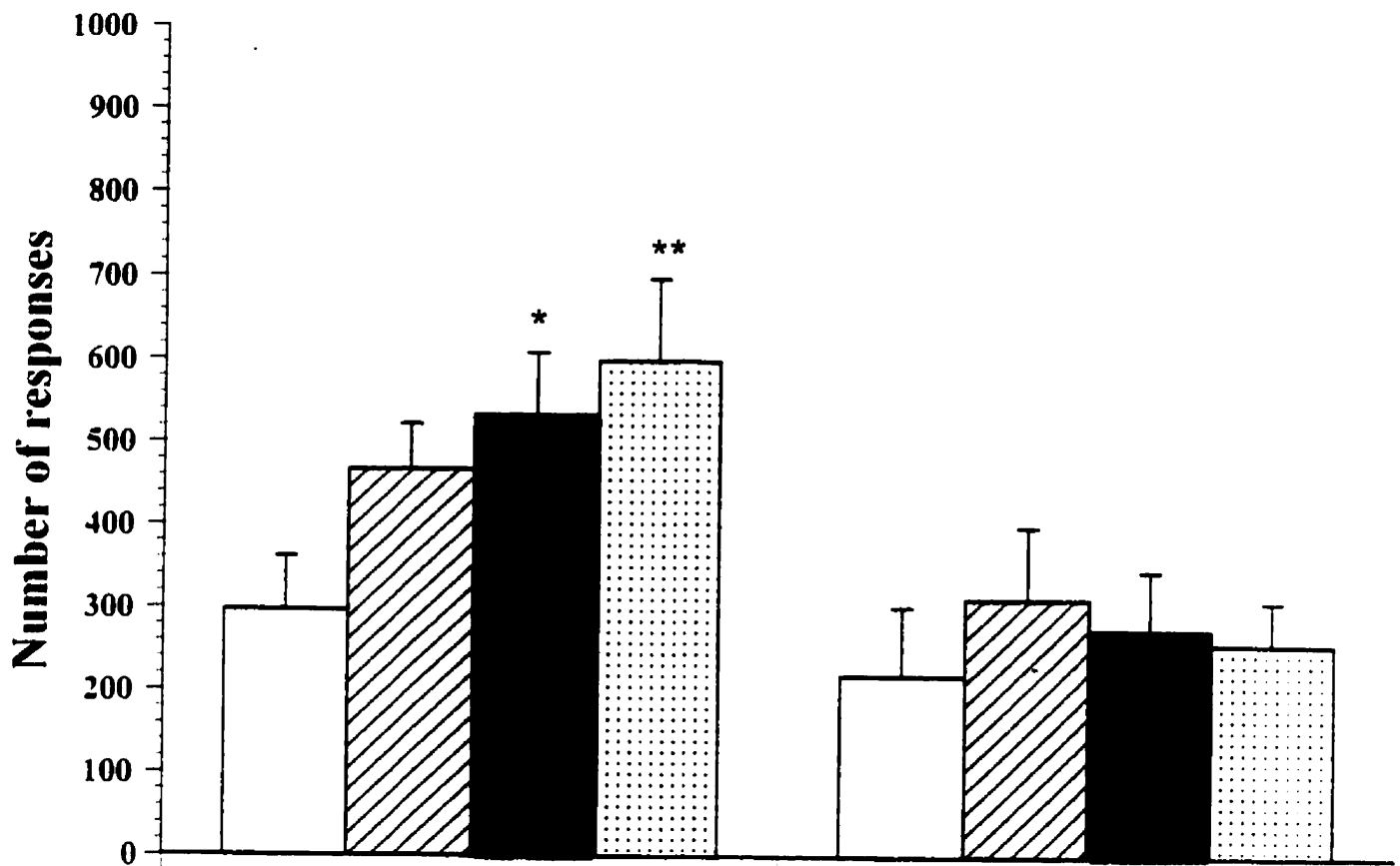
effect of area, $F(1, 21) = 5.58$, $p = .027$, with injections of NPY in the PFH resulting in more responding overall. There was a trend towards a main effect of dose, $F(3, 63) = 2.25$, $p = .09$. For the number of reinforcers earned (bottom panel), a similar two-way ANOVA revealed a significant main effect of area, $F(1, 21) = 6.88$, $p = .015$, with injections in the PFH resulting in a greater number of reinforcers earned overall. As well, there was a main effect of dose, $F(3, 63) = 5.13$, $p = .003$, with all three doses of NPY resulting in the delivery of significantly more pellets.

Four one-way ANOVAs, using dose alone as the factor, were also conducted separately on the number of responses made and reinforcers earned from the PFH- and the N.Acc-implanted rats. The ANOVAs performed on data from the PFH rats revealed a main effect of dose both for number of responses made, $F(3, 45) = 4.19$, $p = .011$, and number of reinforcers earned, $F(3, 45) = 4.99$, $p = .001$. Neither of the two measures was found to be significant following injections into the N.Acc.

Figure 12. Effects of NPY injected into the PFH and the N.Acc on PR operant responding. Top panel: number of responses made. Bottom panel: number of reinforcers earned.

* = $p < .05$ (compared to baseline)(Dunnett's test)

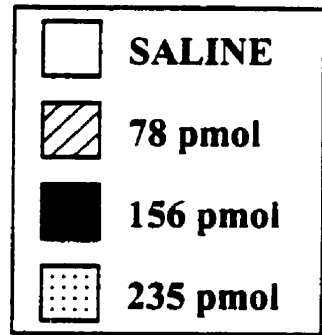
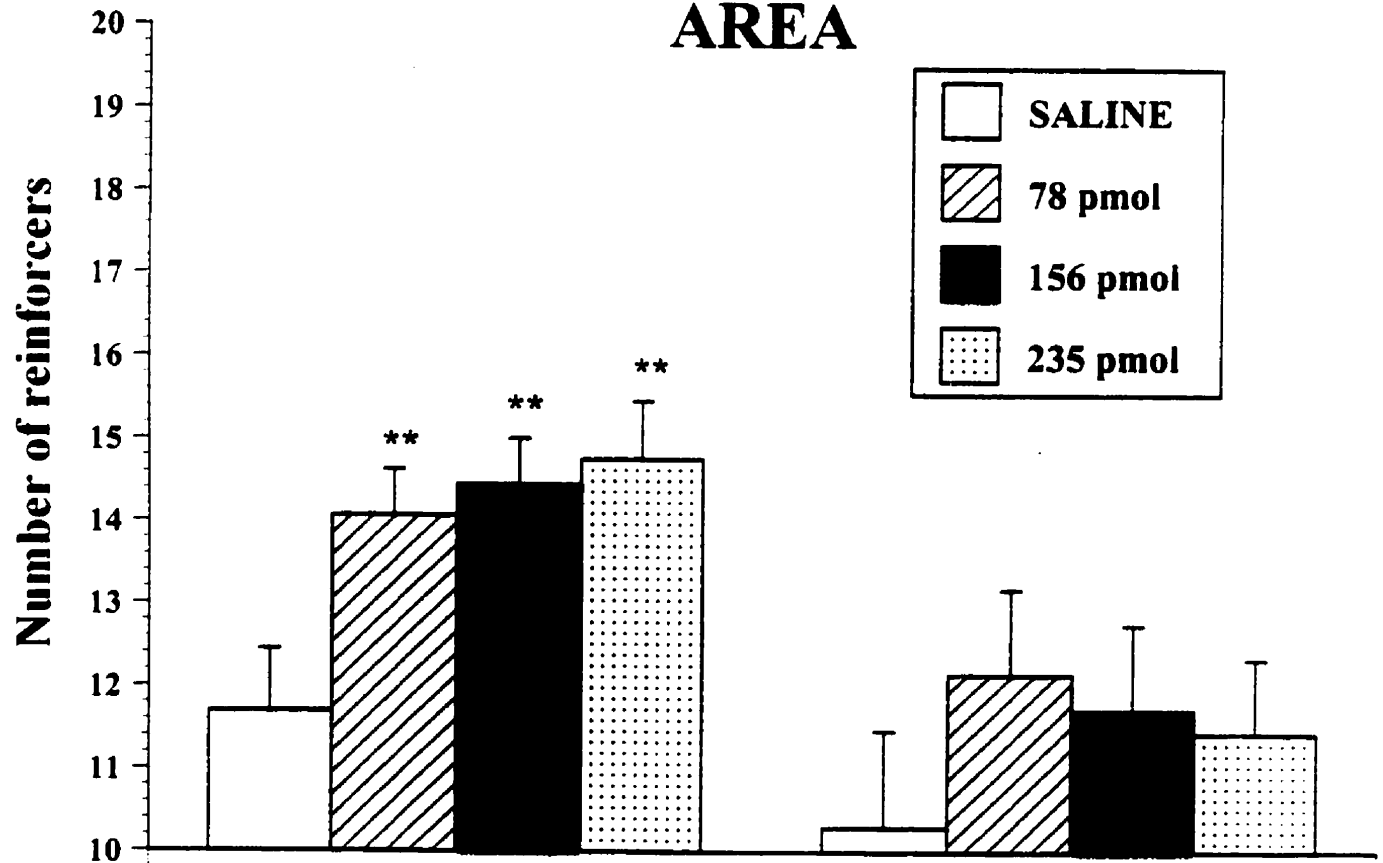
** = $p < .01$ (compared to baseline)(Dunnett's test)



PFH

N.ACC

AREA



PFH

N.ACC

Experiment #3b: Effects of DA antagonism on NPY-induced vs. drug-free PR responding for sucrose pellets

In Experiment #3a, PFH-applied NPY was found to dose-dependently increase the number of responses made by rats responding on a PR operant schedule of reinforcement, resulting in a significantly greater number of reinforcers earned. Typically, drugs that impair the mesolimbic DA system have been found to disrupt the breaking point when animals respond for cocaine (Roberts, 1989; Roberts, 1992), as well as sucrose (Cheeta, Brooks, & Willner, 1995). The purpose of the present experiment was to ascertain whether blockade of the DA system might attenuate the response-stimulating effects of PFH-applied NPY. Additionally, whether DA antagonism might preferentially affect the NPY response as opposed to drug-free responding was also studied.

Procedure

Fifteen rats previously tested in Experiment #3a were used in this experiment. Seven were rats from Experiment #3a that had had cannulae implanted in the PFH which had shown increased PR responding following NPY administration. The other eight were animals with cannulae implanted in the N.Acc, which had shown no effect following NPY administration in Experiment #3a. Both groups had FLU (0, 0.05, 0.1, and 0.2 mg/kg, I.P.) injected 2.5 hrs prior to either a fixed dose of 156 pmol/side of NPY (PFH rats) or no injection. PR testing began 5 min after the NPY injection. Numbers of responses made and reinforcers earned were the dependent measures.

Results

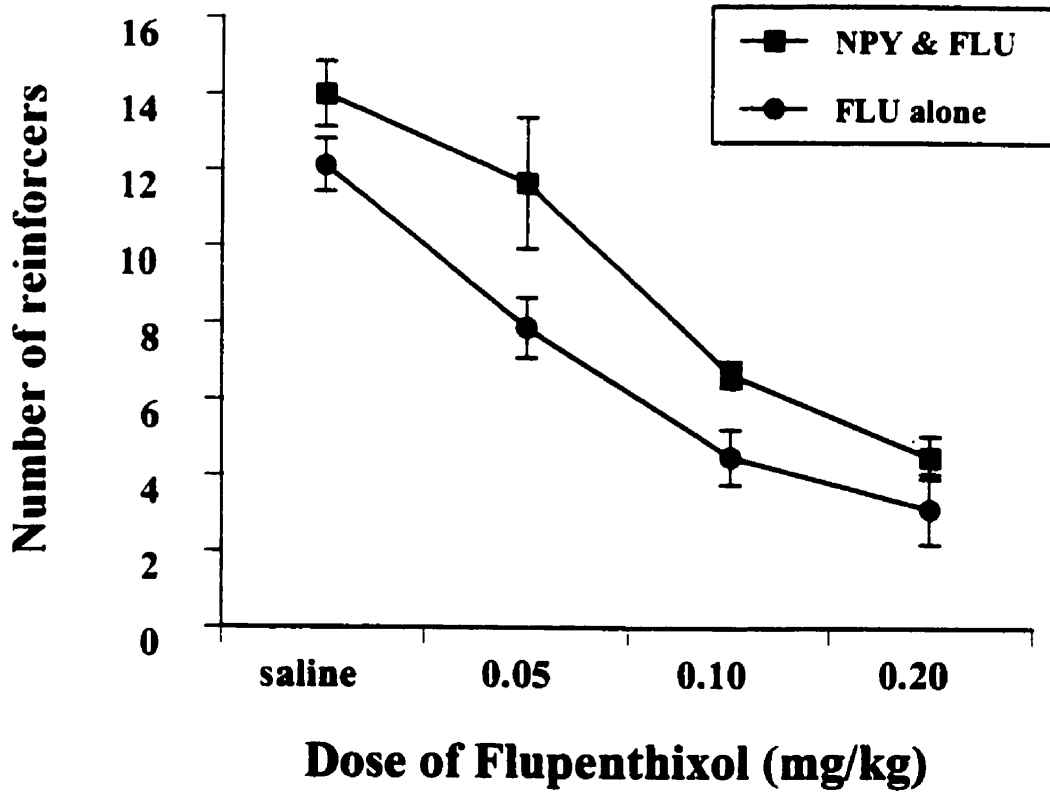
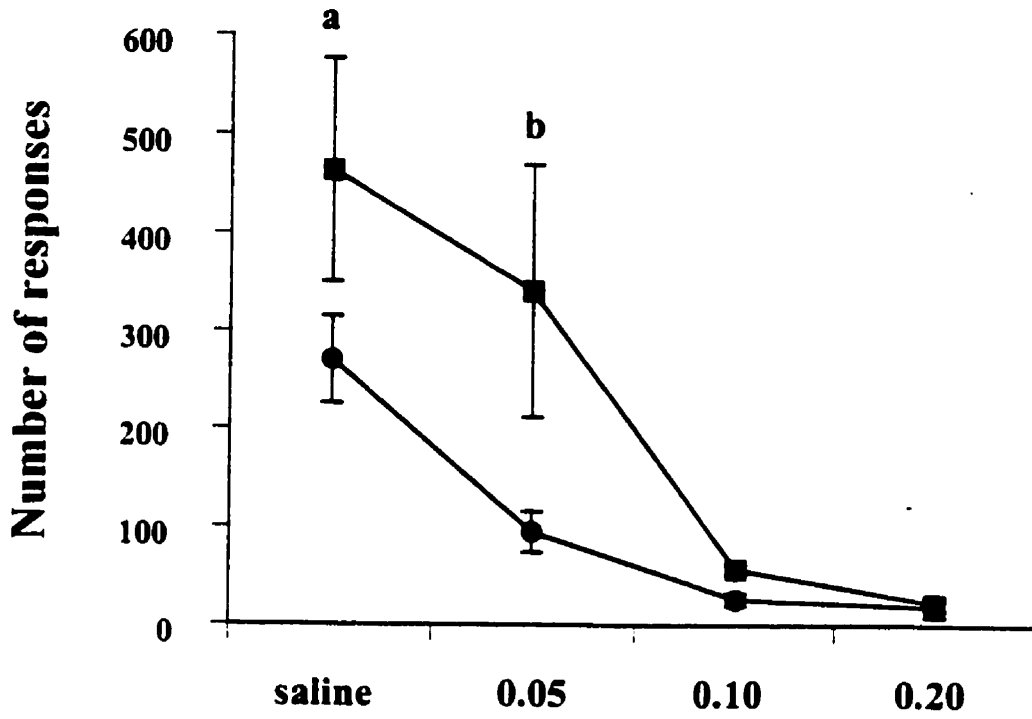
One rat from the PFH-implanted group had inaccurate cannulae placements, so its data were excluded.

The data from the remaining animals are presented in Figure 13. A two-way ANOVA indicated that there was a main effect of NPY for both the numbers of responses made, $F(1, 12) = 5.02$, $p = .045$ (top panel), and reinforcers earned, $F(1, 12) = 11.06$, $p = .006$ (bottom panel), with NPY-induced responding being higher than drug-free responding when data were averaged across all doses of FLU. FLU dose-dependently attenuated both NPY (156 pmol/side)-induced and drug-free PR responding in terms of the numbers of responses made, $F(3, 36) = 22.95$, $p < .001$ (top panel), and reinforcers earned, $F(3, 36) = 46.01$, $p < .001$ (bottom panel). The interaction was significant only for numbers of responses made, $F(3, 36) = 3.077$, $p = .039$ (top panel), with FLU having a greater attenuating effect on drug-free compared to NPY-induced responding at the lowest dose tested (0.05 mg/kg).

Figure 13. Effects of peripheral FLU on NPY(156 pmol/side)-induced (squares) vs. drug free (circles) PR operant responding for sucrose. Top panel: number of responses made. Bottom panel: number of reinforcers earned.

a = p < .01 (Fisher's LSD test)

b = p < .001 (Fisher's LSD test)



Experiment #3c: Effects of NPY on conditioned place preference

NPY has previously been shown to have rewarding effects of its own, as evidenced by its ability to produce a CPP when it is injected in a low dose (24 pmol/side) into the N.Acc (Josselyn & Beninger, 1993). To further substantiate this finding, and to ascertain whether the PFH is also capable of supporting NPY's rewarding effects, the present experiment assessed the effects of a low (24 pmol/side) and a high (156 pmol/side) dose of both N.Acc- and PFH-injected NPY on place conditioning. It was hypothesised that, if PFH NPY was involved exclusively in regulatory mechanisms underlying feeding behaviour, then a place aversion might be seen, based on reports that food deprivation induces conditioned place aversions (Harrington & van der Kooy, 1992; Bechara & van der Kooy, 1992). If both regulatory and non-regulatory aspects of feeding were activated by PFH NPY administration, a CPP might be evident after the administration of a low dose, while not so at higher doses that result in voracious feeding behaviour. As a positive behavioural control for the CPP procedure, a separate group of rats was tested for CPP with AMPH, administered peripherally.

Procedure

Six groups of 12 rats were used in this experiment. Half had bilateral cannulae implanted in the N.Acc, and half had cannulae implanted in the PFH. As well, there was a separate group of 12 unoperated rats that were tested with AMPH (I.P.).

Apparatus. All conditioning took place in four identical boxes, which were constructed from aluminium and Plexiglas and measured 60 cm wide, 30 cm deep, and 40 cm high. Each box was divided into two compartments of equal size, which were

separated by a removable central wall: one compartment was painted black and had a smooth Plexiglas floor, and the other compartment was painted white and had a rough Plexiglas floor. Between these two compartments was a silver-coloured aluminium platform covered with wire, measuring 8 x 31 x 4 cm. This platform served as the transitional zone between the two compartments. Testing was done in a separate room away from the rats' home cages. All rats received the conditioning and testing phases in the same box. Between every session, the floor of each box was washed with soap and water and dried thoroughly.

Testing. Following at least two days post-operative recovery (if indicated), rats were given access to the conditioning box as it would be on the test day, by placing them on the partition in the centre of the apparatus with the middle wall removed for three daily 15 min pre-conditioning sessions. At the end of the three days, conditioning with saline, 24 pmol/side NPY, 156 pmol/side NPY, or 2.5 mg/kg AMPH began. Half of the subjects received NPY or AMPH injections on days 1, 3, 5, and 7, while the other half received them on days 2, 4, 6, and 8. Half of each of these groups received their NPY or AMPH injection paired with a 30 min exposure to the black side of the box, and half with the white side. All received saline control injections, paired with the other side of the box, on alternate days. Two sets of four consecutive conditioning days were separated by two drug-free days. On the test day, the middle walls of the conditioning boxes were removed, and rats were given free access to the entire chamber for 15 min. A camera and VCR recorded the rats' movements between the two sides of the chamber. Time (s) spent in each side was the dependent variable. Timing with two hand-held stopwatches began when a rat's head and two forepaws crossed over into one or the other compartment. The

observer was blind to the treatment conditions until after scoring the videotapes.

In order to assess the feeding effects of NPY in these rats, food intake was measured following conditioning sessions numbers 3 and 4. On these days, immediately following the conditioning session rats were returned to their home cages with a pre-weighed amount of regular chow. Food intake (g) minus spillage was measured after 1 hr.

Results

Two rats with cannulae implants aimed at the N.Acc and three with cannulae aimed at the PFH were determined to have inaccurate cannulae placements, so their data were not included in the analysis.

Figure 14 shows the CPP results of the two groups of rats that received saline injections on both sides of the conditioning apparatus. There was no difference between the amount of time spent on each side of the box either for N.Acc-implanted rats, $t(11) = -1.79$, $p > .10$, or PFH-implanted rats, $t(10) = 0.13$, $p > .10$, indicating that subjects had no pre-existing bias towards either side of the chamber.

The results from the other five CPP groups are displayed in Figure 15. As expected, AMPH (2.5 mg/kg) administration produced a CPP, $t(11) = 6.99$, $p < .001$ (one-tailed), with rats spending, on average, 44% more time on the drug-paired side (far right). A three-way ANOVA using the remaining data, with area, dose, and side as factors, revealed a significant dose x side interaction, $F(1, 40) = 7.02$, $p = .023$, with rats given 24 pmol/side of NPY spending more time in the drug-paired side of the apparatus, regardless of the brain site it was injected into. Rats that received 24 pmol/side NPY in the N.Acc spent, on average, 38% more time on the drug-paired side, $t(11) = 2.01$, $p =$

.035 (one-tailed)(far left), while rats that received 24 pmol/side NPY in the PFH spent, on average, 32% more time on the drug-paired side, $t(10) = 1.86$, $p = .093$ (two-tailed)(middle). The high (156 pmol/side) dose of NPY did not produce any significant effects on CPP either in the PFH or the N.Acc.

The feeding data for the rats that received NPY are shown in Figure 16. A two-way ANOVA using the NPY-saline difference score as the dependent variable revealed a significant main effect of area, $F(1, 40) = 24.66$, $p < .001$, with the PFH-injected rats consuming more chow than the N.Acc-injected rats. There was also a main effect of dose, $F(1, 40) = 7.79$, $p = .008$, with the 156 pmol/side dose of NPY resulting in significantly greater chow intake. The area x dose interaction was also significant, $F(1, 40) = 4.19$, $p = .047$, with intake following the 156 pmol/side dose of NPY in the PFH being significantly greater than for all other groups, and intake following the 24 pmol/side dose of NPY in the PFH being significantly greater than when the same dose was applied to the N.Acc.

Pearson product-moment correlation scores were calculated using the CPP scores and the food intake data for all groups. There was a significant negative correlation between NPY-induced food intake and time spent on the drug-paired side of the conditioning apparatus ($r = -.69$), $t(10) = -2.88$, $p = .018$, for rats receiving 24 pmol/side of NPY in the PFH (Figure 17). This indicates that those rats which ate following NPY administration spent less time on the drug-paired side of the conditioning chamber than did rats which did not eat, or ate less, following NPY administration.

Figure 14. The effects of N.Acc and PFH saline administration on place conditioning.

There were no preferences shown for either side of the conditioning chamber.

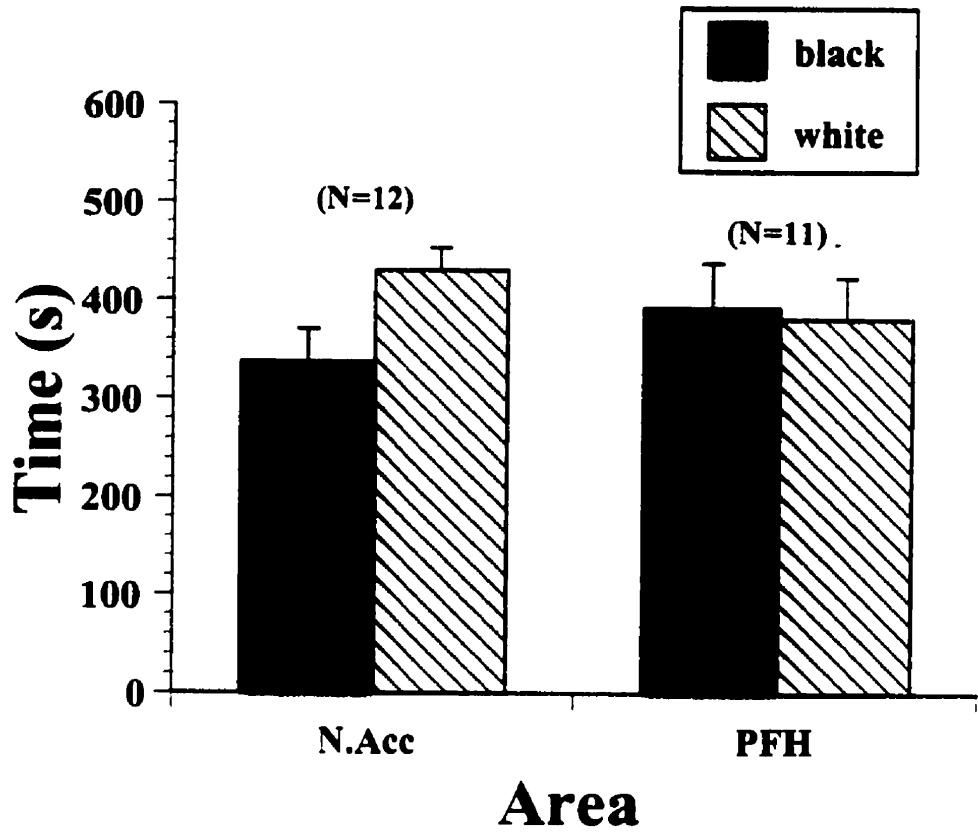


Figure 15. Effects of N.Acc and PFH NPY (24 and 156 pmol/side) and AMPH (2.5 mg/kg) on place conditioning.

**** = $p < .001$ (Student's t -test; one-tailed)**

*** = $p < .05$ (Student's t -test; one-tailed)**

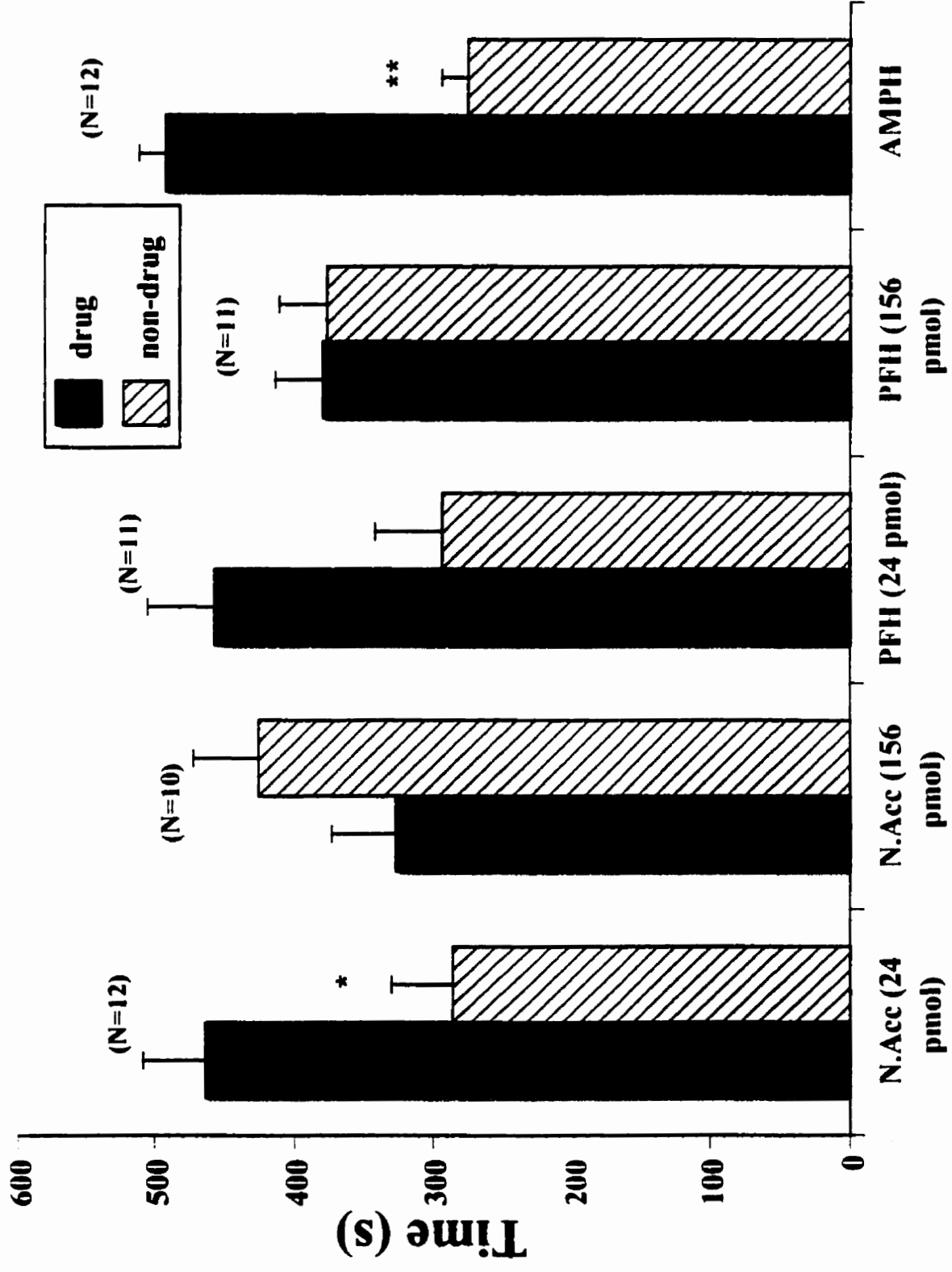


Figure 16. Effects of PFH and N.Acc NPY on 1 hr chow intake in rats being tested for NPY place conditioning.

* = $p < .01$ (Fisher's LSD test)

** = $p < .001$ (Fisher's LSD test)

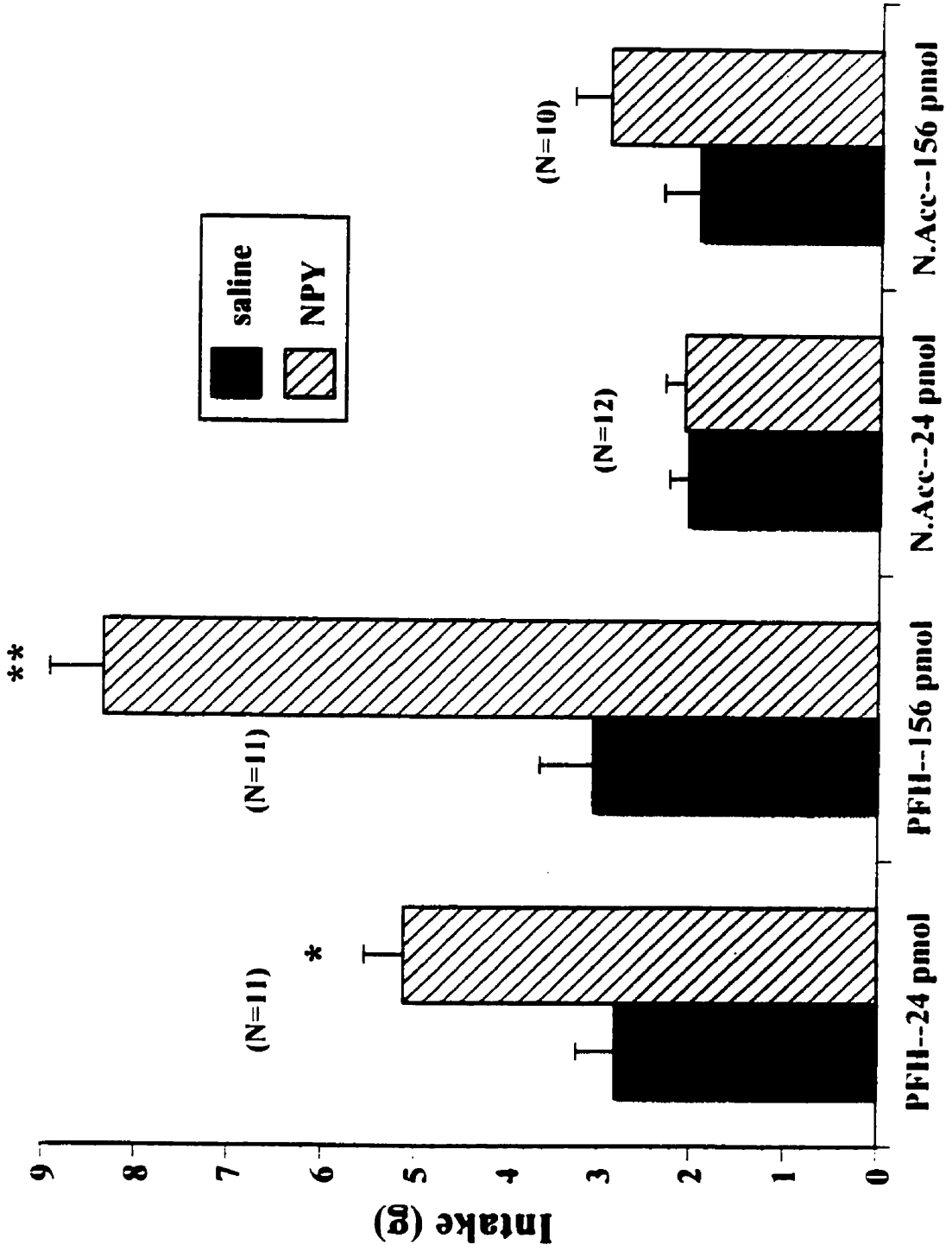
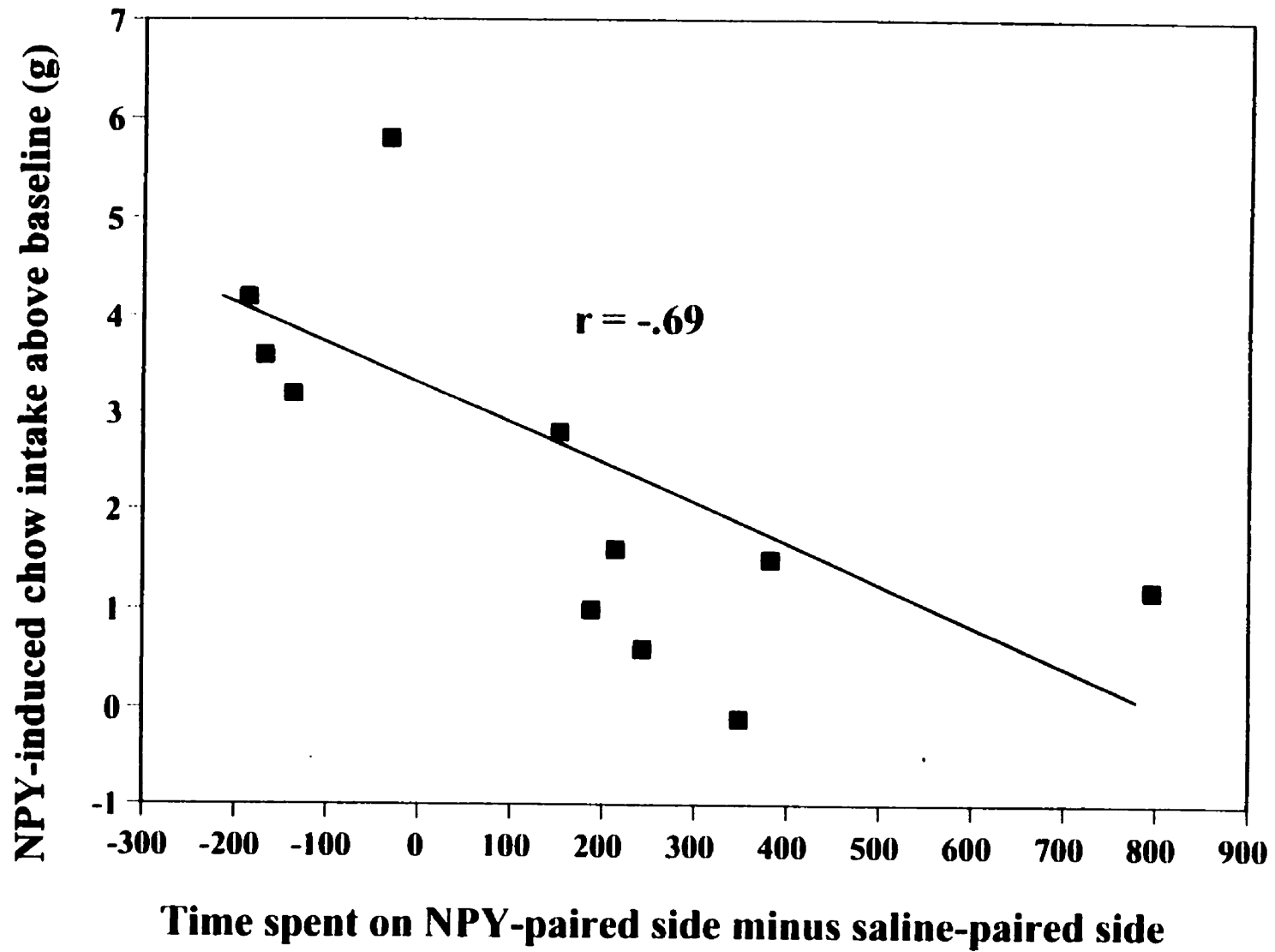


Figure 17. Pearson product-moment correlation between CPP and NPY-stimulated food intake for rats receiving 24 pmol NPY/side in the PFH ($p < .02$).



Experiment #3d: Effects of FLU (I.P.) on NPY-induced CPP

Experiment #3c determined that a low (24 pmol) dose of NPY injected into the N.Acc or the PFH results in a CPP. DA has been implicated in the CPP-producing effect of NPY in the N.Acc (Josselyn & Beninger, 1993), in addition to that produced by other stimuli such as AMPH (Hiroi & White, 1991; Spyraiki, Fibiger, & Phillips, 1982a), heroin (Bozarth and Wise, 1981), and food (Spyraiki, Fibiger, & Phillips, 1982b). The present experiment was designed to examine the involvement of the mesolimbic DA system in the CPP produced by NPY. It was hypothesised that FLU would block both N.Acc and PFH NPY-induced CPP.

Procedure

Thirty-one rats, 16 with bilateral cannulae implanted in the PFH and 15 with cannulae in the N.Acc, were used in this experiment. The procedure was the same as for Experiment #3c, except that 2.5 hrs prior to 24 pmol/side NPY or saline administration subjects were injected with either saline or FLU (0.2 mg/kg, I.P.). All conditions were counterbalanced across subjects. As in the previous experiment, on conditioning days 3 and 4 rats were returned to their home cages immediately following the conditioning session and 1 hr food intake (g) minus spillage was measured.

Results

Three rats with cannulae aimed at the PFH and one with cannulae aimed at the N.Acc were found to have inaccurate cannulae placements, so their data were excluded from the analysis.

The food intake data are shown in Figure 18. A two-way ANOVA using the

NPY-saline difference score as the dependent variable revealed a significant main effect of area, $F(1, 23) = 7.70$, $p = .01$, with PFH NPY stimulating feeding regardless of whether or not subjects received FLU.

NPY failed to produce a significant CPP when it was applied either to the PFH or the N.Acc, $F(1, 23) = .26$, $p > .10$ (Figure 19). It was unrealistic, therefore, to ascertain whether FLU had any effects on NPY-induced CPP. A three-way ANOVA revealed a main effect of FLU, $F(1, 23) = 4.38$, $p = .048$, with those animals that received FLU spending more time overall on both sides of the conditioning box than animals not receiving FLU. The area \times FLU interaction was also significant, $F(1, 23) = 6.82$, $p = .016$, with those animals implanted in the N.Acc spending an equal amount of total time on both sides regardless of FLU administration. Those implanted in the PFH and receiving FLU spent a greater amount of total time on both sides of the apparatus compared to those not receiving FLU, who apparently spent more time in the transitional zone between the two compartments.

Figure 18. Effects of FLU (I.P.) on PFH NPY (24 pmol/side)-induced chow feeding in rats being tested for NPY + FLU place conditioning.

**** = $p > .001$ (Student's t-test)**

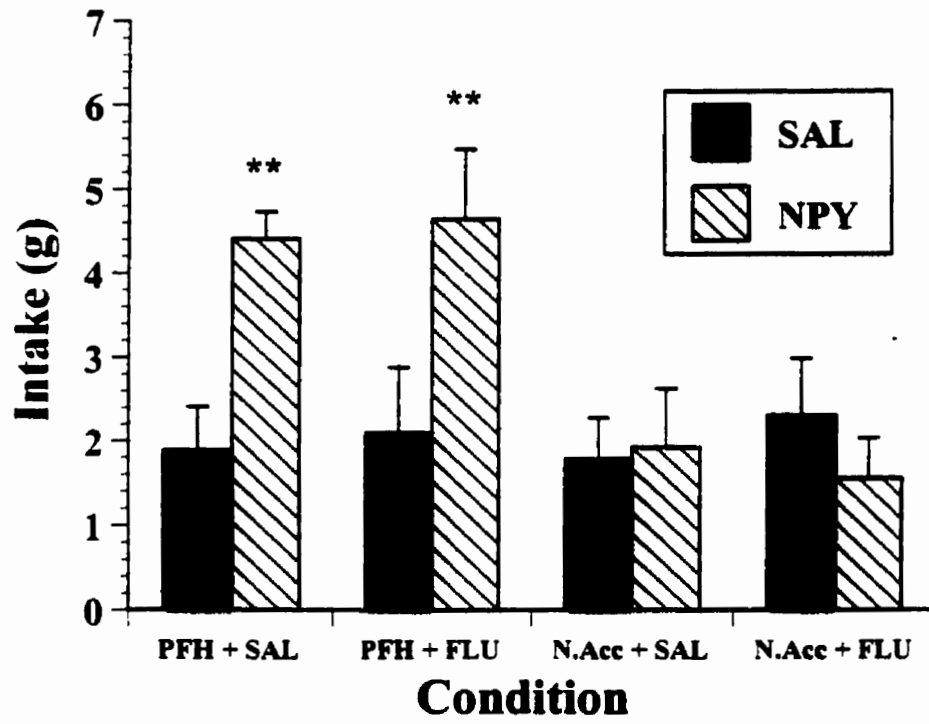
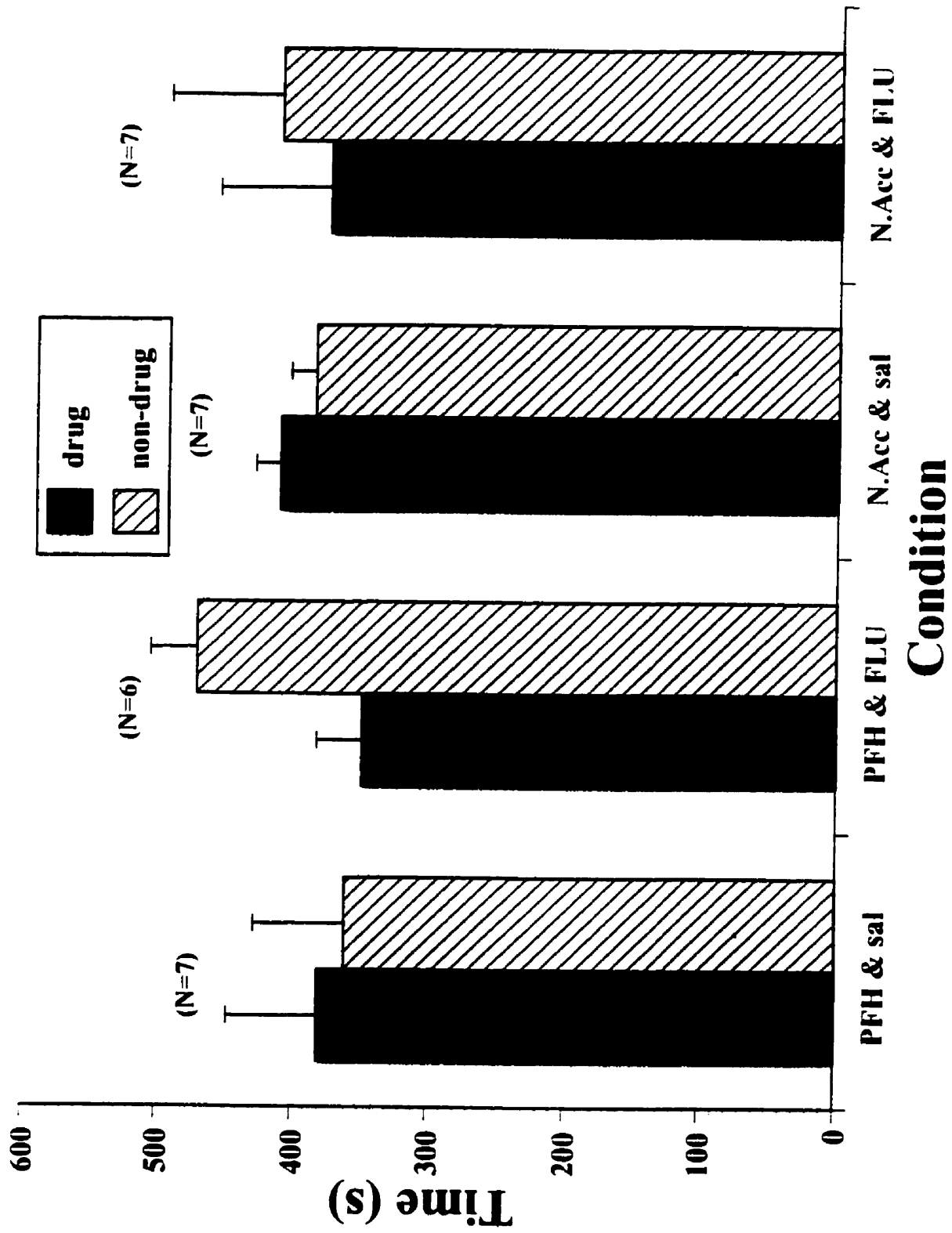


Figure 19. Effects of FLU (I.P.) on NPY-induced CPP.



Summary of Reward Experiments

The results from Experiment #3a show that NPY injected into the PFH, but not the N.Acc, results in increased PR operant responding for sucrose reward pellets. Peripheral FLU's attenuation of this response was no greater than its attenuation of drug-free PR responding, indicating that DA was not preferentially involved in NPY-induced responding (Experiment #3b). Experiment #3c demonstrated that 24 pmol/side of NPY, but not 156 pmol/side, in the N.Acc results in a CPP that is of the same magnitude as that produced by 2.5 mg/kg of AMPH. When 24 pmol/side of NPY is injected into the PFH, a CPP is also seen; however, it did not reach statistical significance when a two-tailed *t*-test was used. There is a negative correlation between the PFH CPP and NPY-stimulated food intake when animals were injected with 24 pmol/side of NPY, suggesting that the pleasurable effects of this dose of NPY in the PFH can be negated when its feeding-stimulatory effects are present at the same time. The results of Experiment #3d were inconclusive, in that 24 pmol/side of NPY failed to produce a CPP in either the PFH or the N.Acc, making it impossible to assess the effects of DA blockade on this response.

These results suggest that NPY-stimulated increases in PR operant responding are related primarily to its effects on food intake in general, and not to any specific effects on reward mechanisms. As well, results of the CPP study (Experiment #3c) show that NPY, given at a low dose in the N.Acc, has rewarding effects that, based on the results of previous experiments, are independent of any feeding effects. The rewarding effects of a low dose of NPY in the PFH may occur when the injections are sub-threshold in their ability to stimulate food intake. The CPP effect of NPY appears to be of a delicate nature, however, as we failed to replicate it in Experiment #3d, even though the

procedure used in both experiments was identical.

Methodological Control Experiments
(Experiments #4a-4c)

Experiment #4a: Effects of N.Acc AMPH on locomotor activity

In order to assess behaviourally whether the N.Acc guide cannulae used in this series of experiments were capable of accessing a DA substrate in the N.Acc, this next experiment examined the effects of N.Acc AMPH on locomotor activity. This measure has previously been well established as a correlate of DA-ergic activation of the N.Acc (Salamone, Cousins, & Snyder, 1997; Di Chiara, 1995; Evans & Vaccarino, 1986). Based on these previous reports, N.Acc AMPH should result in an increase in locomotor activity.

Procedure

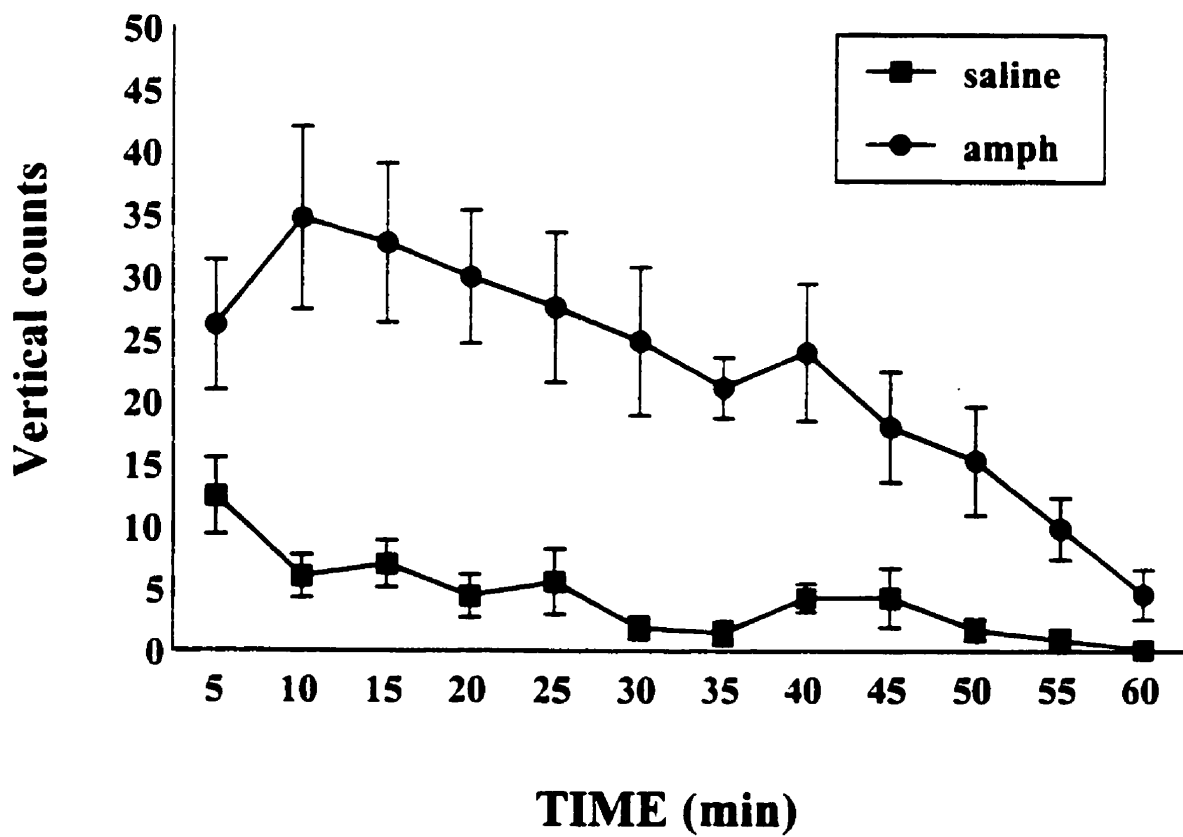
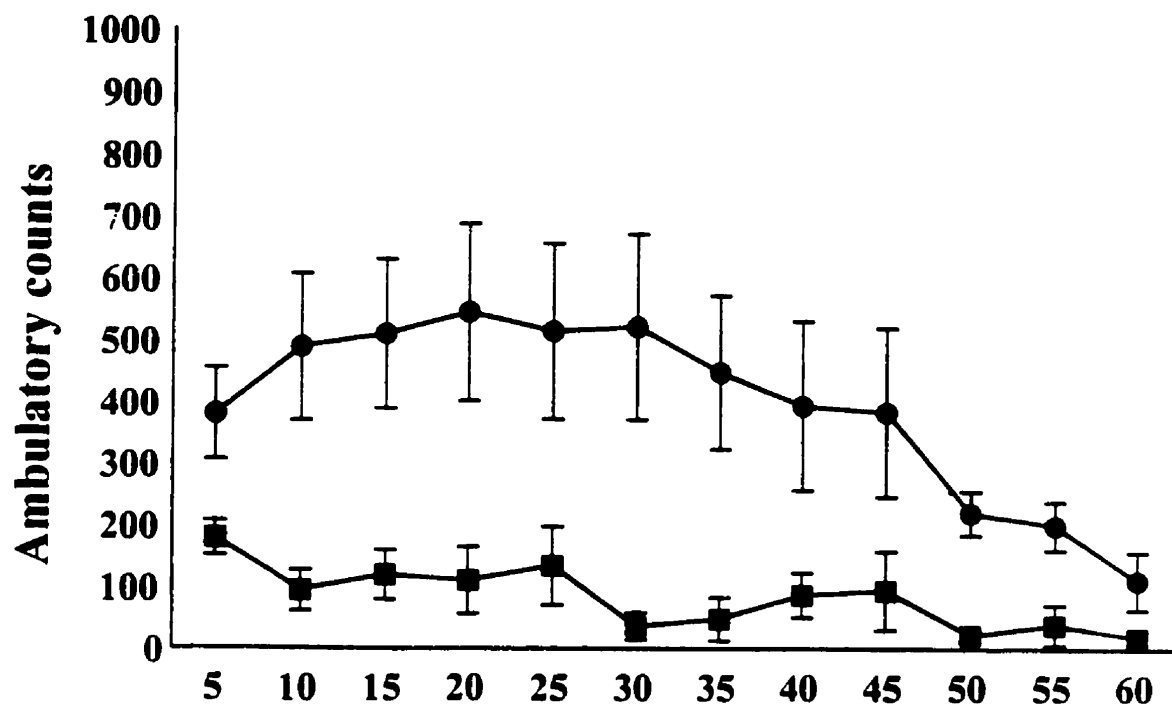
Seven rats had bilateral cannulae implanted, aimed to terminate in the N.Acc. Following at least five days of recovery, testing began. Subjects were placed in the testing apparatus for 30 min, injected with either saline or 10 µg AMPH/side, and returned to the boxes for 1 hr. This dose of AMPH was chosen based on previous reports showing that its administration into the N.Acc reliably increased locomotor activity (Evans & Vaccarino, 1986). Activity measurements were recorded every 5 min.

Apparatus. Activity tests were conducted in four Plexiglas activity chambers (Med Associates Inc., Georgia, VT) measuring 40 cm long, 40 cm wide, and 28 cm high. Ambulatory (horizontal) movement was detected by two arrays of 16 infrared beams, while a third array positioned 10 cm above the cage floor detected vertical movement. Apparatus control and data collection were accomplished with a 386-SX IBM-type computer.

Results

Data from all seven animals are presented in Figure 20. A two-way ANOVA with ambulatory counts as the dependent variable (top panel) revealed significant main effects of drug, $F(1, 6) = 20.42$, $p = .004$, and time, $F(11, 66) = 3.81$, $p < .001$, with AMPH administration resulting in more ambulatory counts overall, an effect which lessened over time. As well, the drug x time interaction was significant, $F(11, 66) = 4.18$, $p < .001$. Similarly, a two-way ANOVA with vertical counts as the dependent measure (bottom panel) revealed significant main effects of drug, $F(1, 6) = 52.28$, $p < .001$, time, $F(11, 66) = 5.87$, $p < .001$, and a significant drug x time interaction, $F(11, 66) = 3.16$, $p = .002$.

Figure 20. Effects of N.Acc AMPH on ambulatory (top panel) and vertical (bottom panel) locomotor activity.



Experiment #4b: Can N.Acc FLU block other DA-mediated behaviours?

In order to test the effectiveness of injection conditions, this experiment was designed to assess whether N.Acc administration of FLU is able to block other DA-mediated behaviours. One such behaviour is the increased locomotor activity seen following peripheral administration of a DA agonist such as AMPH. Based on previous reports (Kelly, Seviour, & Iverson, 1975), it was hypothesised that N.Acc administration of FLU would dose-dependently reduce AMPH-induced locomotor activity.

Procedure

Eleven rats, which had been previously tested in Experiment #1c, were tested in this experiment. Rats were given an injection of FLU (0, 1.25, or 5 $\mu\text{g}/\text{side}$) in the N.Acc and acclimatised to the novel test apparatus for 30 min. Following this period, 1.25 mg/kg of AMPH (I.P.) was administered, subjects returned to the test apparatus, and locomotor activity counts recorded every 15 min for 2 hrs. All treatments were given in a semi-randomised order.

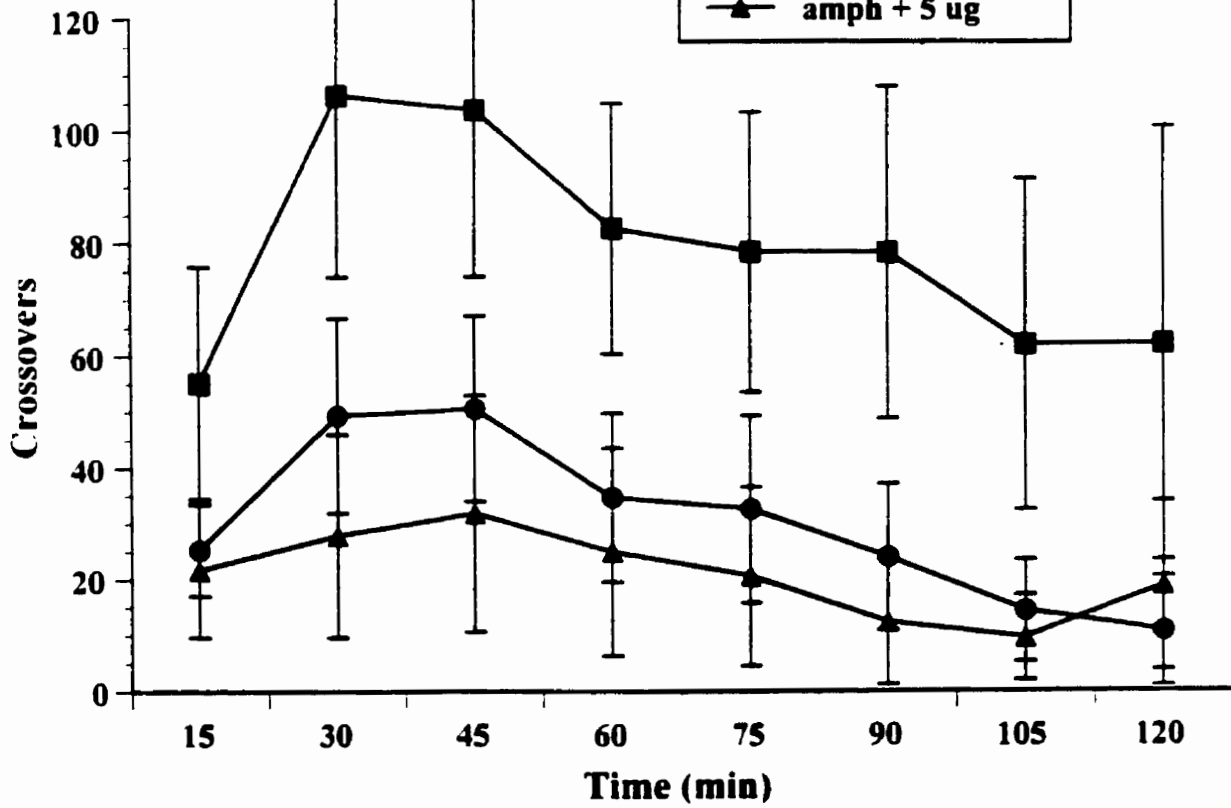
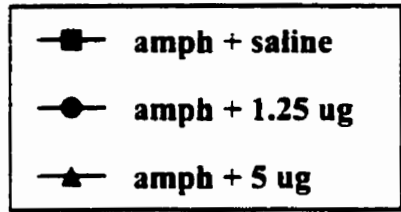
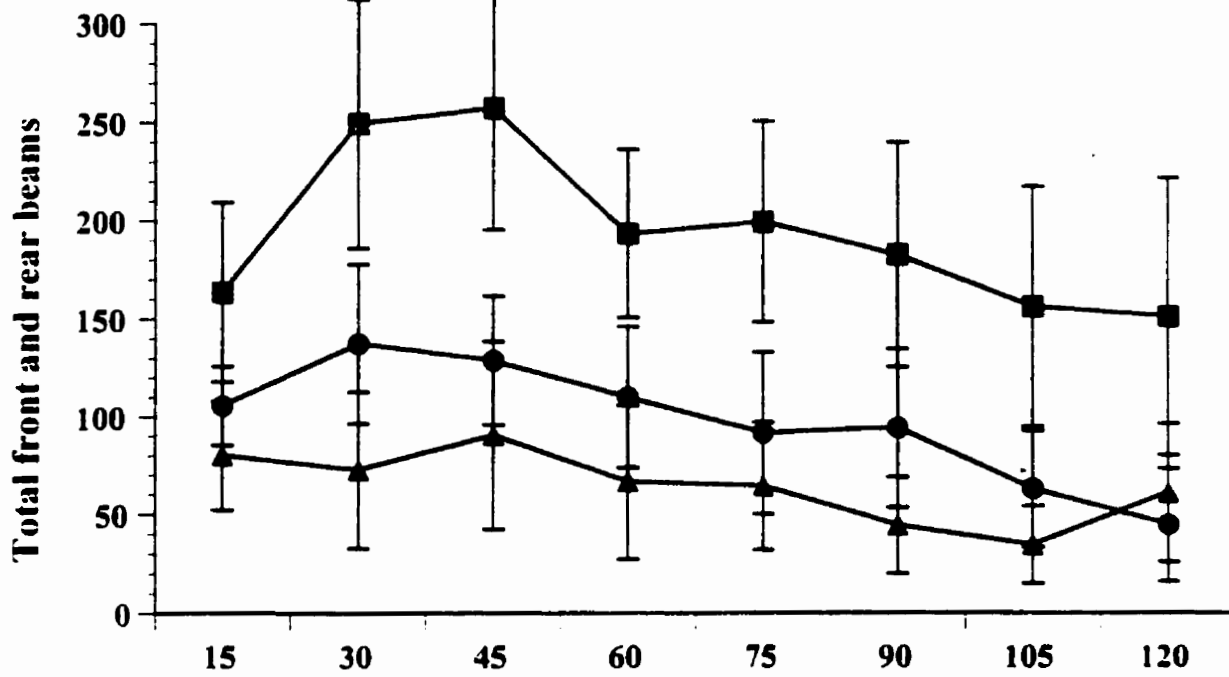
Apparatus. Activity cages consisted of 16 hanging wire mesh cages measuring 25 cm wide, 36 cm deep, and 20 cm high. Two rows of infrared photocell beams, situated across the long axis of the cage 2 cm above the floor, recorded horizontal movement. Apparatus control, and data collection, was performed by a 286 IBM-type computer. The activity cages used in this experiment were different from the ones used in Experiment #4a due to the unavailability of the latter, as well as the ability of the present system to accommodate a greater number of animals at one time.

Results

One animal became ill during the experiment, so its data were not included. The data from the remaining 10 subjects are presented in Figure 21.

A two-way ANOVA done on the total number of front and rear beam crossings (top panel) indicated a trend towards significance for the main effect of drug, $F(2, 27) = 2.99$, $p = .066$, with both doses of FLU attenuating AMPH-induced locomotion. As well, there was a significant main effect of time, $F(7, 189) = 8.74$, $p < .001$ with locomotor activity decreasing towards the end of the test session for all groups. A separate two-way ANOVA on number of crossovers (bottom panel) again revealed a trend towards significance for the main effect of drug, $F(2, 27) = 2.76$, $p = .08$, with both doses of FLU resulting in reduced activity. Again, there was a significant main effect of time, $F(7, 189)$, $p < .001$, with activity levels dropping off toward the end of the test session.

Figure 21. Effects of N.Acc FLU on AMPH-induced locomotor activity. Top panel: total number of front and rear beam breaks. Bottom panel: total number of crossovers.



Experiment #4c: Can N.Acc stimulation with other compounds increase feeding behaviour?

Although Experiment #4a provided behavioural evidence that cannulae placements were accurate by demonstrating that injections aimed at the N.Acc were accessing the mesolimbic DA system, it was still important to ascertain behaviourally whether injections there were able to stimulate feeding behaviour. Recent evidence has shown that the GABA(A) receptor agonist muscimol, when injected into the ventromedial accumbens shell, is a potent inducer of consummatory behaviour in rats (Stratford & Kelley, 1997). The opiate drug morphine has also been shown to elicit significant increases in food intake, primarily when it is injected into the accumbens core region (Bakshi & Kelley, 1993). This experiment assessed the feeding effects of both muscimol and morphine following their injection into the N.Acc.

Procedure

Fifteen rats that had had bilateral cannulae aimed to terminate in the N.Acc, and which had previously undergone place conditioning testing, were pre-satiated in their home cages with regular chow as in Experiment #1a. One hour later, saline, muscimol (100 ng/side), or morphine (5 µg/side) was injected, and rats were returned to their cages. Regular chow intake (g) minus spillage was measured 1 and 2 hrs later. There was at least two days separating test days, and all rats received the treatments in a semi-randomised manner.

Results

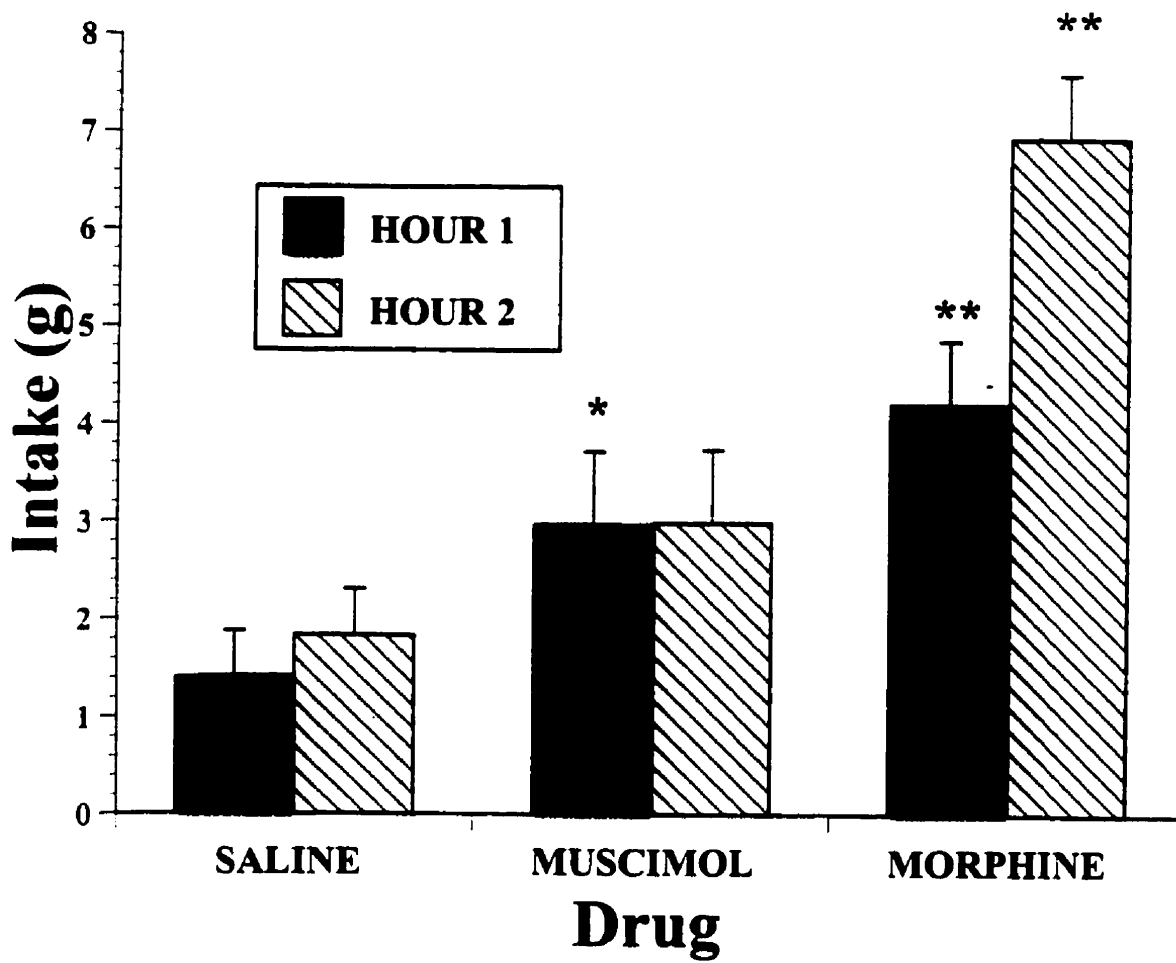
One rat was found to have inaccurate cannulae placements, so its data were excluded from the analysis.

Results from this experiment are shown in Figure 22. Two separate one-way ANOVAs revealed a significant main effect of drug both for 1 hr, $F(2, 26) = 5.89$, $p = .008$, and 2 hr, $F(2, 26) = 17.73$, $p < .001$, intake. Post-hoc analyses using Dunnett's test showed that 1 hr intake following muscimol was significantly higher than saline ($p < .05$) while 2 hr intake did not reach statistical significance. Morphine elicited significant increases in chow intake at both 1 hr ($p < .01$) and 2 hrs ($p < .01$) post-injection.

Figure 22. Effects of N.Acc muscimol (100 ng/side) and morphine (5 μ g/side) on regular chow feeding.

* = $p < .05$ (compared to baseline)(Dunnett's test)

** = $p < .01$ (compared to baseline)(Dunnett's test)



General Discussion:

NPY's involvement in regulatory feeding processes

The results of Experiment #1a clearly indicate that NPY injected into the PFH dose-dependently increases the consumption of a nutritive, yet non-preferred, food type, supporting the notion that NPY in the PFH is involved in processes underlying regulatory feeding behaviour. The administration of FLU, either peripherally or directly into the N.Acc, had no attenuating effect on this response. In fact, the lowest dose of FLU in the N.Acc resulted in a significant increase in PFH NPY chow intake above baseline levels. NPY in the N.Acc did not significantly increase regulatory food intake; however, there was a slight, but statistically insignificant, increase following administration of the 78 pmol dose. According to earlier predictions (see Table 2), these results illustrate a regulatory feeding role for NPY in the PFH.

A role for hypothalamic NPY in regulatory feeding mechanisms has been documented by a number of authors. Stanley and Leibowitz (1984) found that PVN-injected NPY still exerted strong eating-stimulatory effects when food was withheld until 4 hrs post-injection, indicating that food itself acts as an antagonist to NPY's intake-stimulating effects, and that NPY does not simply lose effectiveness over time. This finding lends some credence to the idea that NPY's effects on feeding may depend on its actions on satiety mechanisms (Leibowitz & Alexander, 1991; Paez & Myers, 1991); however, it does not preclude the presence of behavioural activating effects of NPY. In fact, the behavioural activating effects of NPY on feeding behaviour have been posited to result from its effects in the PFH, while the physiological and autonomic effects are believed to stem from its actions in the PVN (Stanley, 1993).

The most conclusive evidence for NPY's role in regulatory feeding comes from its involvement in the behavioural "loop" system with circulating levels of leptin (Gehlert & Heiman, 1997; Rohner-Jeanrenaud et al., 1996). When NPY is injected icv, plasma leptin levels are decreased; similarly, when leptin is injected icv, PVN levels of NPY are reduced (Wang et al., 1997), indicating that this relationship is reciprocal in nature. NPY administration also increases insulin secretion and glucose metabolism when it is injected icv (Marks & Waite, 1996), while leptin administration results in a reduction of hyperglycemia and hyperinsulinemia (Hamann & Matthaei, 1996).

The idea that NPY's effects on regulatory feeding are DA-independent is supported by our results and the findings of Levine & Morley (1984), who demonstrated that peripheral haloperidol decreased NPY-induced feeding, but only at doses that attenuated other behaviours such as drinking. This suggests that the effects of DA antagonism on NPY-induced feeding are non-specific in nature. The doses of peripheral FLU used in Experiment #1b, however, did not have generalised effects on activity. Those doses were chosen based on previous reports demonstrating that they were able to attenuate reward-sensitive measures such as cocaine administration (Roberts & Vickers, 1984) and the locomotor response to amphetamine (Swerdlow, Vaccarino, Amalric, & Koob, 1986), but not spontaneous motor activity (Ahlenius, Hillegaart, Thorell, Magnusson, & Fowler, 1987). Likewise, the doses of FLU injected directly into the N.Acc were chosen based on previous reports showing that doses in this range blocked the feeding induced by the orexigenic agent 8-OH-DPAT while leaving deprivation-induced feeding unaffected (Fletcher, 1991).

The finding in Experiment #4b that the same doses of FLU attenuated AMPH-

induced hyperactivity, an effect that depends on mesolimbic DA release (Salamone et al., 1997; Di Chiara, 1995; Evans & Vaccarino, 1986), refutes the proposition that FLU was not having any effect on behaviour due to the use of an ineffective dose range.

Furthermore, the injections of FLU in both studies were administered to animals that had had cannulae implanted using the same co-ordinates and the same surgical procedures.

This, in addition to subsequent histological verification, makes the possibility of inaccurate cannulae placements in the FLU/NPY study unlikely.

The apparent increase in PFH NPY-induced feeding following the 1.25 μ g dose of FLU in the N.Acc (Experiment #1c) may reflect an effect of FLU on pre-synaptic autoreceptors, resulting in an overall increase in DA in the N.Acc. Such an increase in N.Acc DA, when produced by the administration of very low doses of AMPH, has been shown to result in increases in food intake (Sills et al., 1993). That the higher dose of FLU did not result in such an increase might reflect its ability to block post-synaptic receptors in addition to pre-synaptic ones, resulting in decreased DA activity in the N.Acc. The possibility of the 1.25 μ g dose of FLU increasing DA levels seems unlikely, however, as the same dose in the N.Acc did not result in an increase in AMPH-induced locomotor activity (Experiment #4b), a behavioural measure that is also dependent on DA activity.

Finally, the finding that N.Acc NPY in the present dose range had only a slight, yet non-significant, effect on regulatory feeding behaviour is not surprising given the findings of Stanley et al. (1985). These authors found that, while hypothalamic NPY injections resulted in a robust increase in food intake, several extra-hypothalamic sites were ineffective. Once again, we can be confident that the NPY injections were reaching

their target site, as AMPH injected through cannulae aimed at the same site elicited increased locomotor activity (Experiment #4a), an effect mediated by its effects on N.Acc DA (Salamone et al., 1997; Di Chiara, 1995; Evans & Vaccarino, 1986). Furthermore, that the target site was capable of supporting increased feeding behaviour was confirmed by the effectiveness of both muscimol and morphine to increase food intake following their injection into the N.Acc (Experiment #4c).

Table 2. Summary of experimental paradigms and observed outcomes.

<u>PROCESS</u>	<u>EXPERIMENTAL PARADIGM</u>	<u>PREDICTED OUTCOME OF N.ACC-APPLIED NPY:</u>	<u>PREDICTED OUTCOME OF PFH-APPLIED NPY:</u>	
			<u>IF REGULATORY ONLY:</u>	<u>IF BOTH REGULATORY AND NON-REGULATORY:</u>
REGULATORY FEEDING	Free-feeding (chow)	Slight increase in feeding behaviour—blocked by N.Acc FLU	A robust increase—not blocked by N.Acc FLU	A robust increase—partially blocked by N.Acc -FLU
NON-REGULATORY FEEDING (REWARD)	Free-feeding (sucrose)	A robust increase in feeding when compared to effects on chow feeding—blocked by N.Acc FLU	A robust increase (robust increase from chow feeding)—partially blocked by N.Acc FLU	A robust increase (larger than chow feeding)—partially blocked by N.Acc FLU
REWARD	Progressive ratio operant responding for sucrose	An increase in breaking point—blocked by FLU	A robust increase in breaking point—not blocked by FLU	A robust increase in breaking point—blocked by FLU
REWARD	Conditioned place preference	Produce a CPP—blocked by FLU	No CPP produced. Possibly place aversion	Produce a CPP—blocked by FLU

NPY's involvement in non-regulatory feeding processes

Sucrose is a preferred food type, as baseline intake levels were significantly greater (+83%) than baseline chow intake (Experiment #2a). Although PFH administration of NPY dose-dependently increased sucrose intake, this increase was analogous to NPY's effect on chow intake and was not preferential to sucrose. This suggests that the role of PFH NPY is purely regulatory, as non-regulatory effects would be expected to result in a preferential increase in sucrose intake (see Table 2). Furthermore, as in Experiment #1b, DA did not appear to mediate the regulatory effects of PFH NPY on sucrose intake, as indicated by the fact that the administration of N.Acc FLU did not attenuate this response. N.Acc administration of NPY did not result in any increases in sucrose intake; this finding suggests that the slight feeding-stimulatory effects seen in Experiment #1a may have reflected diffusion of NPY into the lateral ventricles, possibly reaching feeding sensitive sites.

Some authors have found that internal cues (Seeley et al., 1997) and patterns of behaviour (Levine et al., 1991) following NPY administration do not completely mimic those experienced by animals in a food-deprived state. In the present study, although NPY administration increased sucrose intake as compared to baseline, it did not do so any more than chow intake, indicating that NPY's effects are proportionately greater on a regulatory feeding mechanism rather than a non-regulatory, reward-enhancing system. Anecdotally, once the 2 hr sucrose feeding test had been completed, and regular chow was returned to the animals' cages, many rats that had received NPY appeared to change their preferences to the chow, and continue feeding. This observation, albeit inconclusive, lends support to the notion that the animals were attempting to satiate some

homeostatic need that was not met by ingesting sucrose alone. It is interesting to speculate that, although not entirely equivalent to those accompanying deprivation-induced feeding, the internal cues produced by NPY administration may nevertheless correspond to homeostatic-like stimuli. Peripheral cues such as stomach distension and/or learning effects such as the animal's memory of when it last ate are bound to be distinct during the behavioural state produced by NPY and the state of food deprivation. It may be that an organism is able to discern between the two states, even though the two activate similar regulatory, rather than non-regulatory, processes. Indeed, one explanation for the observed increases in activity levels seen with NPY-treated animals vs. food-deprived ones (Levine et al., 1991) might be that the NPY-treated rats simply have more peripheral energy stores from which to gather fuel.

The finding that NPY administration does not affect the consummatory phase of ingestion (Seeley et al., 1995) can also be explained using the same logic. If the signal produced by NPY is of a behavioural-activating nature that signals an organism to eat, then an animal may need to perform an actual approach-like, or appetitive, response in order to satisfy it. Following this reasoning, it is not surprising that NPY does not increase intraoral intake (Seeley et al., 1995), as this reflexive test does not require any form of appetitive response. That is to say, even though NPY has its effects on regulatory aspects of feeding, this does not necessarily mean that its stimulus state completely parallels that of food deprivation.

The observation that NPY administration results in much larger increases in palatable food intake (Stanley & Leibowitz, 1985) than in intake of a non-preferred food type (Brown & Coscina, 1995) may reflect methodological differences in techniques and

cannulae placements, rather than genuine differences in NPY's capability to stimulate intake of various food types. While animals in one study (Stanley & Leibowitz, 1985) were maintained and tested on a wet-mash diet consisting of 37% sucrose and 17% Carnation evaporated milk, animals in the other study (Brown & Coscina, 1995) were maintained and tested on standard Purina rat chow. Therefore, the differences in intake may simply reflect the additive nature of NPY-simulated intake above (already elevated) baseline levels, similar to that observed in the present study.

That NPY in the N.Acc did not increase sucrose consumption is somewhat unexpected given its effects on other DA-mediated behaviours such as CPP (Josselyn & Beninger, 1993) and circling behaviour (Moore et al., 1990). The possibility that NPY injections were not reaching DA terminal areas can be refuted, as AMPH injections in the N.Acc were found to increase locomotor activity (Experiment #4a), an effect mediated by N.Acc DA (Salamone et al., 1997; Di Chiara, 1995; Evans & Vaccarino, 1986). That the injection sites were, in fact, capable of eliciting increased feeding behaviour was verified in Experiment #4c, wherein both muscimol and morphine stimulated intake.

PFH NPY-stimulated sucrose consumption is DA-independent, as injections of FLU into the N.Acc failed to attenuate this response. That the FLU injections were, in fact, capable of inhibiting other DA-mediated behaviour was ascertained by the finding that the same injections into the N.Acc attenuated peripheral AMPH-stimulated locomotor activity (Experiment #4b). Although DA does not appear to play a significant role in the NPY feeding response, this does not preclude the possibility that NPY is acting in conjunction with (an)other neurotransmitter system(s). One candidate is the endogenous opioid system, as the administration of opioid antagonists in satiated

(Borisova, Kadar, & Telegdy, 1991; Kotz, Grace, Briggs, Levine, & Billington, 1995) or food-deprived (Schick, Schusdziarra, Nussbaumer, & Classen, 1991) rats attenuates icv NPY-induced feeding. More specifically, PVN NPY-induced feeding is reduced to baseline levels following injections of naltrexone into the nucleus of the solitary tract, indicating that opioidergic pathways in this area may underlie NPY's orexigenic effects.

The propensity to ingest sucrose following treatments which increase N.Acc DA transmission is dependent on individual differences in endogenous N.Acc DA activity, with, for example, AMPH facilitating sugar consumption in low baseline feeders while inhibiting it in high feeders (Sills et al., 1993; Sills, 1994). The possibility exists, therefore, that the lack of effects seen following NPY administration might reflect the equal distribution of rats across these two types of baseline feeders. The data were examined for this possibility by performing a median split on N.Acc rats in Experiment #2a and analysing their 1 hr sucrose intake, however, and no distinctive relationship between the two (baseline intake and NPY-stimulated intake) was evident ($F(1, 8) = .82$, $p > .10$).

NPY's involvement in reward processes

PR responding for sucrose reward pellets:

The PR operant schedule is a measure that determines an organism's motivation to respond for rewarding stimuli (Roberts & Richardson, 1992). In Experiment #3a, NPY injected into the PFH, but not the N.Acc, was found to dose-dependently increase PR responding for sucrose reward pellets. Peripheral DA blockade with FLU inhibited the PFH NPY-induced increase in PR responding as effectively as it did drug-free responding, indicating that distinct DA-mediated, reward-related effects of NPY are improbable.

These results can be seen as agreeing with the results of a study where raclopride, a D₂/D₃ antagonist, dose-dependently decreased drug-free PR performance in animals responding for 95% sucrose pellets (Cheeta, Brooks, & Willner, 1995). Our data are also consistent with the finding that DA antagonism impairs PR responding maintained by cocaine infusions (Roberts, 1992). One explanation for these results is that DA antagonism impairs motor performance. That possibility seems unlikely, however, since DA antagonism has been shown to increase responding on a continuous or fixed reinforcement schedule when very sweet reinforcers are used (Phillips, Willner, & Muscat, 1991) or when the dose of cocaine used lies on the descending limb of a dose-response curve (Roberts & Vickers, 1984). Furthermore, the doses of FLU tested in Experiment #3b were previously shown to have no effect on spontaneous locomotor activity (Ahlenius, Hillegaart, Thorell, Magnusson, & Fowler, 1987). Our finding that the lowest dose of FLU tested had greater attenuating effects on drug-free vs. NPY-induced responding suggests that partial DA antagonism may be overpowered by an

appropriately strong motivational stimulus, such as NPY.

Our finding that FLU dose-dependently decreased NPY-induced PR operant responding, but had no effect on NPY-induced free-feeding of either chow (Experiments #1b and #1c) or sucrose (Experiment #2b), suggests that DA's involvement in feeding behaviour may be limited to components of that behaviour which are only activated during operant responding. In other words, DA may contribute to the performance of behaviours that are anticipated or learned, such as operant responding, while being less involved in primarily unlearned behaviours such as feeding. This conclusion is in agreement with the finding that DA levels in the N.Acc increase during instrumental performance for food but not during free food consumption (Salamone et al., 1994). These authors concluded that increases in N.Acc DA which accompany operant responding may facilitate the ability of an organism to overcome obstacles that separate it from significant stimuli. It is clear from the present studies that DA is involved in PR operant responding; however, it does not appear to have any effects that are specific to NPY's actions on such behaviour.

NPY and conditioned place preference:

The administration of a low dose (24 pmol/side) of NPY into the N.Acc resulted in a significant CPP. This effect paralleled the CPP produced by peripheral AMPH (2.5 mg/kg). The same dose of NPY injected into the PFH also appeared to result in a CPP; however, it did not reach statistical significance when a two-tailed t -test was used. If more animals had been tested, or if we had been able to make a prediction regarding the direction of the results for injections into the PFH, it is possible that this effect would

have reached statistical significance. A medium dose of NPY (156 pmol/side) into either brain site failed to have any observed effects on place conditioning, indicating that NPY has rewarding effects in both brain structures only when it is given in a low dose. One explanation may be that the 156 pmol/side dose produced a spread of effect to other adjacent areas, counteracting the CPP effects seen with the low (24 pmol/side) dose. It is interesting to note that Moore, Merali and Beninger (1994) found that 24 pmol (0.1 µg) of NPY, but not 235 pmol (1.0 µg), resulted in significant contralateral turning behaviour when it was injected into the striatum, an effect that was blocked by co-administration of FLU. This supports the notion that NPY's effects on the mesolimbic DA system occur in a limited dose range.

The nature of the CPP produced by NPY in the N.Acc, as opposed to that produced by NPY in the PFH, seems to be different. A negative correlation was found between NPY-induced chow intake and time spent on the drug-paired side of the conditioning apparatus for animals that had been injected with 24 pmol of NPY in the PFH, while no such correlation existed for animals injected with the same dose in the N.Acc. This finding may indicate that the rewarding effects of low doses of NPY in the PFH are overshadowed when feeding effects occur concurrently. Hunger has been shown to result in a CPA (Harrington & van der Kooy, 1992; Bechara & van der Kooy, 1992), and would therefore be expected to decrease any rewarding effects of NPY. That no effect of NPY on CPP was observed following the administration of the 156 pmol dose in the PFH can be accounted for in the same manner. It is possible that NPY at higher doses also has rewarding properties, but that either its potent orexigenic effects or other non feeding-related effects obscure these. This type of result has been seen in CPP

tests with other compounds, where a CPP is evident following a low dose, but disappears when a higher dose is used. For example, dorsal raphe injections of a low dose of the orexigenic agent 8-OH-DPAT result in a CPP, while a dose 10 times greater in size had no effect (Fletcher, Ming, & Higgins, 1993). These authors speculated that the hypotensive and hypothermic effects of the higher dose of 8-OH-DPAT might overshadow its rewarding properties, preventing the development of a CPP. It is possible that the orexigenic effects of NPY, or its corollary effects on hypotension (Härfstrand, 1986) and hypothermia (Okita et al., 1990) play a similar role. It is interesting to note that the hypotensive effects of NPY disappear when animals are allowed to eat (Härfstrand, 1986), a condition that was not established during CPP testing in the present study. Furthermore, NPY injected at a dose that results in increased food intake also generates a robust conditioned flavour aversion (Sipols, Brief, Ginter, Saghafi, and Woods, 1992). These authors interpreted this, apparently paradoxical, effect of NPY to be indicative of different populations of central NPY receptors having dissimilar effects on ingestive behaviours. Alternatively, the authors suggest that stimuli that induce excessive amounts of food ingestion may be inherently aversive. Regardless, it is clear from our results that any potentially rewarding effects of PFH NPY do not depend on its effects on feeding.

Although confirming the results of Josselyn and Beninger (1993), the finding that NPY results in a CPP when injected in low doses into the N.Acc, as well as the apparent finding that a low dose of NPY seems to result in a CPP when injected into the PFH, needs to be interpreted with caution. Experiment #3d was designed to assess the effects of peripheral DA antagonism on the NPY CPP. However, the results of Experiment #3c were not replicated, so it was impossible to assess whether DA-ergic mechanisms are

involved in the CPP response. These results, therefore, provide no support for those of Josselyn and Beninger, who found that intra-accumbens FLU blocked the CPP produced by N.Acc NPY. One conclusion that can be drawn from the present study is that the effects of NPY on CPP are fragile. More carefully controlled experimentation is needed before any conclusive statements regarding NPY's rewarding effects, and the contribution of DA to said effects, can be made. Our initial finding of a CPP in N.Acc NPY-treated rats replicates the work of Josselyn et al. (1993) and, therefore, implies that this effect is genuine.

The CPP effect of a low dose of NPY in the PFH or the N.Acc could not be replicated in Experiment #3d. That methodological differences could account for NPY's lack of effect on CPP is unlikely, as all conditions and procedures remained constant throughout Experiments #3c and #3d. While great care was taken while executing all experiments, it remains possible that some subtle factor, such as the time of year at which the test was conducted, or the different cohort of animals used, may have affected the results. Negative results are notably difficult to interpret, however, and this has frequently been raised as one of the main disadvantages of the CPP paradigm (Carr et al., 1989).

NPY's involvement in eating disorders and substance abuse: A revised hypothesis

The finding that NPY levels are disrupted in certain eating disordered populations (Kaye et al., 1990), and that its administration in animals results in voracious feeding behaviour (Brown & Coscina, 1995; Stanley, 1993), combined with the finding that NPY has rewarding effects of its own (Josselyn & Beninger, 1993), led to the proposition that the NPY system may underlie a common diathesis that predisposes individuals towards developing eating and substance abuse disorders. While the collective results of the present thesis do not support a role for NPY in non-regulatory and reward-related behaviours, there remains the possibility that other properties of NPY are common to both disorders. NPY's anxiolytic effects, and its apparent involvement in depressive and anxiety disorders, may be such a property.

NPY has anxiolytic effects in animal models of anxiety such as Montgomery's conflict test and Vogel's drinking conflict test (Heilig et al., 1989). As well, icv NPY and its C-terminal fragments dose-dependently increase preference for the open arms in the elevated plus-maze and inhibit fear-potentiated startle, effects that are likely mediated by Y1 receptors (Broqua et al., 1995). One site of action of NPY's anxiolytic effects appears to be the central nucleus of the amygdala, as injections there reproduce the effects of icv NPY on the elevated plus-maze (Wahlestedt & Heilig, 1995). NPY given icv to olfactory bulbectomized rats, an animal model of depression, results in the reduction of certain depressive symptoms (Song et al., 1996), while cocaine withdrawal, which also produces depressive symptoms, results in decreased N.Acc and cortex levels of NPY (Wahlestedt et al., 1991). These observations led to the proposal that NPY may be involved in the pathophysiology of major depression, a condition that is often

accompanied by heightened levels of anxiety.

Reduced plasma (Nilsson et al., 1996) and CSF (Widerlöv et al., 1988) NPY immunoreactivity is evident in depressed patients, and marked reductions in tissue levels of NPY immunoreactivity are seen in the brains of suicide victims, particularly those with a verified diagnosis of major depression (Widdowson, Ordway, & Halaris, 1992). Plasma levels of NPY are negatively correlated with anxiety symptoms in depressed patients (Widerlöv et al., 1989), indicating that lower levels of this peptide are observed in depressed patients with more severe levels of anxiety. In addition, increased levels of NPY immunoreactivity are found in rat brain tissue after chronic treatment with tricyclic antidepressants (Heilig, Wahlestedt, Ekman, & Widerlöv, 1988).

Lifetime prevalence rates for affective disturbances in bulimic subjects are reported to be as high as 88% (Walsh et al., 1985). Associations have also been noted between depression and substance abuse (Deykin et al., 1986), including alcoholism (Weissman & Myers, 1980). Antidepressant medications are effective in treating certain eating disorders, especially bulimia (Kennedy & Goldbloom, 1991), as well as being effective in the treatment of substance abuse (Batki, Manfredi, Jacob, & Jones, 1993; Kosten et al., 1992). It is possible that, due to its involvement in anxiety and depression, NPY is decreased in those individuals predisposed to developing substance abuse and/or eating disorders. The elevated levels of NPY seen in underweight and weight-restored anorexics (Kaye et al., 1990) may reflect the 'post-treatment' effects of food restriction and weight loss. This explanation is consistent with the self-medication hypothesis of eating disorders and substance abuse, wherein patients are hypothesised to be self-treating their underlying symptoms of depression (Krahn, 1991). It would be interesting

to measure CSF NPY levels in individuals experiencing substance abuse problems. Following the above line of reasoning, those people who are currently abusing substances should show an increase in NPY immunoreactivity, while those who are predisposed to abusing substances but have not yet started to use, or those who are in withdrawal, should exhibit a decrease in NPY levels. This would parallel the finding that NPY levels are significantly reduced in the brains of rats given repeated administrations of cocaine over a two week period (Wahlestedt et al., 1991), an effect believed to reflect the anxiety and depression commonly associated with cocaine withdrawal in humans.

General Conclusions

The present thesis has provided support for the following hypotheses that were outlined in the Introduction (Table 1) and are presented in Table 2:

1. **NPY injected into the PFH is involved primarily in regulatory feeding, as intake of a preferred food type (sucrose) did not increase preferentially as compared to chow intake following NPY administration. Furthermore, although PFH NPY produced a slight increase in PR responding for sucrose, this effect was likely due to its involvement in regulatory feeding, as DA antagonism with FLU failed to differentially decrease NPY-, compared to drug-free, responding.**
2. **DA does not mediate the effects of PFH NPY on regulatory feeding, as the administration of FLU did not block intake of either chow or sucrose. NPY's orexigenic effects are likely due either to its effects on its own receptor system and/or to its effects on another neurotransmitter system.**
3. **NPY has no effects on feeding or on PR responding when it is injected into the N.Acc.**
4. **NPY may have rewarding effects in the N.Acc at a low dose, as the administration of 24 pmol/side of NPY resulted in a CPP. In the PFH, NPY produced an apparent, yet statistically non-significant, CPP. Since these results could not be replicated in a second study, the effects appear to be of a precarious nature.**

5. **NPY in the N.Acc and the PFH at a higher dose (156 pmol/side) does not have rewarding effects, as evidenced by the lack of effect on CPP.**

In summary, the effects of NPY in the PFH and the N.Acc seem to represent a double-dissociation. The PFH underlies NPY's effects on regulatory feeding but has limited effects, if any, on reward-relevant behaviour. Conversely, NPY in the N.Acc can support reward-related, but not feeding, effects. DA does not contribute to NPY's regulatory feeding response in the PFH, while its involvement in the rewarding effects of NPY in the N.Acc is equivocal.

References

Alheid, G.F., McDermott, F.L., Kelly, J., Halaris, A., & Grossman, S.P. (1977). Deficits in food and water intake after knife cuts that deplete striatal DA or hypothalamic NE in rats. Pharmacology, Biochemistry and Behavior, 6, 273-287.

Ahlenius, S., Hillegaard, V., Thorell, G., Magnusson, O., & Fowlet, C.J. (1987). Suppression of exploratory locomotor activity and increase in dopamine turnover following the local application of *cis*-flupenthixol into limbic projection areas of the rat striatum. Brain Research, 402, 131-138.

Allen, Y.S., Adrian, R.E., Allen, J.M., Tatemoto, K., Crow, T.J., Bloom, S.R., & Polak, J.M. (1983). Neuropeptide Y distribution in the rat brain. Science, 221, 877-879.

American Psychiatric Association. (1994). Diagnostic and statistical manual of mental disorders (4th ed.). Washington, DC: Author.

Antelman, S.M., Szechtman, H., Chin, P., & Fisher, A.E. (1975). Tail pinch-induced eating, gnawing and licking behavior in rats: Dependence on the nigrostriatal dopamine system.

Aoki, C. & Pickel, M.V. (1988). Neuropeptide Y-containing neurons in the rat striatum: ultrastructure and cellular relations with tyrosine hydroxylase containing

terminals and with astrocytes. Brain Research, 459, 205-225.

Arnt, J. (1985). Antistereotypic effects of dopamine D₁ and D₂ antagonists after striatal injection in rats. Pharmacological and regional specificity. Naunyn-Schmiedeberg's Archives of Pharmacology, 333, 97-104.

Ault, D.T., Radeff, J.M., & Werling, L.L. (1998). Modulation of [³H]dopamine release from rat nucleus accumbens by neuropeptide Y may involve a *sigma*₁-like receptor. The Journal of Pharmacology and Experimental Therapeutics, 284, 553-560.

Ault, D.T., & Werling, L.L. (1997). Differential modulation of NMDA-stimulated [³H]dopamine release from rat striatum by neuropeptide Y and σ receptor ligands. Brain Research, 760, 210-217.

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

Batki, S.L., Manfredi, L.B., Jacob, P., & Jones, R.T. (1993). Fluoxetine for cocaine dependence in methadone-maintenance: quantitative plasma and urine cocaine/benzoylecgonine concentration. Journal of Clinical Psychopharmacology, 13, 243-250.

Beal, M.F., Frank, R.C., Ellison, D.W., & Martin, J.B. (1986). The effect of

neuropeptide Y on striatal catecholamines. Neuroscience Letters, 71, 118-123.

Bechara, A., & van der Kooy, D. (1992). A single brain stem substrate mediates the motivational effects of both opiates and food in nondeprived rats but not in deprived rats. Behavioral Neuroscience, 106, 351-363.

Berridge, K.C. (1991). Modulation of taste affect by hunger, caloric satiety, and sensory-specific satiety in the rat. Appetite, 16, 103-120.

Blackburn, J.R., Phillips, A.G., & Fibiger, H.C. (1989). Dopamine and preparatory behavior: III. Effects of metoclopramide and thioridazine. Behavioral Neuroscience, 4, 903-906.

Blackburn, J.R., Phillips, A.G., Jakubovic, A., & Fibiger, H.C. (1986). Increased dopamine metabolism in the nucleus accumbens and striatum following consumption of a nutritive meal but not a palatable non-nutritive saccharin solution. Pharmacology, Biochemistry and Behavior, 25, 1095-1100.

Blum, W.F. (1997). Leptin: The voice of the adipose tissue. Hormone Research, 48(suppl.), 2-8.

Bond, J.A., Walker, M.W., Branched, T.A., & Weinshank, R.L. (1995). Cloning and functional expression of a human Y4 subtype receptor from pancreatic polypeptide,

neuropeptide Y and peptide YY. Journal of Biological Chemistry, 270, 26762-26765.

Booth, D.A., Gibson, E.L., Toase, A., & Freeman, R.P.J. (1994). Small objects of desire: The recognition of appropriate foods and drinks and its neural mechanisms. In C.R.Legg and D.A. Booth (Eds), Appetite: Neural and Behavioral Bases (pp. 98-126). New York: Oxford Science Publications.

Borisova, E.V., Kadar, T., & Telegdy, G. (1991). Bimodal effect of neuropeptide Y on feeding, and its antagonism by receptor blocking agents in rats. Acta Physiologica Hungarica, 78, 301-308.

Bozarth, M.S., & Wise, R.A. (1986). Involvement of the ventral tegmental dopamine system in opioid and psychomotor stimulant reinforcement. In L.S. Harris (Ed.), Problems of drug dependence, 1985 (pp. 190-196). NIDA Research Monograph 55, DHS pub. no. (ADM) 85-1393. Washington, D.C.: Government Printing Office.

Brazell, M.P., Mitchell, S.N., Joseph, M.H., & Gray, J.A. (1990). Acute administration of nicotine increases the in vivo extracellular levels of dopamine, 3,4-dihydroxyphenylacetic acid and ascorbic acid preferentially in the nucleus accumbens of the rat: comparison with caudate-putamen. Neuropharmacology, 29, 1177-1185.

Broqua, P., Wettstein, J.G., Rocher, M.N., Gauthier-Martin, B., Junien, J.L. (1995). Behavioral effects of neuropeptide Y receptor agonists in the elevated plus-maze

and fear-potentiated startle procedures. Behavioural Pharmacology, 6, 215-222.

Brown, C.M. (1993). Effects of serotonin on neuropeptide Y-induced feeding when injected into the paraventricular nucleus and the perifornical hypothalamus. Canadian (Master's) Theses, National Library of Canada (Ottawa).

Brown, C.M., & Coscina, D.V. (1995). Ineffectiveness of hypothalamic serotonin to block neuropeptide Y-induced feeding. Pharmacology, Biochemistry and Behavior, 641-646.

Butterfield, P.S., & LeClair, S. (1988). Cognitive characteristics of bulimic and drug-abusing women. Addictive Behaviors, 13, 131-138.

Cabanac, M., Dagnault, A., & Richard, D. (1997). The food-hoarding threshold is not raised by acute intraventricular NPY in male rats. Physiology and Behavior, 61, 131-135.

Carlat, D.J., Camargo, C.A., & Herzog, D.B. (1997). Eating disorders in males: A report on 135 patients. American Journal of Psychiatry, 154, 1127-1132.

Carr, G.D., Fibiger, H.C., & Phillips, A.G. (1989). Conditioned place preference as a measure of drug reward. In J.M. Liebman & S.J. Cooper (Eds.), The Neuropharmacological Basis of Reward (pp. 264-319). New York: Oxford University

Press.

Cheeta, S., Brooks, S., & Willner, P. (1995). Effects of reinforcer sweetness and the D2/D3 antagonist raclopride on progressive ratio operant performance. Behavioural Pharmacology, 6, 127-132.

Cheng, J.T., Chang, C.L., & Tsai, C.L. (1992). Inhibitory effects of neuropeptide Y (NPY) on the in vitro activity of dopamine- β -hydroxylase. Neuroscience Letters, 136, 36-38.

Chronwall, B.M., DiMaggio, D.A., Massari, V.J., Pickel, V.M., Ruggiero, D.A., & O'Donohue, T.L. (1985). The anatomy of neuropeptide-Y-containing neurons in rat brain. Neuroscience, 15, 1159-1181.

Clark, J.T., Kalra, P.S., Crowley, W.R., & Kalra, S.P. (1984). Neuropeptide Y and human pancreatic polypeptide stimulate feeding behavior in rats. Endocrinology, 115, 427-429.

Clark, J.T., Kalra, P.S., & Kalra, S.P. (1985). Neuropeptide Y stimulates feeding but inhibits sexual behavior in rats. Endocrinology, 117, 2435-2442.

Cooper, S.J., & Higgs, S. (1994). Neuropharmacology of appetite and taste preferences. In C.R.Legg and D.A. Booth (Eds), Appetite: Neural and Behavioral Bases

(pp. 212-242). New York: Oxford Science Publications.

Cotton, N. (1979). The familial incidence of alcoholism. Journal of Studies on Alcohol, 40, 89-116.

Crisp, A.H. (1968). Primary anorexia nervosa. Gut, 9, 370-372.

Crisp, A.H., Palmer, R.L., & Kalucy, R.S. (1976). How common is anorexia nervosa? A prevalence study. British Journal of Psychiatry, 128, 49-54.

Currie, P.J., & Coscina, D.V. (1995). Dissociated feeding and hypothermic effects of neuropeptide Y in the paraventricular and perifornical hypothalamus. Peptides, 16(4), 599-604.

Currie, P.J., & Coscina, D.V. (1996). Regional hypothalamic differences in neuropeptide Y-induced feeding and energy substrate utilization. Brain Research, 737, 238-242.

Deykin, E., Levy, J., & Wells, V. (1986). Adolescent depression, alcohol and drug abuse. American Journal of Public Health, 76, 178-182.

Di Chiara, G. (1995). The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug and Alcohol Dependence, 38, 95-137.

Di Chiara, G., & Imperato, A. (1985). Ethanol preferentially stimulates dopamine release in the nucleus accumbens of freely moving rats. European Journal of Pharmacology, 115, 131-132.

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

Drewnowski, A., Yee, D., & Krahn, D.D. (1988). Bulimia on campus: Incidence and recovery rates. American Journal of Psychiatry, 6, 753-755.

Dube, M.G., Xu, B., Crowley, W.R., Kalra, P.S., & Kalra, S.P. (1994). Evidence that neuropeptide Y is a physiological signal for normal food intake. Brain Research, 646, 341-344.

Egawa, M., Yoshimatsu, H., & Bray, G.A. (1991). Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats. American Journal of Physiology, 260, R328-R334.

Ettenberg, A., Koob, G.F., & Bloom, F.E. (1981). Response artifact in the measurement of neuroleptic-induced anhedonia. Science, 213, 357-359.

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

Evans, K.R., & Vaccarino, F.J. (1987). Effects of d- and l-amphetamine on food intake: evidence for a dopaminergic substrate. Pharmacology, Biochemistry and Behavior, 27, 649-652.

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

Fletcher, P.J. (1991). Dopamine receptor blockade in nucleus accumbens or caudate nucleus differentially affects feeding induced by 8-OH-DPAT injected into dorsal or median raphe. Brain Research, 552, 181-189.

Fletcher, P.J., Ming, Z.H., & Higgins, G.A. (1993). Conditioned place preference induced by microinjection of 8-OH-DPAT into the dorsal or median raphe nucleus. Psychopharmacology, 113, 31-36.

Flood, J.F., & Morley, J.E. (1991). Increased food intake by neuropeptide Y is due to an increased motivation to eat. Peptides, 12, 1329-1332.

Fouriezos, G., & Wise R.A. (1976). Pimozide-induced extinction of intracranial self-stimulation: Response patterns rule out motor or performance deficits. Brain Research, 103, 377-380.

Franklin, J.C., Schiele, B.C., Brozek, J., & Keys, A. (1948). Observations on human behavior in experimental semistarvation and rehabilitation. Journal of Clinical Psychology, 4, 28-45.

Gallistel, C.R., & Karras, D. (1984). Pimozide and amphetamine have opposing effects in the reward summation function. Pharmacology, Biochemistry and Behavior, 20, 73-77.

Garfinkel, P.E., Moldofsky, H., & Garner, D.M. (1980). The heterogeneity of anorexia nervosa. Archives of General Psychiatry, 37, 1036-1040.

Gehlert, D.R., & Heiman, M. (1997). Obesity: Leptin-Neuropeptide Y interactions in the control of body weight. Annual Reports in Medicinal Chemistry, 32, 21-30.

Gerald, C., Walker, M.W., Criscione, L., Gustafson, E.L., Batzi-Hartmann, C., Smith, K.E., Vaysse, P., Durkin, M.M., Laz, T.M., Linemeyer, D.L., Schaffhauser, A.O., Whitebread, S., Hofbauer, K.G., Taber, R.I., Branchek, T.A., & Weinshank, R.L. (1996).

A receptor subtype involved in neuropeptide-Y-induced food intake. Nature, 382, 168-171.

Gillard, E.R., Dang, D.Q., & Stanley, B.G. (1993). Evidence that neuropeptide Y and dopamine in the perifornical hypothalamus interact antagonistically in the control of food intake. Brain Research, 628, 128-136.

Glickman, S.E., & Schiff, B.B. (1967). A biological theory of reinforcement. Psychological Review, 74, 81-109.

Goodman, J.H., & Sloviter, R.S. (1993). Cocaine neurotoxicity and altered neuropeptide Y immunoreactivity in the rat hippocampus; a silver degeneration and immunocytochemical study. Brain Research, 616, 263-272.

Gray, T.S., O'Donohue, T.L., & Magnuson, D.J. (1986). Neuropeptide Y innervation of amygdaloid and hypothalamic neurons that project to the dorsal vagal complex in rat. Peptides, 7, 341-349.

Grilo, C.M., Becker, D.F., Levy, K.N., Walker, M.L., Edell, W.S., & McGlashan, T. (1995). Eating disorders with and without substance use disorders: A comparative study of inpatients. Comprehensive Psychiatry, 36, 312-317.

Härfstrand, A. (1986). Intraventricular administration of neuropeptide Y (NPY)

induces hypotension, bradycardia and bradypnoea in the awake unrestrained male rat. Counteraction by NPY-induced feeding behaviour. Acta Physiologica Scandinavica, **128**, 121-123.

Harrington, F., & van der Kooy, D. (1992). Deprivation state determines the motivational effects of neuroleptics in rats. Psychobiology, **20**, 294-299.

Heilig, M., Söderpalm, B., Engel, J.A., & Widerlöv, E. (1989). Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. Psychopharmacology, **98**, 524-529.

Heilig, M., Vècséi, L., Wahlestedt, C., Alling, Ch., & Widerlöv, E. (1990). Effects of centrally administered neuropeptide Y (NPY) and NPY₁₃₋₃₆ on the brain monoaminergic systems of the rat. Journal of Neural Transmission, **79**, 193-208.

Heilig, M., Wahlestedt, C., Ekman, R., & Widerlöv, E. (1988). Antidepressant drugs increase the concentration of neuropeptide Y (NPY)-like immunoreactivity in the rat brain. European Journal of Pharmacology, **147**, 465-467.

Heilig, M., & Widerlöv, E. (1990). Neuropeptide Y: an overview of central distribution, functional aspects, and possible involvement in neuropsychiatric illnesses. Acta Psychiatrica Scandinavica, **82**, 95-114.

Hemby, S.E., Martin, T.J., Co, D., Dworkin, S.I., & Smith, J.E. (1995). The effects of intravenous heroin administration on extracellular nucleus accumbens dopamine concentrations as determined by *in vivo* microdialysis. Journal of Pharmacology and Experimental Therapeutics, 273, 591-598.

Hendry, S.H.C. (1993). Organization of neuropeptide Y neurons in the mammalian central nervous system. In W.F. Colmers & C. Wahlestedt (Eds.), The Biology of Neuropeptide Y and Related Peptides (pp.65-156). New Jersey: Humana Press.

Hernandez, L., & Hoebel, B.G. (1988). Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiology and Behavior, 44, 599-606.

Hiroi, N., & White, N.M. (1991). The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. Brain Research, 552, 141-152.

Hoebel, B. (1984). Neurotransmitters in the control of feeding and its rewards: Monoamines, opiates, and brain-gut peptides. In A.J. Stunkard & E. Stellar (Eds.), Eating and Its Disorders (pp. 15-38). New York: Raven Press.

Hoebel, B. (1985). Brain neurotransmitters in food and drug reward. American Journal of Clinical Nutrition, 42, 1133-1150.

Hökfelt, T., Millhorn, C., Seroogy, K., Tsuruo, Y., Ceccatelli, S., Lindh, B., Meister, B., Melander, T., Schalling, M., Bartfai, T., & Terenius, L. (1987). Coexistence of peptides with classical neurotransmitters. Experientia, 43, 768-780.

Holderness, C.C., Brooks-Gunn, J., & Warren, M.P. (1994). Co-morbidity of eating disorders and substance abuse: Review of the literature. International Journal of Eating Disorders, 16, 1-34.

Humphrey, L. (1986). Structural analysis of parent-child relationships in eating disorders. Journal of Abnormal Psychology, 95, 395-402.

Jaffe, J.J. (1990). Drug addiction and drug abuse. In A.G. Gilman, T.W. Rall, A.S. Nies, & P. Taylor (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics (pp. 522-573). New York: Pergamon Press Inc.

Jewett, D.C., Cleary, J., Levine, A.S., Schaal, D.W., & Thompson, T. (1992). Effects of neuropeptide Y on food-reinforced behavior in satiated rats. Pharmacology, Biochemistry and Behavior, 42, 207-212.

Jewett, D.C., Cleary, J., Levine, A.S., Schaal, D.W., & Thompson, T. (1995). Effects of neuropeptide Y, insulin, 2-deoxyglucose, and food deprivation on food-motivated behavior. Psychopharmacology, 120, 267-271.

Jewett, D.C., Schaal, D.W., Cleary, J., Thompson, T., & Levine, A.S. (1991). Neuropeptide Y (NPY) increases responding of rats under progressive ratio schedules of food reinforcement. Society for Neuroscience Abstracts, 17, 489.

Johnson, J., & Vickers, Z. (1992). Factors influencing sensory-specific satiety. Appetite, 19, 15-31.

Johnston, B.B. (1987). Treatment perspectives in drug misuse: The opiate paradigm. Pharmaceutical Therapies, 35, 359-374.

Josselyn, S.A., & Beninger, R.J. (1993). Neuropeptide Y: Intra-accumbens injections produce a place preference that is blocked by *cis*-flupenthixol. Pharmacology, Biochemistry, & Behavior, 46, 543-552.

Kalra, S.P., Dube, M.G., & Kalra, P.S. (1988). Continuous intraventricular infusion of neuropeptide Y evokes episodic food intake in satiated female rats: Effects of adrenalectomy and cholecystokinin. Peptides, 9, 723-728.

Katzman, M., & Marcus, I. (1991). Eating disorders and substance abuse in men. Journal of General Internal Medicine, 6, 382-383.

Kaye, W.H., Berrettini, W., Gwirtsman, H., & George, D.T. (1990). Altered

cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. Archives of General Psychiatry, 47, 548-556.

Kelly, P.H., Seviour, P.W., Iversen, S.D. (1975). Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Research, 94, 507-522.

Kennedy, S.H., & Goldbloom, D.S. (1991). Current perspectives on drug therapies for anorexia nervosa and bulimia nervosa. Drugs, 41, 367-377.

Kerkerian, L., Salin, P., & Nieoullon, A. (1988). Pharmacological characterization of dopaminergic influence on expression of neuropeptide Y immunoreactivity by rat striatal neurons. Neuroscience, 26, 809-817.

Kerkerian-Le Goff, Forni, C., Samuel, D., Bloc, A., Dusticier, N., & Nieoullon, A. (1992). Intracerebroventricular administration of neuropeptide Y affects parameters of dopamine, glutamate and GABA activities in the rat striatum. Brain Research Bulletin, 28, 187-193.

Kissileff, H.R. (1991). Chance and necessity in ingestive behavior. Appetite, 17, 1-22.

Kleiber, M. (1975). The Fire of Life: an Introduction to Animal Energetics.

New York: Robert R. Krieger.

Kling-Petersen, T., Ljung, E., & Svensson, K. (1994). The preferential dopamine autoreceptor antagonists (+)-UH232 antagonizes the positive reinforcing effects of cocaine and d-amphetamine in the ICSS paradigm. Pharmacology, Biochemistry and Behavior, 49, 345-351.

Kosten, T.A., Kosten, T.R., Gawin, F.H., Gordon, L.T., Hogan, I., & Kleber, H.D. (1992). An open trial of sertraline for cocaine abuse. American Addict, 1, 349-353.

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

Krahn, D.D. (1991). The relationship of eating disorders and substance abuse. Journal of Substance Abuse, 3, 239-253.

Kubota, Y., Inagake, S., Kito, S., Shimada, S., Okayama, T., Hatanaka, H., Pelletier, G., Takagi, H., & Tohyama, M. (1988). Neuropeptide Y-immunoreactive neurons receive synaptic inputs from dopaminergic axon terminals in the rat neostriatum. Brain Research, 458, 389-393.

Kyrkouli, S.E., Stanley, B.G., Hutchinson, R., Seirafi, R.D., & Leibowitz, S.F.

(1990). Peptide-amine interactions in the paraventricular hypothalamus: analysis of galanin and neuropeptide Y in relation to feeding. Brain Research, 521, 185-191.

Kyrkouli, S.E., Stanley, B.G., & Leibowitz, S.F. (1990). Neuropeptide Y: Comparisons with norepinephrine in relation to feeding behavior. Annals of the New York Academy of Sciences, 611, 527-528.

Lacey, E.H., & Evans, C.D.H. (1986). The impulsivist: A multi-impulsive personality disorder. British Journal of Addictions, 81, 641-649.

Larhammar, D. (1996). Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide. Regulatory Peptides, 62, 1-11.

Leibowitz, S.F. (1975). Catecholaminergic mechanisms of the lateral hypothalamus: Their role in the mediation of amphetamine anorexia. Brain Research, 98, 185-191.

Leibowitz, S.F. (1989). Hypothalamic neuropeptide Y, galanin, and amines: Concepts of coexistence in relation to feeding behavior. Annals of the New York Academy of Sciences, 575, 221-235.

Leibowitz, S.F. (1990). Hypothalamic neuropeptide Y in relation to energy balance. Annals of the New York Academy of Sciences, 611, 284-301.

Leibowitz, S.F., & Alexander, J.T. (1991). Analysis of neuropeptide Y-induced feeding: Dissociation of Y1 and Y2 receptor effects on natural meal patterns. Peptides, 12, 1251-1260.

Le Magnen, J. (1985). Hunger. Cambridge University Press: Cambridge.

Levine, A.S., Kuskowski, M.A., Grace, M., & Billington, C.J. (1991). Food deprivation-induced vs. drug-induced feeding: a behavioral evaluation. American Journal of Physiology, 260, R546-R552.

Levine, A.S., & Morley, J.E. (1984). Neuropeptide Y: A potent inducer of consummatory behavior in rats. Peptides, 5, 1025-1029.

Lynch, W.C., Hart, P., & Babcock, A.M. (1994). Neuropeptide Y attenuates satiety: evidence from a detailed analysis of patterns ingestion. Brain Research, 636, 28-34.

Maldonado-Irizarry, C.S., Stellar, J.R., & Kelley, A.E. (1994). Effects of cocaine and GBR-12909 on brain stimulation reward. Pharmacology, Biochemistry, and Behavior, 48, 915-920.

Mark, G.P., Smith, S.E., Rada, P.V., & Hoebel, B.G. (1994). An appetitively

conditioned taste elicits a preferential increase in mesolimbic dopamine release.

Pharmacology, Biochemistry and Behavior, 48, 651-660.

Martel, P., & Fantino, M. (1996). Influence of the amount of food ingested on mesolimbic dopaminergic system activity: A microdialysis study. Pharmacology, Biochemistry and Behavior, 55, 297-302.

Matos, F.F., Guss, V., & Korpinen, C. (1996). Effects of neuropeptide Y (NPY) and [D-Trp³²]NPY on monoamine and metabolite levels in dialysates from rat hypothalamus during feeding behavior. Neuropeptides, 30, 391-398.

McGregor, I.S., Atrens, D.M., & Jackson, D.M. (1992). Cocaine facilitation of prefrontal cortex self-stimulation: a microstructural and pharmacological analysis. Psychopharmacology, 106, 239-247.

Menéndez, J.A., McGregor, I.S., Healey, P.A., Atrens, D.M., & Leibowitz, S.F. (1990). Metabolic effects of neuropeptide Y injections into the paraventricular nucleus of the hypothalamus. Brain Research, 516, 8-14.

Michel, M.C. (1991). Receptors for neuropeptide Y: multiple subtypes and multiple second messengers. Trends in Pharmacological Sciences, 12, 392-394.

Miner, J.L. (1992). Recent advances in the central control of intake in ruminants.

Journal of Animal Science, 70, 1283-1289.

Mittleman, G., Rushing, P.A., & Winders, S.E. (1993). Effects of phenylpropanolamine on regulatory and nonregulatory ingestion in adult rats. Physiology and Behavior, 54, 515-521.

Mittleman, G., Castañeda, E., Robinson, T.E., & Valenstein, E.S. (1986). The propensity for nonregulatory ingestive behavior is related to differences in dopamine systems: Behavioral and biochemical evidence. Behavioral Neuroscience, 100, 213-220.

Moltz, J.H., & McDonald, J.K. (1985). Neuropeptide Y: direct and indirect action on insulin secretion in the rat. Peptides, 6, 1155-1159.

Moore, E. (1990). Effects of intrastriatal neuropeptide Y and amphetamine on circling behavior in the rat. Unpublished master's thesis, Queen's University, Kingston, Ontario, Canada.

Moore, E., Merali, Z., & Beninger, R.J. (1990). Neuropeptide Y and behaviour: Effects of intrastriatal NPY on circling behaviour of rats. Polish Journal of Pharmacology and Pharmaceuticals, 42, 521-525.

Moore, E., Merali, Z., & Beninger, R.J. (1994). Neuropeptide Y: Intrastriatal injections produce contralateral circling that is blocked by a dopamine antagonist in rats.

Pharmacology, Biochemistry and Behavior, **48**, 681-688.

Morris, Y.A., & Crews, D. (1990). The effects of exogenous neuropeptide Y on feeding and sexual behavior in the red-sided garter snake (*Thamnophis sirtalis parietalis*). Brain Research, **530**, 339-341.

Myers, R.D., Lankford, M.F., & Paez, X. (1992). Norepinephrine, dopamine, and 5-HT release from perfused hypothalamus of the rat during feeding induced by Neuropeptide Y. Neurochemical Research, **17**, 1123-1132.

Myers, R.D., Lankford, M.F., & Roscoe, A.K. (1996). Neuropeptide Y perfused in the preoptic area of rats shifts extracellular efflux of dopamine, norepinephrine, and serotonin during hypothermia and feeding. Neurochemical Research, **21**, 637-648.

Nader, K., Bechara, A., & van der Kooy, D. (1997). Neurobiological constraints on behavioral models of motivation. Annual Reviews in Psychology, **48**, 85-114.

Nakajima, M., Inui, A., Okita, M., Hirose, Y., Miura, M., Himori, N., & Baba, S. (1990). Effects of neuropeptide Y and its related peptides on feeding and learning behaviors in the mouse. Annals of the New York Academy of Sciences, **611**, 486-488.

Nilsson, C., Karlsson, G., Blenow, K., Heilig, M., & Ekman, R. (1996). Differences in the neuropeptide Y-like immunoreactivity of the plasma and platelets of

human volunteers and depressed patients. Peptides, 17, 359-362.

Okita, M., Inui, A., Miura, M., Hirose, Y., Nakajima, M., & Baba, S. (1990). Central actions of neuropeptide Y and its related peptides in the dog, with special reference to their effects on the hypothalamic-pituitary-adrenal axis, feeding behavior, and thermoregulation. Annals of the New York Academy of Sciences, 611, 483-485.

Paez, X., & Myers, R.D. (1991). Insatiable feeding evoked in rats by recurrent perfusion of neuropeptide Y in the hypothalamus. Peptides, 12, 609-616.

Paez, X., Nyce, J.W., & Myers, R.D. (1991). Differential feeding responses evoked in the rat by NPY and NPY(1-27) injected intracerebroventricularly. Pharmacology, Biochemistry & Behavior, 38, 379-384.

Paxinos, G., & Watson, C. (1986). The Rat Brain in Stereotaxic Coordinates (2nd ed.). Orlando: Academic Press, Inc.

Pellegrino, L.J., Pellegrino, A.S., & Cushman, A.J. (1979). A Stereotaxic Atlas of the Rat Brain. New York: Plenum Press.

Phillips, A.G., Atkinson, L.J., Blackburn, J.R., & Blaha, C.D. (1993). Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditional stimulus for food: an electrochemical study. Canadian Journal of Physiology and

Pharmacology, 71, 387-393.

Phillips, A.G., & Nikaido, R.S. (1975). Disruption of brain stimulation-induced feeding by dopamine receptor blockade. Nature, 258, 750-751.

Phillips, G., Willner, P., & Muscat, R. (1991). Suppression or facilitation of operant behaviour by raclopride dependent on concentration of sucrose reward. Psychopharmacology, 105, 239-246.

Pich, E.M., Messori, B., Zoli, M., Ferraguri, F., Marrama, P., Biagini, G., Fuxe, K., & Agnati, L. (1992). Feeding and drinking responses to neuropeptide Y injections in the paraventricular nucleus of aged rats. Brain Research, 375, 265-271.

Remesar, X., Rafecas, I., Fernández-López, J.A., & Alemany, M. (1997). Leptin. Medicinal Research Reviews, 17, 225-234.

Richardson, N.R., & Gratton, A. (1996). Behavior-relevant changes in nucleus accumbens dopamine transmission elicited by food reinforcement: An electrochemical study in rat. Journal of Neuroscience, 16, 8160-8169.

Richardson, N.R., & Roberts, D.C.S. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. Journal of Neuroscience, 66, 1-11.

Roberts, D.C.S. (1989). Breaking points on a progressive ratio schedule reinforced by intravenous apomorphine increase daily following 6-hydroxydopamine lesions of the nucleus accumbens. Pharmacology, Biochemistry & Behavior, 32, 43-47.

Roberts, D.C.S. (1992). Neural substrates mediating cocaine reinforcement: The role of monoamine systems. In J.M. Lakoski, M.P. Galloway & F.J. White (Eds.), Cocaine: Pharmacology, Physiology, and Clinical Strategies (pp.73-90). Boca Raton: CRC Press.

Roberts, D.C.S., & Bennett, S.A.L. (1993). Heroin self-administration in rats under a progressive ratio schedule of reinforcement. Psychopharmacology, 111, 215-218.

Roberts, D.C.S., & Richardson, N.R. (1992). Self-administration of psychomotor stimulants using progressive ratio schedules of reinforcement. In A. Boulton, G. Baker, & P.H. Wu (Eds.), Neuromethods, Vol.24: Animal Models of Drug Addiction (pp.233-269). New Jersey: Humana Press.

Roberts, D.C.S., & Vickers, G.J. (1984). Atypical neuroleptics increase self-administration of cocaine: An evaluation of a behavioral screen for antipsychotic drug activity. Psychopharmacology, 82, 135-139.

Rolls, E.T. (1975). The Brain and Reward. Oxford: Pergamon Press.

Rowland, N.E. (1988). Peripheral and central satiety factors in neuropeptide Y-induced feeding in rats. Peptides, 9, 989-992.

Sahu, A., Kalra, P.S., & Kalra, S.P. (1988). Food deprivation and ingestion induce reciprocal changes in neuropeptide Y concentrations in the paraventricular nucleus. Peptides, 9, 83-86.

Salamone, J.D., Cousins, M.S., McCullough, L.D., Carriero, D.L., & Berkowitz, R.J. (1994). Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. Pharmacology, Biochemistry and Behavior, 49, 25-31.

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

Salin, P., Kerkerian, L., & Nieoullon, A. (1990). Expression of neuropeptide Y immunoreactivity in the rat nucleus accumbens is under the influence of the dopaminergic mesencephalic pathway. Experimental Brain Research, 81, 363-371.

Sargent, J., Liebman, R., & Silver, M. (1985). Family therapy for anorexia

nervosa. In D. Garner & P. Garfinkel (Eds.), Handbook of psychotherapy for anorexia and bulimia (pp. 280-307). New York: Guilford Press.

Schaffhauser, A.O., Stricker-Krongrad, A., Brunner, L., Cumin, F., Gerald, C., Whitebread, S., Criscione, L., & Hofbauer, K.G. (1997). Inhibition of food intake by neuropeptide Y Y5 receptor antisense oligodeoxynucleotides. Diabetes, 46, 1792-1798.

Schick, R.R., Schusdziarra, V., Nussbaumer, C., & Classen, M. (1991). Neuropeptide Y and food intake in fasted rats: effect of naloxone and site of action. Brain Research, 552, 232-239.

Seeley, R.J., Benoit, S.C., & Davidson, T.L. (1995). Discriminative cues produced by NPY do not generalize to the interoceptive cues produced by food deprivation. Physiology and Behavior, 58, 1237-1241.

Seeley, R.J., Payne, C.J., & Woods, S.C. (1995). Neuropeptide Y fails to increase intraoral intake in rats. American Journal of Physiology, 268, R423-R427.

Selby, M.J., & Moreno, J.K. (1995). Personal and familial substance misuse patterns among eating disordered and depressed subjects. The International Journal of the Addictions, 30, 1169-1176.

Sills, T.L. (1994). Individual Differences in Sugar Intake and Responsiveness to

Amphetamine Treatments: Role of Nucleus Accumbens Dopamine and Cholecystinin Mechanisms. Unpublished doctoral dissertation, University of Toronto, Toronto, Ontario, Canada (University Microfilms No. 5785 MICR).

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

Sipols, A.J., Brief, D.J., Ginter, K.L., Saghafi, S., & Woods, S.C. (1992). Neuropeptide Y paradoxically increases food intake yet causes conditioned flavor aversions. Physiology and Behavior, 51, 1257-1260.

Song, C., Earley, B., & Leonard, B.E. (1996). The effects of central administration of neuropeptide Y on behavior, neurotransmitter, and immune functions in the olfactory bulbectomized rat model of depression. Brain, Behavior, and Immunity, 10, 1-16.

Spyraki, C., Fibiger, H.C., & Phillips, A.G. (1982a). Dopaminergic substrates of amphetamine-induced place preference conditioning. Brain Research, 253, 185-193.

Spyraki, C., Fibiger, H.C., & Phillips, A.G. (1982b). Attenuation by haloperidol of place preference conditioning using food reinforcement. Psychopharmacology, 77, 379-382.

Stanley, B.G. (1993). Neuropeptide Y in multiple hypothalamic sites controls eating behavior, endocrine and autonomic systems for body energy balance. In W.F. Colmers & C. Wahlestedt (Eds.), The Neurobiology of Neuropeptide Y and Related Peptides. New Jersey: Humana Press.

Stanley, B.G., Chin, A.S., & Leibowitz, S.F. (1985). Feeding and drinking elicited by central injection of neuropeptide Y: Evidence for a hypothalamic site(s) of action. Brain Research Bulletin, 14, 521-524.

Stanley, B.G., & Leibowitz, S.F. (1984). Neuropeptide Y: Stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sciences, 35, 2635-2642.

Stanley, B.G., & Leibowitz, S.F. (1985). Neuropeptide Y injected in the paraventricular hypothalamus: A powerful stimulant of feeding behavior. Proceedings of the National Academy of Science, U.S.A., 82, 3940-3943.

Stanley, B.G., Magdalin, W., Seirafi, A., Thomas, W., & Leibowitz, S.F. (1993). The perifornical area: The major focus of a patchily distributed hypothalamic neuropeptide Y sensitive feeding system(s). Brain Research, 604, 304-317.

Steinman, J.L., Fujikawa, D.G., Wasterlain, C.G., Cherkin, A., & Morley, J.E. (1987). The effects of adrenergic, opioid and pancreatic polypeptidergic compounds on

feeding and other behaviors in neonatal leghorn chicks. Peptides, 8, 585-592.

Stellar, J.R., Corbett, D., & Hamilton, A.L. (1985). A forebrain map of dopamine's relevance to lateral hypothalamic stimulation reward as based on intracranial neuroleptic injection. Society for Neuroscience Abstracts, 11, 48.

Stewart, R.B., & Grupp, L.A. (1984). A simplified procedure for producing ethanol self-selection in rats. Pharmacology, Biochemistry, and Behavior, 21, 255-258.

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

Strober, M. (1991). Family-genetic studies of eating disorders. Journal of Clinical Psychiatry, 52(Suppl.), 9-12.

Taylor, A.V., Peveler, R.C., Hibbert, G.A., & Fairburn, C.G. (1993). Eating disorders among women receiving treatment for an alcohol problem. International Journal of Eating Disorders, 14, 147-151.

Toates, F. (1994). Comparing motivational systems—an incentive motivation perspective. In C.R.Legg and D.A. Booth (Eds), Appetite: Neural and Behavioral Bases (pp. 305-327). New York: Oxford Science Publications.

Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigrostriatal dopamine system. Acta Physiologica Scandinavica, **82** (Suppl.), 95-122.

Vaccarino, F.J. (1994). Nucleus accumbens dopamine-CCK interactions in psychostimulant reward and related behaviors. Neuroscience and Biobehavioral Reviews, **18**, 207-214.

Valenstein, E.S. (1964). Problems of measurement and interpretation with reinforcing brain stimulation. Psychological Review, **71**, 415-437.

Vallejo, M., Carter, D.A., Biswas, S., & Lightman, S.L. (1987). Neuropeptide Y alters monoamine turnover in the rat brain. Neuroscience Letters, **73**, 155-160.

Wahlestedt, C., & Heilig, M. (1995). Neuropeptide Y and related peptides. In F.E. Bloom & D.J. Kupfer (Eds.), Psychopharmacology: The Fourth Generation (pp. 543-551). New York: Raven Press.

Wahlestedt, C., Karoum, F., Jaskiw, G., Wyatt, R.J., Larhammar, D., Ekman, R., & Reis, D. (1991). Cocaine-induced reduction of brain neuropeptide Y synthesis dependent on medial prefrontal cortex. Proceedings of the National Academy of Science, U.S.A., **88**, 2078-2082.

Wahlestedt, C., & Reis, D.J. (1993). Neuropeptide Y-related peptides and their receptors—are the receptors potential therapeutic drug targets? Annual Reviews of Pharmacology and Toxicology, 32, 309-352.

Walsh, B.T., Roose, S.P., Glassman, A.H., Gladis, M., & Sadik, C. (1985). Bulimia and depression. Psychosomatic Medicine, 47, 123-131.

Wang, Q., Bing, C., Al-Barazanji, K., Mossakowaska, D.E., Wang, X.M., McBay, D.L., Neville, W.A., Taddayon, M., Pickavance, L., Dryden, S., Thomas, M.E.A., McHale, M.T., Gloyer, I.S., Wilson, S., Buckingham, R., Arch, J.R.S., Trayhurn, P., & Williams, G. (1997). Interactions between leptin and hypothalamic neuropeptide Y neurons in the control of food intake and energy homeostasis in the rat. Diabetes, 46, 335-341.

Weiss, F., Markou, A., Lorang, M.T., & Koob, G.F. (1992). Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration. Brain Research, 593, 314-318.

Weissman, M., & Myers, J. (1980). Clinical depression in alcoholism. American Journal of Psychiatry, 37, 372-373.

Westfall, T.C., & Vickery, L. (1994). Neuropeptide Y induced alterations in

dopamine synthesis in slices of rat striatum and nucleus accumbens. Society for Neuroscience Abstracts, 20, 851.

White, B.D., Dean, R.G., & Martin, R.J. (1990). Adrenalectomy decreases neuropeptide Y mRNA levels in arcuate nucleus. Brain Research Bulletin, 25, 711-715.

White, J.D., Olchovsky, D., Kershaw, M., & Berelowitz, M. (1990). Increased hypothalamic content of preproneuropeptide-Y messenger ribonucleic acid in streptozotocin-diabetic rats. Endocrinology, 126, 765-772.

Widdowson, P.S. (1997). Regionally-selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to the Y5 'feeding' receptor. Brain Research, 758, 17-25.

Widdowson, P.S., Ordway, G.A., Halaris, A.E. (1992). Reduced neuropeptide Y concentrations in suicide brain. Journal of Neurochemistry, 59, 73-80.

Widerlöv, E., Heilig, M., Ekman, R., & Wahlestedt, C. (1989). Neuropeptide Y—Possible involvement in depression and anxiety. In V. Mutt, K. Fuxe, T. Hökfelt, & J.M. Lundberg (Eds.), Neuropeptide Y (pp.331-342). New York: Raven Press.

Widerlöv, E., Lindström, L.H., Wahlestedt, C., & Ekman, R. (1988). Neuropeptide Y and peptide YY as possible cerebrospinal fluid markers for major

depression and schizophrenia, respectively. Journal of Psychiatric Research, 22, 69-79.

Wise, R.A., & Bozarth, M.A. (1987). A psychomotor stimulant theory of addiction. Psychological review, 94, 469-492.

Wise, R.A., Spindler, J., de Wit, H., & Gerber, G.J. (1978). Neuroleptic-induced 'anhedonia' in rats: pimozide blocks the reward quality of food. Science, 201, 262-264.

Yeary, J.R., & Heck, C.L. (1989). Dual diagnosis: Eating disorders and psychoactive substance dependence. Journal of Psychoactive Drugs, 21, 239-249.

Yoshihara, T., Honma, S., Mitome, M., & Honma, K. (1996). Methamphetamine stimulates the release of neuropeptide Y and noradrenaline from the paraventricular nucleus in rats. Brain Research, 707, 119-121.

Zahm, D.S., & Brog, J.S. (1992). On the significance of subterritories in the "accumbens" part of the rat ventral striatum. Neuroscience, 50, 751-767.

Zarjevski, N., Cusin, I., Vettor, R., Rohner-Jeanrenaud, F., & Jeanrenaud, B. (1993). Intracerebroventricular administration of neuropeptide Y to normal rats has divergent effects on glucose utilization by adipose tissue and skeletal muscle. Diabetes, 43, 764-769.

Zhang, Y., Proence, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J.M. (1994). Positional cloning of the mouse *obese* gene and its human homologue. Nature, 372, 425-432.