Karolinska Institutet

Thesis for doctoral degree (Ph.D.) 2008

FOOD AVAILABILITY, PHYSICAL ACTIVITY AND BODY WEIGHT

Ricard Nergårdh

Thesis for doctoral degree (Ph.D.)
2008

FOOD AVAILABILITY, PHYSICAL ACTIVITY AND BODY WEIGHT

- Role of Dopamine, Neuropeptide Y and Orexin

Ricard Nergårdh

Karolinska Institutet

From the Department of Physiology and Pharmacology
Karolinska Institutet, Stockholm, Sweden

FOOD AVAILABILITY, PHYSICAL ACTIVITY AND BODY WEIGHT

- Role of Dopamine, Neuropeptide Y and Orexin

Ricard Nergårdh

M.D.

Karolinska Institutet

Stockholm 2008

Published by Karolinska Institutet.

ISBN 978-91-7409-051-2

Printed by

www.reproprint.se
Gårdsvägen 4, 169 70 Solna

“Therapeutics is an empirical stage cared for by practical doctors and clinicians, and it is by means of a combination with physiology that it must rise to be a science,..”

Rudolf Virchow mid-19th

ABSTRACT

During the last decades our knowledge about neuroendocrine control of energy balance has increased tremendously. Numerous neuropeptides and hormones with pronounced effects on feeding and body weight have been identified and put into schemes as “anorexic” or “orexigenic” signals. So far this has not rendered new insights into how to explain or treat human pathology such as obesity or anorexia nervosa. Although different in many aspects, obese patients and patients suffering from anorexia nervosa share the feature of abnormal body weight. In this thesis I try to elucidate the role of NPY, orexin and dopamine—all three known to have pronounced effects on ingestive behavior and body weight—under different experimental conditions, with emphasis on availability of food and physical activity. The aim is to get a better understanding of human body weight pathology such as anorexia nervosa and obesity.

If food supply is restricted to only 1 hour each day, rats that have access to running wheels run excessively and lose control over body weight, which rapidly falls. This provides a model of activity based anorexia. In this model NPY mRNA is up regulated in the arcuate nucleus. We show that treatment with NPY increases the fall in body weight by increasing wheel running and decreasing food intake in this model.

While appetitive ingestive behavior is complex, with a wide representation in the central nervous system, consummatory ingestive behavior is stereotyped and involves mainly the brainstem. The intra oral intake test separates the consummatory phase of ingestive behavior. We use this test to characterize the effects of NPY on ingestive behavior and compare the effect of NPY to the known effects of CCK on ingestive behavior and on c-fos pattern in the brainstem. While CCK decreases both appetitive and consummatory ingestive behavior NPY decreases the consummatory phase (an effect additive to that of CCK), and increases the appetitive phase. Both peptides activate neurons in the nucleus of the solitary tract, also indicating similar effects on consummatory ingestive behavior, but there is no evidence that they interact at this level.

To evaluate the role of dopamine D_1 and D_5 receptors on two major readouts of energy expenditure, namely physical activity and core temperature, two full dopamine D_1 receptor family agonists, the isochroman A 68930 and the benzazepine SKF 82958 were compared. The compounds differ in several behavioral aspects and in the pattern of immediately early gene expression they induce. Quantitative receptor autoradiography shows that A 68930 is more potent than SKF 82958 at displacing the selective dopamine D_1 antagonist [^{3}H]SCH 23390. This difference agrees with the difference observed in cAMP formation in cells transfected with the D_1 receptor. In contrast, SKF 82958 is more potent than A 68930 in cells transfected with the D_5 receptor. We suggest that the balance between signaling via dopamine D_1 and D_5 receptors determines the functional effects of agonists at D_1/D_5 receptors.

The increased activity seen in activity based anorexia predominantly occurs during the normally sedentary light phase of a 24 h light-darkness cycle. Treatment with the D_1 antagonist SCH 23390 prevented the development of this running pattern and also prevented the c-fos and orexin induction in the lateral hypothalamus typically seen in activity based anorexia. Quantitative receptor autoradiography shows [^{3}H]SCH 23390 binding in the lateral hypothalamus and we propose that the D_1 antagonist, by acting in in this brain structure, alters orexin signaling and thereby reduces light phase running.

The experiments in this thesis show that the effects of NPY, dopamine and orexin are highly dependent upon environmental factors. Labeling them as “anorexic” or “orexigenic” is therefore in most cases an over-simplification. Developing new treatment strategies for diseases like obesity and anorexia nervosa will require a deeper knowledge about how the environment, and especially the availability of food and need for physical activity, influences ingestive behavior, energy expenditure and body weight.

LIST OF PUBLICATIONS

I. Neuropeptide Y facilitates activity-based-anorexia
Nergårdh R, Ammar A, Brodin U, Bergström J, Scheurink A, Södersten P
Psychoneuroendocrinology. 2007 Jun; 32(5):493-502

II. Intake inhibition by NPY and CCK-8: A challenge of the notion of NPY as an "Orexigen"
Ammar AA, Nergårdh R, Fredholm BB, Brodin U, Södersten P
Behav Brain Res. 2005 Jun 3; 161(1):82-7

III. Differences between A 68930 and SKF 82958 could suggest synergistic roles of D_1 and D_5 receptor
Nergårdh R, Oerther S, Fredholm BB
Pharmacol Biochem Behav. 2005 Nov;82(3):495-505

IV. Dopamine D_1 receptor blockade reduces physical activity induced by food restriction
Nergårdh R, Ammar AA, Boersman GJ, Scheurink A, Fredholm BB, Södersten S
Brain Research submitted for publication

CONTENTS

LIST OF ABBREVIATIONS

AgRP	Agouti-related peptide
A 68930	(1R,3S)-1-aminomethyl-5,6-dihydroxy-3-phenylisochroman HCl
ABA	activity-based-anorexia
AcbC	nucleus accumbens core
AcbSh	nucleus accumbens shell
ACTH	adrenocorticotropic hormone
ANOVA	analysis of variance
ARC	nucleus arcuatus
BMI	body mass index
CART	cocaine and amphetamine regulated transcript
CCK-8	cholecystokinin octapeptide
CLIP	corticotrophin-like intermediate lobe peptide
CPu	caudate putamen
DA	dopamine
DP	dark phase
ENK	preproenkephalin
IEG	immediate early gene
Lepr	leptin receptor gene
LH	lateral hypothalamus
LP	light phase
LR	leptin receptor
MC_4R	melanocortin 4 receptor
MSH	melanocyte-stimulating hormone
NA	noradrenaline
NPY	neuropeptide Y
NTS	nucleus of the solitary tract
OD	optical density
OR	orexin receptor
POMC	proopiomelanocortin
PVN	paraventricular nucleus
SCH 23390	(*R*(+)-7-chloro-8-hydroxy-1-phenyl-2,3,4,5,-tetrahydro-1H-benzazepine maleate
SKF 82958	(±)-6-chloro-7,8-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine HBr
VMH	ventromedial hypothalamus
VTA	ventral tegmental area

1 INTRODUCTION

Body weight is often looked upon as tightly controlled feature and has been proposed to be under homeostatic control. This idea has become widely accepted in recent decades. Clinical observations and lesion studies have identified certain brain regions as “centers” for feeding or satiety (Anand and Brobeck 1951; Stellar 1954). Region-specific expression of neuropeptides and their receptors in these areas have been observed. Some of these peptides have a pharmacological effect on feeding and body weight, and are therefore conceptually integrated into complex schemes as either “anorexic”- or “orexigenic” neuropeptides (Coll, Farooqi and O'Rahilly 2007). According to the paradigm of a homeostatic control over body weight, hormones from stored fat and pancreas, along with glucose, lipids and amino acids from ingested macronutrients, feed back on brain networks to excite or inhibit eating such that body weight remains stable over time. There are many recent reviews on this topic (López et al. 2007) and on the anatomy of the neural substrates engaged during eating (Grill and Kaplan 2002; Elmquist et al. 2005).

The original concept of homeostasis (Bernard 1865; Cannon 1932) applies to control of body temperature, potassium concentration, and blood sugar levels. In these cases a tight control is essential for survival in the very short term. Only small fluctuations can be accepted and if the control breaks down death occurs within minutes or hours because essential life processes can only take place within a very specific milieu. There is therefore no such thing as an increase in blood potassium concentration over time, no matter what happens in the animal’s external environment.

When the history of body mass index (BMI) in the Western world is examined, however, it is obvious that body weight has **not** been stable over time. Figure 1 shows that the average BMI of English men has changed considerably during the last 180 years (Flood 1998). It is questionable whether this variation of BMI is compatible with a homeostatic regulation of body weight in the Bernard-Cannon sense. Rather, the marked variation in BMI during the last two centuries argues against homeostatic regulation of body weight, at least under the conditions of life in Western Europe after the industrial revolution.

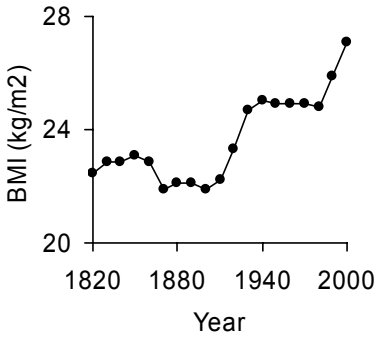

Fig. 1 The average BMI of English men has changed markedly during the last 180 years [Flood 1998; DEFRA].

However, abundance of food is probably rare in nature. When it does occur, most animals (and indeed many humans) will eat and gain weight (Hill et al. 2003; Periwal and Chow 2006). Becoming obese will take some time, however, and in most cases environmental factors such as seasonal variations in food availability and temperature will prevent this. It is therefore hard to envision any evolutionary pressure to develop systems to prevent overeating (i.e., that energy intake exceeds energy expenditure). In fact, the opposite seems more likely; when food is abundant it's an advantage to be able to overeat. A decrease in body weight in response to food shortage is also probably a normal phenomenon in nature. How the animal behaves under these conditions will have a direct impact on survival and later opportunity to reproduce. Therefore, there ought to be a strong evolutionary pressure in favor of behavior patterns that promote survival in situations with lack of food and reduced body weight (Wang, Hung and Randall 2006b).

The overall aim of this thesis is to get a better understanding of the pathology of weight-related conditions such as anorexia nervosa and obesity in humans. There are indeed many things that separate those two diagnoses but they share a fundamental similarity in that body weight is seemingly out of control. According to the homeostatic concept of body weight control, obesity is often looked upon as pathology in the body weight control systems. Anorexia nervosa, on the other hand, is often considered to be primarily a psychiatric disorder and is therefore described in totally different terms. A huge research field has evolved over the last decade, dramatically increasing our knowledge about different neuroendocrine systems involved in body weight control.

However, all this research has not yet led to a better understanding and treatment of obesity or indeed anorexia nervosa. To get further insight into the physiological roles of some of the neurotransmitters and neuroendocrine systems implicated in feeding and body weight control (e.g. dopamine, NPY and orexin) I choose to study them under conditions of food restriction.

In this thesis, I try to understand how food supply influences behavior. I concentrate on physical activity since obese patients have low levels of physical activity and patients with anorexia nervosa characteristically have very high levels of physical activity. In both these clinical conditions the level of physical activity significantly contributes to morbidity and probably even to mortality. Physical activity is also an easily accessible readout that can be studied in well controlled animal experiments.

1.1 NEUROENDOCRINE SUBSTRATE OF INGESTIVE BEHAVIOR

A full review of the neuroendocrine mechanisms involved in metabolism, energy balance and ingestive behavior is beyond the scope of this thesis. The following section will only give a brief description of some of the hormones, neuropeptides and transmitter substances necessary for understanding the relevance of the experiments performed. A brief description of how they interact is also provided. A scheme outlining some of these principles is shown in figure 2. The system I try to describe can for convenience be divided into an afferent arm, an integrating and measuring function, and an executing arm. The afferent arm gives different cues about energy stores, the surrounding environment, and so on. Afferent information is then measured and processed. Based on this, the executive arm adjusts the animal's behavior. Leptin is one part of the afferent arm whereas dopamine (DA) is part of the executive arm. NPY and the melanocortin system are in between the afferent and the executive arm, constituting part of the more elusive measuring and processing system. All these endogenous substances and systems are described later in this section.

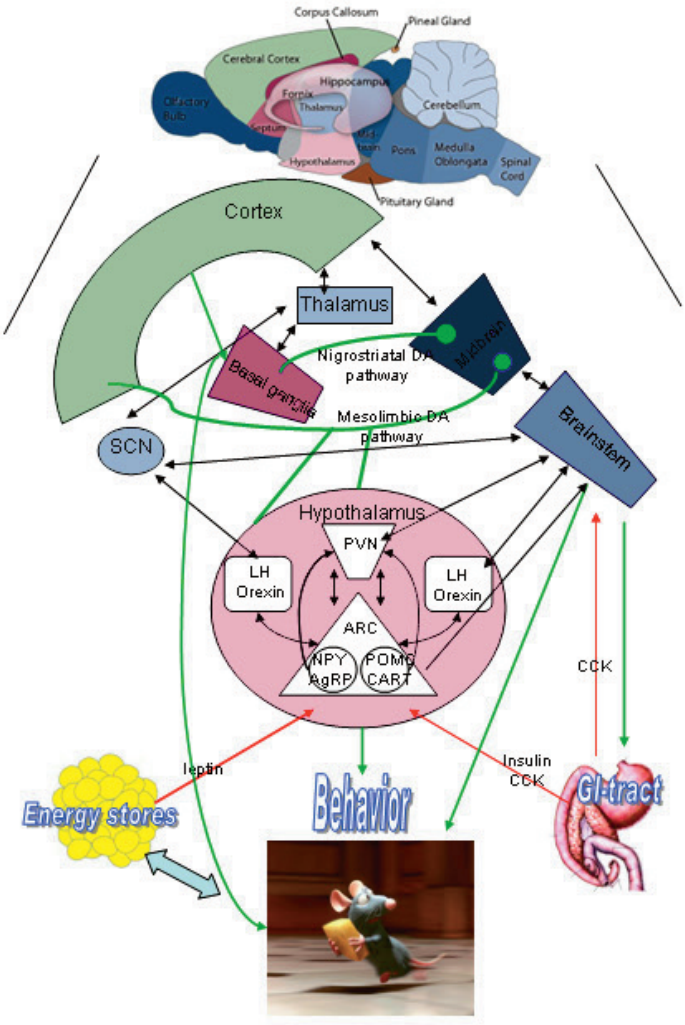

Fig. 2 A schematic drawing outlining some key projections in the neuroendocrine network discussed in this thesis. Red arrows indicates the afferent arm, black arrows indicate the integrative component where this information is processed. The efferent arm, executing behavior, is indicated by green arrows.

1.1.1 Leptin

Almost 40 years ago, the existence of a hormone that reflects body energy storage was suggested by Coleman and Hummel (Coleman and Hummel 1969). Their ideas came from studies on two different mouse strains, the ob/ob mice and the db/db mice. These mouse strains exhibit sustained hyperphagia, reduced energy expenditure and severe

obesity. Linking the circulation of the ob/ob mouse with that of either wild type or db/db mice promoted weight loss in the ob/ob mice. Linking the db/db mouse with that of either ob/ob or wild type mice had no effect on the db/db phenotype. This strongly suggested that ob/ob mice lacked a hormone that db/db mice had, but were insensitive to. In 1994, it was shown that the ob/ob mouse had a mutation in the ob gene encoding a 167 amino acid long peptide that was named leptin (Zhang et al. 1994). Soon thereafter, the leptin receptor (LR) was cloned and a mutation in the gene for this receptor explained the phenotype of the db/db mouse (Tartaglia et al. 1995).

Leptin is synthesized and released to the bloodstream in response to energy intake (Wang et al. 1998) and storage (Rosenbaum et al. 1996). The level of circulating leptin, however, largely reflects energy stores (e.g., amount of body fat). Leptin, being a peptide, does not pass the blood brain barrier, but enters the CNS via a satiable transport mechanism (Banks et al. 1996) and exerts its effects in the brain via the LR (Tartaglia et al. 1995). Alternative splicing of the LR gene gives several LR isoforms. Each isoform, however, contains identical extracellular and transmembrane domains with variations only in the intracellular sequences (Louis and Myers 2007). Of all the isoforms of the LR only LRb is altered in the db/db mice and it is only the LRb isoform that modulates known intracellular signaling of leptin action (Louis and Myers 2007).

Leptin is a vital link between metabolic and endocrine pathways. Prolonged (e.g., days) changes in energy status are required to alter leptin levels. This means leptin is well positioned to function as an indicator of energy stores (Louis and Myers 2007). Adequate circulating leptin levels are necessary to permit energy-intensive endocrine outputs like growth and reproduction (Louis and Myers 2007). During negative energy balance and starvation, leptin levels fall. Preventing the starvation-induced fall in leptin with exogenous leptin substantially blunts the changes in gonadal, adrenal and thyroid axes typical of starvation and prevents the starvation-induced delay in ovulation in female mice. In fact many of the physiological responses to starvation can be prevented by exogenously administered leptin (Ahima et al. 1996).

1.1.2 Neuropeptide Y

In 1982, the 36 amino acid long peptide NPY was isolated from porcine brain tissue by Tatemoto, Carlquist and Mutt (1982). The novel peptide was found to be widely distributed throught the central (Allen et al. 1983) and peripheral nervous system

(Lundberg et al. 1984) and it was soon realized that NPY is co-expressed with NA (Lundberg et al. 1982; Hökfelt et al. 1983). NPY activates at least five different G-protein coupled NPY receptors (Lin, Boey and Herzog 2004; Eva et al. 2006).

Since food intake is stimulated by NA acting in the hypothalamus (Leibowitz and Brown 1980) and since NPY is co-expressed in NA neurons, Leibowitz and others early on tried injecting NPY ICV and looking for effects on food intake. They found a marked increase (Clark et al. 1984; Stanley and Leibowitz 1984) after treatment with NPY. Since then, NPY is considered the most potent "orexigens" ever found. However, NPY has many other effects, including effects on circadian rhythms (Yannielli and Harrington 2001), sexual behavior (Clark, Kalra and Kalra 1985), blood pressure (Lundberg et al. 1982) and on seizures (Dubé 2007). It is clear that it is an oversimplification to look upon NPYs effect on feeding as merely an orexigenic signal (Cabanac and Swiergiel 1989). In experiments separating the appetitive phase (e.g., searching for and approaching the food) from the consummatory phase (e.g., chewing and swallowing) it is clear that NPY stimulates the appetitive phase while in many circumstances the consummatory phase is suppressed (Seeley, Payne and Woods 1995; Ammar et al. 2000, 2005).

1.1.3 α-MSH

α-MSH is derived from the proopiomelanocortin (POMC) transcript so named because it gives rise to β-endorphin, MSH, ACTH and CLIP. The melanocortins act on at least five different G-protein coupled receptors, MC_1R-MC_4R. α–melanotocyte stimulating hormone (α-MSH), together with other melanocyte-stimulating hormones, stimulates melanocytes in the skin to increase pigmentation. That signaling via melanocortin receptors could influence feeding and body weight control was deduced from studies of mice carrying certain alleles of the Agouti coat color gene, such as "Viable yellow" with a phenotype including yellow fur and obesity (Manne, Argeson and Siracusa 1995). The melanocortin most strongly implicated in feeding and body weight control is α-MSH acting via the MC_4R.

1.1.4 AgRP

This peptide was first described in 1997 by two groups working independently (Ollmann et al. 1997; Shutter et al. 1997). The Agouti protein, encoded by the Agouti

coat color gene, is mainly expressed in the skin where it functions as an antagonist on MC_1R in controlling pigmentation. α-MSH, one of the products of the POMC transcript, activates MCRs and the functional balance between α-MSH and the Agouti protein is one of the first described instances where an endogenous antagonist controls physiological function. Over-expression of Agouti protein causes a characteristic coat color and obesity. Shutter et al. (1997) isolated a cDNA encoding Agouti-related transcript (ART) that was shown to be expressed in the arcuate nucleus (ARC) of the hypothalamus and up-regulated in both ob/ob and db/db mice. Ollman et al. (1997) showed, almost at the same time, that Agouti-related peptide (AgRP), the peptide encoded by the ART, was a potent and selective antagonist at MC_3R and MC_4R but had no effect on MC_1R. Over-expression of AgRP in mice therefore causes obesity without altering skin pigmentation.

1.1.5 Orexin

In 1998 Sakurai et al. published a paper describing a novel petide isolated from the lateral hypothalamus (LH), a brain area long known to be involved in feeding behavior and often described as a "feeding center". The peptide activated a previously cloned orphan G-protein coupled receptor. The mRNA encoding the peptide was strictly localized to neurons in the LH. When the peptide was administered centrally to rats, it stimulated food intake. It was also shown that starvation increased the synthesis of the peptide. Based on this the authors named this new neuropeptide "orexin". It was later realized that the orexigenic effect of orexins was, at least in part, dependent upon NPY (Yamanaka et al. 2000). During the 10 years since the orexins were first described we have come to realize that, like most other neuropeptides, orexins have several roles and are not exclusively involved in the control of feeding.

In 1999 Lin et al. showed that a well known canine model of narcolepsy, the canarc-1 mutant dog, arose through an autosomal recessive mutation in the gene encoding the orexin receptor (OR). The same year Chemelli et al. (1999), showed that knock out mice lacking the orexins showed a phenotype very similar to the phenotype of the canarc-1 mutant dog. It was also shown that drugs effective in treating narcolepsy in humans activate orexin neurons in LH (Chemelli et al. 1999). These and other studies have identified orexin as a key peptide in modulating states of alertness (Yamanaka et al. 2003) and sleep-wake states (Ohno and Sakurai 2008). It should be

kept in mind that Lin et al. (1999) named the novel peptide hypocretin and both names, orexin and hypocretin, are used.

1.1.6 Dopamine

The catecholamine DA is present in both peripheral and central nervous system (just as many neuropetides). At first believed to be merely a step in the formation of noradrenalin and adrenaline, DA was recognized to have a biological function of its own in the late 1950s (Carlsson 1959). In the CNS, DA synthesizing cells are present in the mesencephalon, the diencephalon, the olfactory bulb and the retina (Björklund and Lindvall 1984). Mesencephalic DA-cells, originating from the retrorubal nucleus, substantia nigra and the ventral tegmental area, project in the nigrostriatal pathway and the medial forebrain bundle to striatal, limbic and cortical areas. Mesencephalic DA-neurons also project to the pituitary gland, locus coeruleus and areas in the spinal cord. Some aspects of DA-transmission are given here as a background for the experiments in this thesis.

DA exerts its effects via five different G protein coupled receptors. These can be grouped into D_1-like (D_1 and D_5) and D_2-like (D_2, D_3 and D_4) on the basis of their ability to increase or decrease cAMP formation, respectively (Gingrich and Caron 1993). Dopaminergic receptors are widespread throughout the central and peripheral nervous systems, where they influence a variety of physiological, behavioral, and endocrine functions (Sibley 1999). Within the basal ganglia and elsewhere, DA receptors are found in both presynaptic and postsynaptic locations, with the D_1 and D_2 receptors being the predominant subtypes. The D_3, D_4 and D_5 receptors are less abundant than the D_1 and D_2 subtypes and are expressed in a more restricted distribution pattern within the brain (Sibley 1999). DA receptors are clinically important drug targets for the treatment of a number of disorders, such as Parkinson's disease, schizophrenia, and hyperprolactinaemia. Many drugs of abuse are also active at DA receptors.

For the D_1 family a substantial amount of data indicates different roles for individual D1-like receptors in mediating distinct elements of DA influenced behaviors (Waddington et al. 1995). Different distribution (Luedtke et al. 1999; Sibley 1999; Khan et al. 2000) and differences in the phenotype of D_1 and D_5 knock-out mice (Holmes et al. 2001; Waddington et al. 2001) all indicate functionally different roles for

the members of the DA D_1 receptor family (Waddington et al. 1995, 2001; Nicola, Surmeier and Malenka 2000).

In the early 1950s it was first shown by Anand and Brobeck that destruction of the lateral hypothalamus (LH) produced aphagia (Anand and Brobeck 1951; Teitelbaum and Epstein 1962). It was later shown by Ungerstedt (1971), that the hypophagia was largely due to destruction of dopaminergic axons in the medial forebrain bundle traveling through the LH in its way to the striatum. Those studies were the beginning of a search for the role of DA in control of ingestive behavior and a huge literature on this subject has evolved. In brief, two main models have been proposed to explain the role of DA in ingestive behavior: the sensorimotor hypothesis and the anhedonia hypothesis. Both these hypotheses try to explain the fact that DA depletion renders a rat aphagic. According to the sensorimotor hypothesis, DA plays a role mainly in the motor control and the aphagic state is due to an inability to move (Le Moal and Simon 1991). According to the anhedonic hypothesis, the DA depleted rat will not eat because eating gives it no pleasure (Wise et al. 1978). In resent years the hypothesis of incentive salience has been added to explain the causal contribution made by mesolimbic DA systems to reward. In an excellent recent review Berridge (Berridge 2007) describes and discusses this issue in depth. In short the hypothesis of incentive salience, where a reward contains multiple component types like wanting, learning and liking, DA mediates the wanting component. According to this DA add incentive value to conditioned stimuli. This is in contrast to the anhedonic hypothesis where DA is merely necessary for hedonic "liking".

1.1.7 CCK-8

Early in the last century, Ivy and co-workers purified a substance from the upper intestine mucosa. This substance could constrict the gall bladder and they therefore named it "cholecystokinin" (CCK) (Ivy and Oldberg 1928). CCK was assumed to be produced in the proximal part of the small intestine with the purpose of emptying the gall bladder when fat passed into the duodenum. In 1971, Mutt and Jorpes reported the amino acid sequence of CCK. Since then, it is recognized that CCK is synthesized in several molecular forms containing 58, 39, 33 and 8 amino acids due to post translational modifications. The carboxyl terminal octapeptide sequences are identical for all these forms. Within duodenum and proximal jejunum, CCK is expressed by mucosal cells. CCK is also found in neurons of the myenteric plexus and in nerves

innervating the urinary bladder and uterus. CCK-8 is the major molecular form in the brain. CCK acts on two different G-protein-coupled receptors, CCKA and CCKB (Moran et al. 1986; Moran et al. 1992). The CCKB receptor subtype is the one implicated in the central effects of CCK. Release of CCK is stimulated by intraduodenal content, independent of its content of protein and fat (Mamoun et al. 1995). Once released, CCK induces satiety, an effect mediated via CCKA receptors (Qian et al. 1999) on vagal afferent neurons (Lindén and Södersten 1990).

1.1.8 Leptin, NPY, α-MSH, AgRP, orexin and DA are members of a neuroendocrine network influencing behavior

Leptin, NPY, α-MSH, AgRP, orexin and DA are all key players in a complex neuroendocrine network that adapts the animal's behavior to a changing environment. The basic interactions are described in this section. Needless to say, this summary is highly selective with the aim of providing a background for the experiments in this thesis. Figure 2 outlines the basic structure of this network.

Circulating levels of leptin in the blood correlate with the amount of body fat and function as an indicator of energy status. Neurons in ARC and the median eminence have dense expression of LR (Wang et al. 1997) and are postulated to "sense" the leptin signal. Neurons in the ARC also express many of the neuropeptides implicated in the control of energy intake and expenditure, indicating a central role for the ARC in relaying information about peripheral energy status to the brain. There are two subsets of LR-expressing neurons in the ARC: NPY/AgRP expressing neurons and POMC/CART expressing neurons. Both those neuron types react to changes in circulating leptin levels. Falling leptin concentrations cause an activation of NPY/AgRP neurons and an increase in NPY synthesis (Schwartz et al. 1996; Benoit et al. 2004). Conversely, POMC/CART neurons are stimulated by leptin, and falling leptin levels decrease POMC expression (Coll, Farooqi and O'Rahilly 2007). It is not clear whether CART influences energy balance, but there is evidence that POMC peptides influence feeding behavior, as both mice and humans with POMC deficiency develop hyperphagia and obesity (Coll et al. 2004). Both NPY/AgRP and POMC/CART neurons in ARC project to the paraventricular hypothalamic nucleus (PVN). This is a region known to be involved in control of feeding, with dense expression of the NPY receptor subtypes Y1 and Y5, i.e., those most implicated to play

a role in feeding-related behaviors (Eva et al. 2006; Kamiji and Inui 2007), and MC_4R. MC_4R are believed to mediate the effects of melanocortins and AgRP on feeding (Balthasar et al. 2005).

There is an increasing amount of evidence showing that leptin, by stimulating leptin receptors in the ARC, suppresses the expression of NPY in ARC and the release of NPY in PVN under situations when food is abundant. Infusion of NPY into the PVN also increases food intake (Stanley and Leibowitz 1984). Hence, this is one of the plausible sites of action for the enhancing effects of NPY on ingestive behavior. The POMC/CART neurons, on the other hand, are inhibited by falling leptin levels. Post-translational modification of POMC generates a range of smaller active peptides called melanocortins of which α-MSH is the one most strongly implicated in feeding. Melanocortins are agonists at melanocortin receptors and there is a dense expression of MC_4R in PVN. AgRP, synthesized and released from NPY-positive cells in ARC, functions as an antagonist at MC_4R. By reducing the concentration of agonist, α-MSH, and increasing the concentration of antagonist, AgRP, signaling via $MC_4R$is reduced. Deletion of MC_4R in mice and humans results in obesity (Coll et al. 2004) and MC_4R mutations are responsible for up to 5% of severe childhood obesity (Larsen et al. 2005). It has also recently been shown that especially the MC_4R expressed on neurons in PVN are important in this context (Balthasar et al. 2005). Hence AgRP acting in PVN (and possibly amygdale) induce feeding by acting as a competitive antagonist on MC_4R constitutively activated by α-MSH. However, it is important to keep in mind that the PVN is not the only region implicated in feeding control that is sensitive to NPY and melanocortins. It was shown early on that infusion of NPY into the fourth ventricle also enhanced ingestive behavior (Corp et al. 1990) and NPY-positive cells in the brainstem project to various brain regions, including the PVN. POMC neurons also project to the brainstem and MC_4R are densely expressed in the nucleus of the solitary tract (NTS).

NPY is also implicated in circadian rhythms. This was first suggested by Alberto and Ferris as early as 1984. Microinjection of NPY into the suprachiasmatic region of the hypothalamus phase-shifted the circadian activity rhythm of hamsters housed in constant light. Xu et al have shown that NPY expression in the brain is under influence of rhythms (Xu et al. 1999)generated in the SCN (Ralph et al. 1990; Reppert and Weaver 2002). This basic rhythm is entrained to different environmental cues through

complex neuronal networks involving several hypothalamic regions, including the LH (Reppert and Weaver 2002; Elmquist et al. 2005). Circulating leptin levels, together with blood glucose and other signals of nutritional state, influence these rhythms and affect behavior.

DA release and DA receptor expression in LH (Fetissov et al. 2002) as well as orexin synthesis (Yamanaka et al. 2003) are altered by nutritional cues. The orexin system is proposed to integrate circadian-photic and nutritional metabolic influences to regulate the timing and consolidation of behavioral states, like arousal and motivation, and nutritional states (Selbach and Haas 2006). Orexin neurons in the LH fire spontaneously during active waking and are silent during sleep (Selbach and Haas 2006). Hypofunction of the orexin system, possibly as a consequence of an autoimmune attack against orexin neurons, causes narcolepsy, a debilitating inability to separate and consolidate sleep and waking (Peyron et al. 2000). It is also interesting to note that LH has long been implicated in the regulation of body temperature and that lesions in this region produce hyperthermia (Corbett, Kaufman and Keesey 1988), just as starvation does, or for that matter D_1 receptor activation (Salmi, Jimernez and Ahlenius 1993). DA influences orexin neurons in LH (Alberto et al. 2006) and methamphetamine, a DA releasing agent, induces c-Fos (an immediate early gene used as a marker of neural activity, see next section) in orexin neurons and the c-Fos expression correlates positively with wakefulness (Estabrooke et al. 2001). The cellular mechanism by which DA modulates orexin neurons remains largely unknown. However, it has recently been shown that DA modulates excitatory presynaptic terminals impinging onto orexin neurons (Alberto et al. 2006). D_1-like receptors increase the frequency of this spontaneous excitatory transmission onto orexin neurons, whereas D_2-like receptors diminish it. This is expected to alter the orexin neurons' firing rate (Li et al. 2002).

There are numberous functional connections between the orexin and the NPY systems. Feeding induced by ICV injection of orexin is reduced by pre-treatment with the Y1 receptor antagonist BIBO3304 (Yamanaka et al. 2000). ICV injection of orexin also induces NPY mRNA expression in ARC. Orexin-positive cells make close, synapse-like contacts with cells in the intergeniculate leaflet (IGL) and some of those cells express NPY and project to the SCN (Nixon and Smale 2004). The IGL is known to be involved in feeding-schedule-induced modulations of mammalian circadian

systems (Challet, Pévet and Malan 1996) and it has been proposed by Nixon et al (2004) that increased activity in orexin-positive cells maintains arousal when the animals otherwise should sleep. Orexins are consequently candidates for relaying information about the arousal state to the NPY cells in the IGL; those cells in turn project to the SCN, altering circadian rhythm.

1.2 IMMEDIATE EARLY GENES AS MARKERS FOR NEURONAL ACTIVITY

c-fos and NGFI-A are members of the family of so-called immediate early genes (IEG). These genes share the feature that they are induced rapidly—often within minutes—and strongly by various stimuli. Genes in this family often encode proteins that act as transcription factors (Morgan and Curran 1991, 1995). c-Fos biosynthesis has been shown to track stimuli that increase intracellular calcium following the occupation of different receptors in a way that is related to frequency of excitatory neural input (Luckman, Dyball and Leng 1994). In general, however, a lot of knowledge about the function of IEGs in the CNS is still lacking. Numerous genes do have binding sites for IEG products in their promoter regions, indicating a role in transcription of other genes. Most IEGs, like c-fos, are expressed constitutively at low levels, but some, like NGFI-A, have a rather high basal expression, making it possible to detect both reduction and induction of their expression. c-fos is a member of a family of fos and jun genes that contain a leucin-zipper motif. Heterodimers of Fos bind to specific DNA sequences as so-called activator proteins (AP) and regulate transcription. NGFI-A belongs to a zink-finger family and encodes a transcription factor that binds to a specific DNA sequence. At this site it competes with the transcription factor SP-1. An up-to-date review on the subject of how IEGs can be used to map neural circuits involved in the control of different aspects of ingestive behavior is provided by Watts et al (2006). In present thesis I use IEGs as markers of neural activity in order to map neuronal circuits activated under different conditions and after various pharmacological treatments.

1.3 ANIMAL BEHAVIOR

To explain the rationale for the experiments I will give a brief summary of relevant animal behavior.

The behavior of an animal is the result of a stimulus and has a goal. The stimulus can be external, e.g., an environmental factor such as food, or internal, e.g., hormones. In most, or even all cases, it is a combination of both external and internal stimuli that leads to a behavior. In other words; the brain is not a generator of behavior: it mediates between environmental changes and behavior in order to adapt the behavior of the animal to the new environment (Watts et al. 2006; Schulkin 2007). All behavior also consists of a temporally arranged series of sensory – motor events (Watts and Swanson. 2002). According to this an animal's behavior in a specific situation can be predicted if the stimuli that are relevant for the animal can be understood. Animal experiments can therefore be used to discern cause and effect relationships between environmental changes and changes in animal behavior. This is one of the basic assumptions upon which the experiments of this thesis rely. By studying the neuroendocrine changes that mediate the effects of environmental stimuli on behavior, we hope to get a better understanding of how the brain mediates between milieu and behavior.

In the beginning of the 20th century, ideas on how to categorize animal behavior were presented by Craig (1917). According to Craig (1917), an appetite is a state of agitation which continues as long as a certain stimulus is absent. When the stimulus is received it triggers a consummatory reaction, after which the appetitive behavior ceases ("...and is succeeded by a state of relative rest"). Precisely where in the sequence appetitive behavior ends and consummatory reactions begin is arbitrary, according to Craig (1917).

1.3.1 Ingestive behavior

Feeding is necessary from birth to death for all animals. Feeding involves many different behaviors: decisions on where and when to look for food, hunting for it, smelling and tasting it, chewing and swallowing it, deciding when to stop eating, and much more{Levine, Kotz & Gosnell, 2003}}. There are also important differences in feeding-related behaviors between animal species, from the hoarding behavior of hamsters to the specialized filter-feeding mechanism of whales. Taking the complexity of feeding behavior into account it is no surprise that many brain areas are involved (Druce and Bloom 2006). The past decade has seen enormous interest in central mechanisms involved in body weight control. Recent research has led us to conclude that the hypothalamic feeding center hypothesis, formulated in the 1950s, is probably

an oversimplification and that neural networks involved in feeding behaviors and body weight control extend throughout the brainstem and forebrain (Grill and Kaplan 2002). In this thesis the ingestive part of feeding behavior is studied.

1.3.1.1 Appetitive and consummatory ingestive behavior

Ingestive behavior can be divided in to two distinct phases; the appetitive phase where food is searched for, and the consummatory phase where food is chewed and swallowed (Craig 1917). While appetitive ingestive behavior is very complex and involves many different brain areas, consummatory ingestive behavior is highly stereotyped and takes place even in decerebrated rats (Grill and Smith 1988) strongly suggesting that consummatory ingestive behavior is controlled in the brainstem. The intraoral intake test allows us to study the consummatory phase of ingestive behavior in isolation. A sucrose solution is delivered inside the oral cavity so that all the rat has to do to ingest the solution is to swallow. The method was first developed to monitor taste reactivity in rats (Grill and Norgren 1978). Later the method has also been used to study consummatory ingestive behavior (Grill et al. 1987). A detailed description of the test is given in the material and methods section.

1.3.2 Activity based anorexia

Rats offered the opportunity to run in running wheels will do so. To compensate for increased energy expenditure due to increased physical activity they will eat more (Scheurink et al. 1999). However, if the food supply is restricted, rats run progressively more as the level of food deprivation is increased. The rats run excessively and lose control over body weight if food is available for only 1-2 h each day (Routtenberg and Kuznesof 1967). This well established model is often referred to as activity-based-anorexia (ABA) (Spear and Hill 1962; Routtenberg and Kuznesof 1967; Dixon, Ackert and Eckel 2003).

1.4 OBSERVATIONS NOT CONSISTENT WITH A HOMEOSTATIC CONTROL OF BODY WEIGHT

Our knowledge of neuroendorine systems involved in feeding and body weight control has increased dramatically over the past 10 years. So far, however, this has not led to effective treatments for human pathology including obesity and anorexia nervosa, both

characterized by abnormal body weight. In the case of obesity, energy intake exceeds energy expenditure and the opposite is true for anorexia nervosa. With obesity reaching epidemic proportions, there is now huge interest in research aimed at understanding how increases in body weight can be controlled. Anorexia nervosa, a disease often looked upon as a primarily psychiatric disorder, is described in totally different terms. Many features of both anorexia nervosa and obesity, as well as the behavior of rats in the ABA model, are inconsistent with the idea of a homeostatic control of body weight and will be described briefly in this section and discussed further in the result and discussion section.

1.4.1 Obesity

In Western countries food availability has increased tremendously in the past few decades. This is paralleled by a decrease in physical activity resulting in a sedentary lifestyle (Yancey et al. 2004). At the same time we see an obesity epidemic with a rapidly increasing number of obese people (Allison et al. 1999; Abelson and Kennedy 2004; Hedley et al. 2004). It has been shown many times that the vast majority of obese people have no defects in known neuroendocrine systems such as leptin, NPY or orexin signaling. The hyperphagia, leading to obesity, continues despite high circulating levels of leptin, insulin- and blood glucose. This is inconsistent with the idea of a homeostatic control of body weight. In fact the hypothesis of a homeostatic control of body weight argues against even the possibility of a phenomenon like an obesity epidemic.

1.4.2 Anorexia nervosa

Patients suffering from anorexia nervosa have depleted energy stores, low circulating leptin levels (Hebebrand et al. 2007) and they are hyperactive. The low levels of leptin result, as expected, in high levels of NPY in CNS (Kaye 1996; Goldstone et al. 2002), yet the patients don't eat. This is inconsistent with the idea of a homeostatic control of body weight and indeed with the idea that NPY is an orexigenic signal. It has also been shown that stimulating the hypothalamic-pituitary-ovarian axis with LHRH induces follicular maturation, ovulation, a functional luteal phase and menstruation in amenorrhoeic women with anorexia nervosa (Nillius, Fries and Wide 1975). These findings indicate that there are no primary defects in the neuroendocrine system underlying the disease; rather, the neuroendocrine function is intact but hibernating in anorexia nervosa, as in animals and humans during starvation.

1.4.3 Activity based anorexia

The behavior of the rats in ABA is well in line with the fact that most animals increase their physical activity in states of starvation in order to increase food searching activities (Wang, Hung and Randall 2006a). It is however hard to explain this behavior from the hypothesis that body weight is under homeostatic control.

1.4.4 A constant BMI for 40000 years and then...

As mentioned in the first section the average human BMI has increased in the past few decades, probably as a result of a dramatic shift in our living environment. This argues against a neuroendocrine control system preventing overeating and subsequent development of obesity. This does not, however, imply that body weight cannot be kept rather constant over prolonged time periods under specific conditions, and I will give an example of this.

Australian aborigines who are not influenced by a Western life style are reported to have a BMI less then 20 kg/m^2 (O'Dea, White and Sinclair 1988). In fact a group of 11 women and seven men, who were 34 (15-68) years old had a BMI of 16.7 (13.4-19.3). Yet they showed no clinical or biochemical signs of malnutrition. Even more interesting, they retained a capacity to consume large amounts of food if offered the chance. Thus, intake of as much as 2-3 kg of a high protein diet in one day was often recorded (O'Dea et al. 1988). Both aboriginal men and women spent 3.5 (0-7) hours each day catching and preparing food (White 1985). Men could travel very far either alone or in small groups and return to their camp after one or several days hunting prey. Women spent as much time as men foraging for food although by they used different strategies. “One woman some 55-60 yrs old returned to the camp with a 28.5 kg load of firewood herself 32 kg bw” (White 1985). Australian aborigines are reported to have maintained a relatively constant life style for perhaps more than 40 000 years (Blainey 1993). In addition, their BMI did not increase with age. Thus, when a high level of physical activity is required for the acquisition of food, body weight is low, body fat is depleted, and the correlation between BMI and age disappears. Under these conditions, with an environment that prevents obesity, BMI can be kept constant.

Based on these observations we are trying to get a better understanding of how NPY, orexin and DA influence ingestive behavior by studying them under conditions of reduced access to food.

2 AIMS

The overall aim of this thesis is to get a better understanding of human body weight pathology, such as obesity and anorexia nervosa. Using relevant animal models, we attempt to answer the following questions:

1 Does NPY play a role in the development of ABA?

2 Does NPY interact with CCK-8 in inhibiting consummatory ingestive behavior?

3 Can the balance between D_1/D_5 receptor signaling explain differences between the two D1 family selective agonists A 68930 and SKF 82958?

4 Are D_1 receptors involved in the development of ABA?

3 MATERIAL AND METHODS

Methods that are considered standard are described in detail in the original papers and are only listed in Table 1.

Table 1. General material and methods used in the studies in this thesis

Methods	paper
Running wheel activity	I, III, IV
Body weight measurements	I
Tissue preparation	I, II, III, IV
In situ hybridization	I, II, III, IV
Autoradiography	III, IV
cAMP accumulation measurements	III
Cell culture	III
Tranfection of cells	III

3.1 ADDITIONAL COMMENTS ON MATERIAL AND METHODS

3.1.1 Surgery

Pentobarbital sodium anesthesia (60 mg/kg) was used for surgical procedures (Mebumal, NordVacc, and Stockholm, Sweden). A 2-4 weeks postoperative period of recovery was allowed.

3.1.2 Ovariectomy

The lumbar dorsum was shaved bilaterally and the skin prepared for aseptic surgery with 70% ethanol. A small dorsal flank incision penetrating the abdominal cavity was made bilaterally. The paraovarian fatty tissue was identified and retracted. The exposed ovary was removed. A hemostat pressure was applied for 1-2 minutes. The incision was closed with non-absorbable suture.

3.1.3 Intracerebroventricular cannulation

The head of the rat was placed in a stereotaxic instrument. An incision was made on the top of the skull. A hole was drilled in the skull and a cannula of 22 gauge (Plastic One, Roanoke, VA, USA) was implanted aiming at the left lateral ventricle at a position of bregma -1 mm, lateral 1.4 mm and ventral 3.5 mm (Paxinos and Watson, 1998).
The cannula was anchored to the skull with acrylic cement and stainless steel screws.

3.1.4 Intraoral cannulation

The technique developed by Grill and Norgren (1978) was used. The animal's head was placed in a stereotaxic frame. An incision was made in the midline of the scalp. Three holes were drilled in the skull and screws were fixed in the bone. A teflon washer was placed over a 5 cm long PE-100 tubing and anchored in the correct position by heating the end of the tubing. The blunted end of an injection needle was connected to the other end of the tubing. The end of the needle was then placed between the cheek and the gum at a point slightly rostral to the first upper molar. The needle was advanced subcutaneously ending at the incision at the top of the head. The tube is cut off at an appropriate length and a 20 mm steel tube is inserted and anchored with acrylic cement to the screws.

3.1.5 Intraoral intake

The method was first developed to monitor taste reactivity in rats (Grill and Norgren 1978). Later the method has also been used to study consummatory ingestive behavior (Grill et al. 1987). The test equipment includes polyethylene tubing (PE 100), connecting steel tubes (1 mm outer diam) a peristaltic pump (Alitea XV, Ventur Alitea, Stockholm, Sweden), a plexiglas test arena (35 cm in diam) and lids with swivels (Harvard Apparatus, X-tube, and Cambridge, MA, USA) to connect the polyethylene tubing to the rat. The rat is placed in the test arena and PE tubing is connected to the intraoral cannula. As the pump is turned on, consummatory ingestive behavior is considered initiated if the solution is ingested by the rat. Passive rejection occurs if the rat lets the solution drop from the mouth. When rejection occurs, the infusion is interrupted and restarts after 30 min. If the rat rejects the solution once again in 60 s the test is terminated.

3.1.6 Combined intraoral and bottle intake

Both intra oral and bottle intake was measured in some experiments. Intra oral intake of a sucrose solution was trained as stated above for 4-6 days and the rat was then trained to ingest from a bottle for 4-6 days. The intra oral and the bottle intake were then combined for an additional 3 days. Under these circumstances the intra oral intake stabilized at 14-20 ml and the intake from the bottle at about 3-4 ml.

3.1.7 Spontaneous motor activity

In paper III spontaneous motor activity was observed in a square open-field arena (680 × 680 × 450 mm), equipped with two rows of photocells (8 × 8), sensitive to infrared light (Regler och mätteknik, Kungsbacka, Sweden). The number of photocell beam interruptions was collected on a PC. Separate groups of animals for the two conditions ("naïve" and "habituated"), and doses of A 68930 or SKF 82958, were used for the open-field observations. The "naïve" animals were also used for the temperature experiments. A precise description of how the measurements were done and the data analyzed is given in paper III.

The photocell-equipped open field arena allows measurement of motor activity. Breaking of light beams is recorded as counts by the computer. The following variables were calculated: locomotor activity (all interruptions of photo beams at the lower level); peripheral locomotion (interruptions of photo beams provided that the photo beams spaced 25 mm from the wall at the lower level also had been activated); rearing (all interruptions of photo beams at the upper level); forward locomotion (successive interruptions of photocells in the lower rows when the animal was moving in the same direction).

3.1.8 Core temperature

Core temperature measurements were made in a temperature-controlled room (ambient temperature 21.0 ± 0.4°C). Recordings were made by means of a commercially available telethermometer (YSI-2100, Yellow Springs Instruments Co, Yellow Springs, OH, USA) and an accompanying probe (YSI-402). A precise description of how the measurements were done and the data analyzed is given in paper III.

3.1.9 Transfection of cells and measurements of cAMP formation

In paper II CHO cells transfected with either D_1 or D_5 receptors were used as a functional assay for D_1 and D_5 receptor activation, respectively. Since we did not use stably transfected cell lines there ought to be variations in transfection efficacy between assays. We therefore calculated a dose-response curve for DA in every assay and that curve was used to define top and bottom values for each assay. To be able to compare EC_{50} values between assays, the EC_{50} value for the agonist was related to the EC_{50} value of DA in the same assay. Details on transfection and measurements of cAMP are given in paper IIII.

3.1.10 In situ hybridization

For in situ hybridization 14 μm coronal sections were thaw-mounted on poly-L-lysine coated slides. In situ hybridization was performed as described by Svenningson et al (1998) and full details are given in paper I, II and III.. Sections were exposed on an autoradiographic film for approximately four weeks. Mean optical density in a given region was then measured using a PC-based system. To characterize cell populations in paper III co-localization of IEG expression and preproenkephalin mRNA was studied. Consecutive sections 6 μm thick were hybridized with the c-fos probe or the Enk probe. Sections were then dipped in emulsion (Amersham Pharmacia Biotech Inc.) and exposed for 20 weeks. Sections were compared based on morphology and individual neurons appearing on both sections were identified using a microscope with a digital camera attached to it. Cholinergic interneurons were identified by size. A detailed description of the technique and how the data were analyzed is given in paper III.

3.1.11 Receptor autoradiography

To construct competitive binding curves against [^{3}H]SCH 23390 brains were rapidly dissected out and frozen on dry ice. Sections were thaw-mounted on poly-L-lysine coated slides. The binding assay was performed as described in detail in paper III. In brief, slides were incubated with 1 nM [^{3}H]7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-ol (NEN^{TM} Life Science Products, Inc. Boston, MA, USA) and increasing concentrations of D_1/D_5 agonist, either SKF 82958 or A 68930. Slides were apposed to Hyperfilm-^{3}H (Amersham Pharmacia) for 6 months at 4ºC together with [^{3}H] standards (Amersham Pharmacia). Non-specific binding was defined by 100 μM DA.

4 RESULTS AND DISCUSSION

4.1 NPY AND THE DEVELOPMENT OF ABA

In paper I, we investigate if NPY contributes to the development of ABA. In other words: can treatment with NPY increase running activity and decrease food intake and body weight in food-restricted rats? This question is relevant since rats expressing ABA as well as patients suffering from anorexia nervosa have high levels of NPY in CNS (Kaye 1996; Goldstone et al. 2002). This is inconsistent with the prevailing view of NPY as an "orexigen".

In order to answer this question we first confirmed that the amount of wheel running is influenced by food restriction. We found that the decline in body weight over time was different between rats whose daily access to food was limited to different degrees. This simple experiment appears to have been done only once before (Terry et al. 2005) and suggests that loss of body weight is not directly related to the amount of food deprivation. Other factors, e.g., energy expenditure and physical activity, are likely to contribute. Brief daily access to food causes an increase in physical activity (Mistlberger 1994), but food intake is not directly related to the amount of physical activity (King, Tremblay and Blundell 1997). Also, there is not an immediate compensatory increase in food intake with an increase in energy expenditure (Stubbs and Tolkamp 2006). Perhaps for these reasons, the trend in the decline of body weight over time in rats that had access to running wheels was different among groups of rats that had different time limits on their daily food access. These observations underline the complexity of the relationship between energy intake and energy expenditure and suggest that manipulation of one or two of the neuroendocrine mechanisms mediating a behaviour such as food intake is unlikely to affect body weight more than marginally (Stubbs and Tolkamp 2006). As expected rats lost control over body weight if food was available for only 1 hour a day.

There was also an expected (Lewis et al. 1993) increase in NPY mRNA in the ARC, an area where NPY is thought to play a key role in the neuroendocrinology of body weight regulation (Elmquist et al. 2005). Injecting rats that were food restricted to 2 hours a day with ICV NPY increased running and decreased food intake compared to vehicle-injected rats (Fig 3). As a consequence, the NPY treated rats lost body weight.

Rats treated with vehicle however were able to maintain their body weight. Hence in a situation of reduced availability of food, NPY may not act as an "orexigen", but instead mediate other behavioral adaptations. The

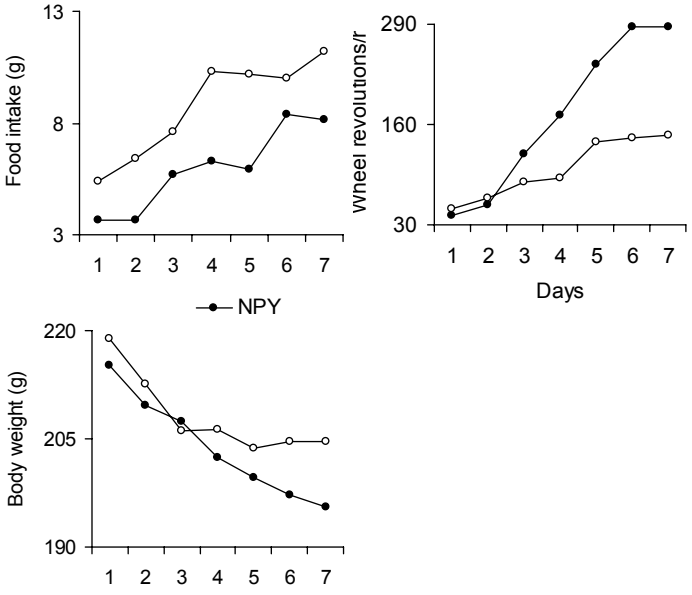

Fig. 3 . Body weight, food intake and wheel running in female rats with access to food during 2 hours/day and access to a running wheel. The rats were infused with 10 µg NPY or aCSF intracerebroventricularly at 09.00 a.m. Measures of variability are omitted tofacilitate visual inspection. There were 5-6 rats/group.

results in paper I and those of others (Day, Keen-Rhinehart and Bartness 2005) show that NPY can stimulate behaviors displayed in anticipation of food, such as physical activity, at the expense of reducing eating. A recent study supports this by showing that an NPY receptor antagonist reverses the stimulatory effect of food deprivation on wheel running for a food reward (Keen-Rhinehart and Bartness 2007). The physiological importance of this behavioral shift is obvious during starvation when the search for food is a priority.

4.2 CHALLENGE OF THE NOTION OF NPY AS AN "OREXIGEN"

Based on the results from paper I and other previous results (Ammar et al. 2000) showing that NPY decreases consummatory ingestive behavior (Sederholm, Ammar and Södersten 2002) we decided to compare NPY's effects on ingestive behavior to

those of CCK-8. CCK-8 is a well known suppressor of consummatory ingestive behavior as well as intake from a bottle (Mamoun, Bergström and Södersten 1997). We tested the hypothesis that NPY could interact with CCK-8 in inhibiting intra oral intake and that the site of interaction could be the NTS. NTS is the first central relay for ingestion-related neural afferents from the GI tract (Berthoud et al. 2001), receiving afferents via the vagus nerve. We also tested the effect of both peptides on intake from a bottle, a test that does not distinguish between appetitive and consummatory ingestive behavior.

In paper II we show that both CCK-8 and NPY have inhibitory effects on intraoral intake of a sucrose solution. Combined treatment with CCK-8 and NPY produced an even greater suppression of intra oral intake but this combined effect did not reach significance compared to the effect of CCK-8 alone. There was, as expected, a significant stimulatory effect of NPY on intake from a bottle and a significant inhibitory effect of CCK-8 in this test. In other words it seems as if NPY influences appetitive and consummatory ingestive behavior in opposite directions. CCK-8 and NPY exerted similar activating effects on c-fos expression in the NTS but there was no evidence of interaction at this level.

Appetitive ingestive behavior is a complex behaviour and many types of appetitive responses are dependent on forebrain function: consummatory ingestive behavior is not (Grill and Smith 1988). The findings in paper II are well in line with this and suggest that the effects of NPY on consummatory ingestive behavior, just like the CCK-8 effect, could be mediated via NTS in the brain stem.

4.3 EFFECTS OF D_1/D_5 RECEPTOR ACTIVATION ON PHYSICAL ACTIVITY AND BODY TEMPERATURE

There are several agonists and antagonists that can discriminate between the D_1- like and D_2-like receptors, but few agents are selective for individual members of the DA D_1 family of receptors (e.g., D_1 and D_5 receptors). Growing numbers of observations also show important behavioral differences between supposed DA D_1-family selective agonists (Desai, Terry and Katz 2003; Makihara et al. 2004). The isochronal A 68930 and the benzazepine SKF 82958 are two full DA D_1 receptor agonists. Responses to these compounds are different in several important aspects. SKF 82958 is reported to

increase physical activity (Le Moine et al. 1997) and A 68930 is reported to decrease physical activity (Salmi and Ahlenius 2000). In paper III, we have examined the effects of these two agonists on spontaneous locomotor activity of rats in an open-field arena. Since it is well known that a novel environment has effects on locomotion we investigated the effect of the two agonists in rats habituated to the test arena and in animals naïve to the test arena. We found distinct differences between the two agonists effectd on locomotion.

In naïve animals, A 68930 suppressed locomotor activity whereas SKF 82930 had no effect. Both agonists suppressed rearing in the naïve animals. In rats habituated to the test arena on the other hand, A 68930 had no effect on locomotor activity or rearing, while SKF 82930 produced a statistically significant increase in both locomotor activity and rearing. Since it is well known that DA D_1 receptor stimulation has effects on core temperature (Clark and Lipton 1985), we used this as an autonomic readout of DA receptor activation and show that the compounds differ also in this respect. We have related these differences in actions to expression of IEGs in the rat brain and observed differences between the two agonists' ability to induce c-fos and NGFI-A: both the magnitude and the pattern of IEG induction differed. To see if this could be due to activation of different cell populations, consecutive sections were hybridized to NGFI-A and ENK. The rationale for this is that in striatum, GABAergic medium-sized spiny neurons expressing ENK are those that project to substantia nigra pars reticulata and the entopeduncular nucleus via the external part of globus pallidus and subthalamic nucleus (Gerfen and Young 1988). This subpopulation of medium sized spiny GABAergic neurons has been shown to express functionally important DA D_2 receptors (Le Moine et al. 1990) but not D_1 receptors. DA D_1 receptors, on the other hand, have been shown to be strongly segregated to neurons expressing substance P/dynorphin but not preproenkephalin (Gerfen et al. 1990). Both agonists induced IEG expression in neurons that did not express Enk. We could not detect any induction of NGFI-A in Enk-positive medium-sized spiny neurons (strongly arguing against D_2 activation there), or in the large cholinergic interneurons.

To characterize the pharmacological differences, we performed quantitative receptor autoradiography on brain slices, looking for region-specific differences in competitive binding against SCH 23390. The idea was to compare binding curves from regions relatively rich in D_5 receptors, e.g., cortex and hippocampus, and regions relatively

poor in D_5 receptors, e.g., CPu (Ariano et al. 1997). Since binding experiments cannot tell us if the ligand is an agonist or an antagonist we also compared the functional agonist profile of the two agonists on cAMP formation in CHO cells transfected with D_1 or D_5 receptors.

The binding experiment provided no evidence for more than one binding site for either of the two agonists in any region measured. The largest difference between the two agonists' ability to displace SCH 23390 binding was seen in DG, where A 68930 was 13 times more potent than SKF 82958. The smallest difference was noted in CPu, where A 68930 was 9 times more potent than SKF 82958 in displacing SCH 23390. Thus, there are only subtle differences in the ability of A 68930 and SKF 82958 to displace SCH 23390 in different areas of the brain. There were, on the other hand, clear differences in their ability to induce cAMP formation in CHO cells transfected with D1 or D5 receptors. The functional assay also confirmed that the compounds are full agonists.

SCH 23390 binds with essentially the same affinity to both D_1 and D_5 receptors. The approximately 10-fold difference in potency of the two compounds as displacers of SCH 23390 agrees well with the finding that A 68930 was almost 10 times more potent in inducing cAMP formation in the D_1 transfected CHO cells. The autoradiography data thus only suggests that throughout the brain, SCH 23390 finds many more D_1 than D_5 receptors. This has important functional consequences as it is known that the potency of an agonist in a functional context depends not only on the affinity of the agonist to the receptor but also on the number of receptors present (Arslan, Kull and Fredholm 1999; Johansson et al. 2001; Kenakin 2002). Therefore, in order to activate both D_5 and D_1 receptors at a given dose, the agonist must be more potent at D_5 than at D_1 receptors (just like SKF 82930), because the latter are so much more abundant. In our functional assay this means that A 68930 will be an almost pure D_1 agonist, whereas SKF 82958, just like DA, will activate both D_1 and D_5 receptors to an approximately equal extent. This explains why SKF 82958 also in the behavioral readout has a profile more similar to DA. When examining the effect on core temperature we found that A 68930 is much more potent than SKF 82930. This then indicates that the acute effect on core temperature is almost exclusively mediated by D_1 and not D_5 receptors. We propose that D_5 receptors are not involved in body

temperature control and that this could provide a way to study pure D_1 effects of D_1-agonists that are not selective for D_1/D_5 receptors.

4.4 OREXIN AND THE DEVELOPMENT OF ABA, ROLE OF DA D_1 RECEPTORS

As described in the introduction, ABA is characterized by an increase in wheel running and a decrease in body weight. In paper IV we characterize the running pattern in ABA further, showing that the increased activity predominantly occurs during the normally sedentary LP of a 24 hour light darkness cycle (Fig. 11). This means that both the spatial and the temporal area available for the search for food are increased.

In mammals circadian rhythms are generated by the suprachiasmatic nucleus (SCN) (Ralph et al. 1990; Saper, Scammell and Lu 2005) and entrained to various environmental cues through complex neuronal networks involving several hypothalamic regions, including the LH (Reppert and Weaver 2002; Elmquist et al. 2005). In paper IV, we show that c-fos and the neuropeptide orexin are up-regulated in LH (Fig. 12). The orexin system integrates circadian-photic and nutritional metabolic influences to regulate the timing and consolidation of behavior like arousal and motivation (Selbach and Haas 2006). DA is one of the key players in this system and exerts some of its effects by acting in LH (Alberto et al. 2006). It has recently been shown that DA modulates excitatory presynaptic terminals impinging onto orexin neurons (Alberto et al. 2006). D_1-like receptors increase the frequency of this spontaneous excitatory transmission onto the orexin neurons, whereas D_2-like receptors diminish it. This is expected to alter their firing rate (Li et al. 2002). The cellular mechanism by which DA modulates the activity of orexin neurons remains, however, largely unknown. D_1 receptors are known to influence both locomotion and learning of new behavior. We and others (Salmi, Jimernez and Ahlenius 1993; Nergårdh, Oerther and Fredholm 2005) have also shown that D_1 receptor activation lowers body temperature, one of the characteristics of anorexia nervosa, and that this effect can be antagonized by the D_1 receptor antagonist SCH 23390 (Salmi et al. 1998). In paper IV we therefore try to modify the behavioral readout of starvation in the ABA model using the D_1 receptor family antagonist SCH 23390. Our hypothesis was that DA D_1/D_5 signaling is necessary for some of the behavioral changes induced by food restriction and that LH could be a site of action. Treatment with SCH 23390 prevented the

increase in LP running typical of ABA (Fig. 4a). It also prevented the induction of c-fos and orexin in the LH (Fig. 4b and c).

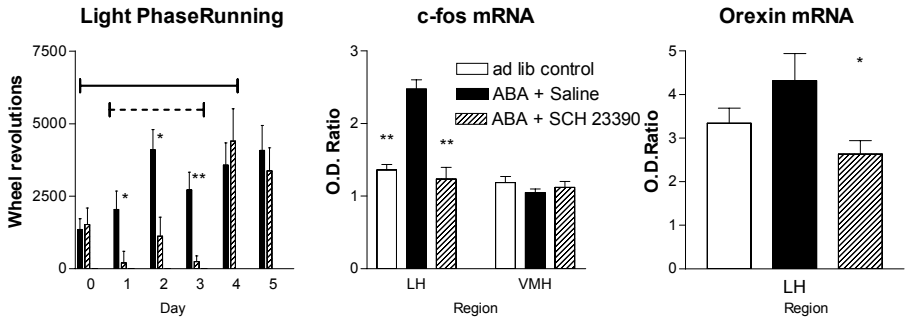

Fig. 4 Effects of SCH 23390 on the development of ABA (a). The solid line indicates days of food restriction. Dotted line indicates days when SCH 23390 was administrated. c-fos (b) and orexin (c) mRNA in LH in ad lib fed rats (n=5), in food restricted rats expressing ABA (n=5) and in food restricted SCH 23390 treated rats (n=4). Values are presented as mean ± S.E.M.

Our data support the idea that increased activity in orexin cells, induced by the negative energy balance in ABA, is necessary for maintaining arousal and activity during the normally sedentary time of the LD cycle and that this activity can be modified with a DA D_1 antagonist. A direct effect of SCH 23390 in the LH is supported by the presence in this structure of [^{3}H]SCH 23390 binding sites, displaceable by D_1 agonists, the isochroman A 68930 and the benzazepine SKF 82958. The potency of these compounds suggest that the receptors are D_1 rather then D_5 (Nergårdh et al. 2005). This suggests an important role for orexins in linking energy balance to vigilance state (Yamanaka et al. 2003).

5 GENERAL DISCUSSION

There is strong evidence that leptin and anorexigenic signals downstream of leptin do have effect on food intake. Indeed, the pronounced effects on body weight in leptin-deficent animals and humans are suggestive of a system with little redundancy. The marked obesity seen in leptin deficiency indicates that leptin signaling is a common path orchestrating many of the other signaling pathways. However, the fact that leptin deficient animals will become obese in environments that offer a constant supply of food and water does not necessarily mean that leptin has a role in preventing the animal from overeating, and the same is true for signaling pathways downstream of leptin. We propose that there is no evolutionary advantage in keeping body weight constant per se and therefore no neuroendocrine homeostatic control over body weight has developed. We propose an alternative explanation: when food supply is poor and body weight therefore decreases, neuroendocrine networks, in which leptin is a key player, adapt the animal's behavior in order to cope with the new environment. We would expect the behavior to be complexly regulated, and indeed this is reflected in the abundance of CNS regions and transmitter systems involved. It is therefore crucial that manipulations of those systems are studied under relevant conditions.

A peptide that increases food intake in rats housed in a cage with free access to food and water can of course play a much more complex role when it is given or endogenously released in a more complex natural environment. The experiments in this thesis are compatible with a scheme where peptides such as NPY have other roles than merely to regulate food intake. We introduced a measure of complexity by adding a running wheel. Obviously, the complexity added by introducing a running wheel is minor compared to the complex living environment of a free rat. Yet the minor manipulation of reducing the access to food and adding a running wheel shifts the effects of NPY on body weight from increase to decrease. Subtle factors such as whether the milieu is novel or familiar will change the effect of D1 agonists from motor stimulant till motor suppressive. The overall aim of all behavioral changes is probably to balance the energy scale so that in the long run, energy expenditure can be paid for by energy intake. In the short perspective this means that body weight must be allowed to fluctuate to allow a long term balance. There is simply no point in having an upper limit to the size of deposits in your bank account, but in times of crisis a credit card, or

saved capital can be of great help. This is illustrated by the abundant connections between the neuroendocrine systems involved in energy balance and the ones involved in reproduction (Crown, Clifton and Steiner 2007). Reproduction without enough energy reserves to insure the survival of offspring would be counterproductive. Therefore complex neuroendocrine mechanisms limit reproductive activity to situations where energy balance is positive and adequate nourishment of offspring is ensured.

However, this does not mean that body weight cannot be kept constant over long periods of time. In a milieu where a high level of physical activity is necessary to get food, a long term balance can be reached between energy intake and energy expenditure, even without an internal system to prevent obesity, and body weight can remain rather constant over time. The environmental factor prevents obesity. We find support for this idea in Aboriginals in Australia. In an environment with a constant need for physical activity (energy expenditure) and a limited supply of food (energy intake) the environment, not leptin, prevents an increase in BMI. It is, however, important to emphasize that leptin is by no means less important under these conditions. Without a functional system for sensing and signaling energy status to the CNS, necessary behavioral adaptations to handle the environment would be impossible. In fact the only situation under which a defect in this system is compatible with life is under conditions of very easy access to food with no need to search or hunt for it. It is therefore interesting to note that the prevalence of the ob/ob genotype is far higher in research labs than in nature. The fact that humans lacking leptin are hyperphagic, severely obese and respond dramatically to leptin administration are well in line with this. In the absence of leptin, neuroendocrine networks involved in energy-behavioral adaptation signal that energy stores are low. When exogenous leptin is given, the neuroendocrine signal system permits other energetically costly activities like growth and reproduction. We believe that this is also paralleled by a behavioral shift from food intake-oriented behavior. However, this does not mean that leptin prevents overeating. In fact it has been shown repeatedly that obese people do not eat significantly less if treated with leptin, a phenomenon often explained in terms of “leptin resistance”. This explanation underlines the difference in view: we propose that leptin is merely at the top of its physiological dose–response curve. There is simply no point in adding more of an agonist when signaling is already at its peak. This is a well known phenomenon in pharmacology and the concept of spare receptors has shown that this “peak” can be reached with fairly low concentrations of agonist. It is possible that some of the

signaling pathways downstream of leptin, like NPY, orexin and melanocortins, play a significant role only in states of low energetic reserves. This idea is supported by the fact that, although serum levels of LH are reduced by fasting in normal animals, a similar response is not seen in NPY knock out mice.

Humans and animals lacking leptin are obese. This is also true for humans and animals with genetic deletion of MC_4R. In fact it has been reported that up to 5% of cases of severe childhood obesity and 0.5 – 2.5% of adult obesity can be explained by MC_4R mutations (Larsen et al. 2005; Hinney et al. 2006) and are associated with the most common form of monogenic obesity. How should this be interpreted? How is this genotype expressed in milieus where food supply is restricted? Balthasar et al (Balthasar et al. 2005) have suggested that energy balance can be viewed as having the following three elements: (1) a sensory afferent arm receiving input from the gut, adipose tissue, and metabolic factors, (2) an integrative component where this sensory information is processed along with input from higher centers in the brain, and (3) an efferent arm that is postulated to split at some level to control food intake and energy expenditure. I agree with this description but propose that the afferent arm is far more variable and sophisticated. Since the readout of most experiments (food intake and body weight) is most closely linked to the afferent arm it is necessary, for understanding the physiological role of these systems, to study them under relevant conditions. Failure to do so could lead to futile treatment strategies like treating obese patients with leptin.

6 ACKNOWLEDGEMENTS

This work has mainly been carried out at Karolinska Institutet but also at University of Groningen. I would like to express my sincere gratitude to everyone who has contributed to this thesis. In particular I thank:

My supervisor, Professor Bertil B. Fredholm for introducing me to research and welcoming me to the very exciting milieu of your research group. Thank you for all the discussions, great ideas and careful analysis of my studies. Thank you also for your patience and understanding for my absence during periods of clinical work and education.

My co-supervisor Professor Per Södersten for your great knowledge and for all interesting and fun discussions. Thank you also for showing how clinical problems can be addressed by experimental research and how the results can be implemented in the clinic.

Karin Lindström-Törnqvist for helping me with laboratory work and for giving access to your working space and computer.

Björn Kull for all help and for sharing your great laboratory knowledge and skill.

Peter Salmi for sharing your knowledge on animal studies.

Per Svenningsson for all help.

Allan Johnsson for many interesting discussions and for your great generosity in sharing knowledge and ideas.

Janet Holmén for excellent help in proof-reading manuscripts and this thesis.

My co-authors for knowledge and technical skills: Ahmed Ammar, Sandra Oerther, Gretta Boersman, Ulf Brodin, Jonas Bergström, Anthon Scheurink,

All other members, former and present, of the Fredholm group: Eva Lindgren, Giulia Arslan, Gunnar Schulte, Agneta Wallman-Johansson, Ulrika Ådén, Linda Halldner-Henriksson, Stina Johansson, Olga Björklund, Björn Johansson.

All other members, former and present of the Södersten group and the staff at Anorexicentrum, AB Mando.

Nina Perrin, present head of Barnmedicin 1 at Karolinska University Hospital, for your support, interest and help and for being an excellent manager during my specialist training in pediatrics.

Svante Norgren, head of the DEMO-section at Karolinska University Hospital, for interest and help.

My colleagues and friends at Barnens sjukhus, Karolinska Universitetssjukhuset for support and interest.

My late mother Birgitta and my father Arne for your love and support.

My sister Anna and my brother Kristofer with their families for support.

My wonderful wife Petra and our sons Jakob and David for your love, care and patience. I love you!

7 REFERENCES

Abelson, P, and D. Kennedy. 2004. The obesity epidemic. *Science* 304:1413.

Ahima, R.S., D. Prabakaran, C. Mantzoros, D. Qu, B. Lowell, E. Maratos-Flier, and J.S. Flier. 1996. Role of leptin in the neuroendocrine response to fasting. *Nature* 382:250-252.

Albers, H.E, and C.F. Ferris. 1984. Neuropeptide Y: role in light-dark cycle entrainment of hamster circadian rhythms. *Neuroscience Letters* 50:163-168.

Alberto, C.O., R.B. Trask, M.E. Quinlan, and M. Hirasawa. 2006. Bidirectional dopaminergic modulation of excitatory synaptic transmission in orexin neurons. *Journal of Neuroscience* 26:10043-10050.

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

Allison, D.B., K.R. Fontaine, J.E. Manson, J. Stevens, and T.B. VanItallie. 1999. Annual deaths attributable to obesity in the United States. *JAMA* 282:1530-1538.

Ammar, A.A., R. Nergårdh, B.B. Fredholm, U. Brodin, and P. Södersten. 2005. Intake inhibition by NPY and CCK-8: A challenge of the notion of NPY as an "Orexigen". *Behavioural Brain Research* 161:82-87.

Ammar, A.A., F. Sederholm, T.R. Saito, A.J. Scheurink, A.E. Johnson, and P. Sodersten. 2000. NPY-leptin: opposing effects on appetitive and consummatory ingestive behavior and sexual behavior. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 278:R1627-R1633.

Anand, B.K, and J.R. Brobeck. 1951. Hypothalamic control of food intake in rats and cats. *Yale Journal of Biology and Medicine* 24:123-140.

Ariano, M.A., J. Wang, K.L. Noblett, E.R. Larson, and D.R. Sibley. 1997. Cellular distribution of the rat D1B receptor in central nervous system using anti-receptor antisera. *Brain Research* 746:141-150.

Arslan, G., B. Kull, and B.B. Fredholm. 1999. Signaling via A2A adenosine receptor in four PC12 cell clones. *Naunyn-Schmiedeberg's Archives of Pharmacology* 359:28-32.

Balthasar, N., L.T. Dalgaard, C.E. Lee, J. Yu, H. Funahashi, T. Williams, M. Ferreira, V. Tang, R.A. McGovern, C.D. Kenny, L.M. Christiansen, E. Edelstein, B. Choi, O. Boss, C. Aschkenasi, C.Y. Zhang, K. Mountjoy, T. Kishi, J.K. Elmquist, and B.B. Lowell. 2005. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123:493-505.

Banks, W.A., A.J. Kastin, W. Huang, J.B. Jaspan, and L.M. Maness. 1996. Leptin enters the brain by a saturable system independent of insulin. *Peptides* 17:305-311.

Benoit, S.C., D.J. Clegg, R.J. Seeley, and S.C. Woods. 2004. Insulin and leptin as adiposity signals. *Recent Progress in Hormone Research* 59:267-285.

Bernard, C. 1865. *An introduction to the study of experimental medicine*. New York: Dover Publications.

Berridge, K.C. 2007. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology* 191:391-431.

Berthoud, H.R., T. Earle, H. Zheng, L.M. Patterson, and C. Phifer. 2001. Food-related gastrointestinal signals activate caudal brainstem neurons expressing both NMDA and AMPA receptors. *Brain Research* 915:143-154.

Björklund, A, and O. Lindvall 1984. Dopamine Containing Systems in the CNS. In A. Björklund and T. Hökfelt, eds., *Handbook of chemical neuroanatomy*. Amsterdam: Elsevier., pp 55-122.

Blainey, J. 1993. *Triumph of the nomads. A history of aboriginal Australia*. Woodstock: The Overlook Press.

Cabanac, M, and A.H. Swiergiel. 1989. Rats eating and hoarding as a function of body weight and cost of foraging. *American Journal of Physiology* 257:R952-R957.

Cannon, W.B. 1932. *The wisdom of the body*. London: Kegan Paul, Trench, Trubner & Co.

Carlsson, A. 1959. The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacological Reviews* 11:490-493.

Challet, E., P. Pévet, and A. Malan. 1996. Intergeniculate leaflet lesion and daily rhythms in food-restricted rats fed during daytime. *Neuroscience Letters* 216:214-218.

Chemelli, R.M., J.T. Willie, C.M. Sinton, J.K. Elmquist, T. Scammell, C. Lee, J.A. Richardson, S.C. Williams, Y. Xiong, Y. Kisanuki, T.E. Fitch, M. Nakazato, R.E. Hammer, C.B. Saper, and M. Yanagisawa. 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell* 98:437-451.

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

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

Clark, W.G, and J.M. Lipton. 1985. Changes in body temperature after administration of amino acids, peptides, dopamine, neuroleptics and related agents: II. *Neuroscience and Biobehavioral Reviews* 9:299-371.

Coleman, D.L, and K.P. Hummel. 1969. Effects of parabiosis of normal with genetically diabetic mice. *American Journal of Physiology* 217:1298-1304.

Coll, A.P., I.S. Farooqi, B.G. Challis, G.S. Yeo, and S. O'Rahilly. 2004. Proopiomelanocortin and energy balance: insights from human and murine genetics. *Journal of Clinical Endocrinology and Metabolism* 89:2557-2562.

Coll, A.P., I.S. Farooqi, and S. O'Rahilly. 2007. The hormonal control of food intake. *Cell* 129:251-262.

Corbett, S.W., L.N. Kaufman, and R.E. Keesey. 1988. Thermogenesis after lateral hypothalamic lesions: contributions of brown adipose tissue. *American Journal of Physiology* 255:E708-E715.

Corp, E.S., L.D. Melville, D. Greenberg, J. Gibbs, and G.P. Smith. 1990. Effect of fourth ventricular neuropeptide Y and peptide YY on ingestive and other behaviors. *American Journal of Physiology* 259:R317-R323.

Craig, W. 1917. Appetites and Aversions as Constituents of Instincts. *Proceedings of the National Academy of Sciences of the United States of America* 3:685-688.

Crown, A., D.K. Clifton, and R.A. Steiner. 2007. Neuropeptide signaling in the integration of metabolism and reproduction. *Neuroendocrinology* 86:175-182.

Day, D.E., E. Keen-Rhinehart, and T.J. Bartness. 2005. Role of NPY and its receptor subtypes in foraging, food hoarding, and food intake by Siberian hamsters. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 289:R29-R36.

Desai, R.I., P. Terry, and J.L. Katz. 2003. Comparison of the discriminative-stimulus effects of SKF 38393 with those of other dopamine receptor agonists. *Behavioural Pharmacology* 14:223-228.

Dixon, D.P., A.M. Ackert, and L.A. Eckel. 2003. Development of, and recovery from, activity-based anorexia in female rats. *Physiology & Behavior* 80:273-279.

Druce, M, and S.R. Bloom. 2006. The regulation of appetite. *Archives of Disease in Childhood* 91:183-187.

Dubé, C. 2007. Neuropeptide Y: potential role in recurrent developmental seizures. *Peptides* 28:441-446.

Elmquist, J.K., R. Coppari, N. Balthasar, M. Ichinose, and B.B. Lowell. 2005. Identifying hypothalamic pathways controlling food intake, body weight, and glucose homeostasis. *Journal of Comparative Neurology* 493:63-71.

Estabrooke, I.V., M.T. McCarthy, E. Ko, T.C. Chou, R.M. Chemelli, M. Yanagisawa, C.B. Saper, and T.E. Scammell. 2001. Fos expression in orexin neurons varies with behavioral state. *Journal of Neuroscience* 21:1656-1662.

Eva, C., M. Serra, P. Mele, G. Panzica, and A. Oberto. 2006. Physiology and gene regulation of the brain NPY Y1 receptor. *Frontiers in Neuroendocrinology* 27:308-339.

Fetissov, S.O., M.M. Meguid, T. Sato, and L.H. Zhang. 2002. Expression of dopaminergic receptors in the hypothalamus of lean and obese Zucker rats and food intake. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 283:R905-R910.

Flood, R. 1998.DEFRA.

Gerfen, C.R., T.M. Engber, L.C. Mahan, Z. Susel, T.N. Chase, F.J. Monsma Jr, and D.R. Sibley. 1990. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 250:1429-1432.

Gerfen, C.R, and W.S. Young. 1988. Distribution of striatonigral and striatopallidal peptidergic neurons in both patch and matrix compartments: an in situ hybridization histochemistry and fluorescent retrograde tracing study. *Brain Research* 460:161-167.

Gingrich, J.A, and M.G. Caron. 1993. Recent advances in the molecular biology of dopamine receptors. *Annual Review of Neuroscience* 16:299-321.

Goldstone, A.P., U.A. Unmehopa, S.R. Bloom, and D.F. Swaab. 2002. Hypothalamic NPY and agouti-related protein are increased in human illness but not in Prader-Willi syndrome and other obese subjects. *Journal of Clinical Endocrinology and Metabolism* 87:927-937.

Grill, HJ, AC Spector, GJ Schwartz, JM Kaplan, and F.W. Flynn 1987. Evaluating taste effects on ingestive behavior. In FM Toates and N.E. Rowlands, eds., *Feeding and drinking*. Amsterdam: Elsevier., pp 151-158.

Grill, H.J, and J.M. Kaplan. 2002. The neuroanatomical axis for control of energy balance. *Frontiers in Neuroendocrinology* 23:2-40.

Grill, H.J, and R. Norgren. 1978. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. *Brain Research* 143:263-279.

Grill, H.J, and G.P. Smith. 1988. Cholecystokinin decreases sucrose intake in chronic decerebrate rats. *American Journal of Physiology* 254:R853-R856.

Hebebrand, J., T.D. Muller, K. Holtkamp, and B. Herpertz-Dahlmann. 2007. The role of leptin in anorexia nervosa: clinical implications. *Molecular Psychiatry* 12:23-35.

Hedley, A.A., C.L. Ogden, C.L. Johnson, M.D. Carroll, L.R. Curtin, and K.M. Flegal. 2004. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. *JAMA* 291:2847-2850.

Hill, J.O., H.R. Wyatt, G.W. Reed, and J.C. Peters. 2003. Obesity and the environment: where do we go from here? *Science* 299:853-855.

Hinney, A., T. Bettecken, P. Tarnow, H. Brumm, K. Reichwald, P. Lichtner, A. Scherag, T.T. Nguyen, P. Schlumberger, W. Rief, C. Vollmert, T. Illig, H.E. Wichmann, H. Schäfer, M. Platzer, H. Biebermann, T. Meitinger, and J. Hebebrand. 2006. Prevalence, spectrum, and functional characterization of melanocortin-4 receptor gene mutations in a representative population-based sample and obese adults from Germany. *Journal of Clinical Endocrinology and Metabolism* 91:1761-1769.

Hökfelt, T., J.M. Lundberg, K. Tatemoto, V. Mutt, L. Terenius, J. Polak, S. Bloom, C. Sasek, R. Elde, and M. Goldstein. 1983. Neuropeptide Y (NPY)- and FMRFamide neuropeptide-like immunoreactivities in catecholamine neurons of the rat medulla oblongata. *Acta Physiologica Scandinavica* 117:315-318.

Holmes, A., T.R. Hollon, T.C. Gleason, Z. Liu, J. Dreiling, D.R. Sibley, and J.N. Crawley. 2001. Behavioral characterization of dopamine D5 receptor null mutant mice. *Behavioral Neuroscience* 115:1129-1144.

Ivy, A.C, and Eric Oldberg. 1928. A hormone mechanism for gall-bladder contraction and evacuation. *American Journal of Physics* 86:599-613.

Johansson, B., L. Halldner, T.V. Dunwiddie, S.A. Masino, W. Poelchen, L. Giménez-Llort, R.M. Escorihuela, A. Fernández-Teruel, Z. Wiesenfeld-Hallin, X.J. Xu, A. Hårdemark, C. Betsholtz, E. Herlenius, and B.B. Fredholm. 2001. Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. *Proceedings of the National Academy of Sciences of the United States of America* 98:9407-9412.

Kamiji, M.M, and A. Inui. 2007. Neuropeptide y receptor selective ligands in the treatment of obesity. *Endocrine Reviews* 28:664-684.

Kaye, W.H. 1996. Neuropeptide abnormalities in anorexia nervosa. *Psychiatry Research* 62:65-74.

Keen-Rhinehart, E, and T.J. Bartness. 2007. NPY Y1 receptor is involved in ghrelin- and fasting-induced increases in foraging, food hoarding, and food intake. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* 292:R1728-R1737.

Kenakin, T. 2002. Drug efficacy at G protein-coupled receptors. *Annual Review of Pharmacology and Toxicology* 42:349-379.

Khan, Z.U., A. Gutiérrez, R. Martin, A. Penafiel, A. Rivera, and A. De La Calle. 2000. Dopamine D5 receptors of rat and human brain. *Neuroscience* 100:689-699.

King, N.A., A. Tremblay, and J.E. Blundell. 1997. Effects of exercise on appetite control: implications for energy balance. *Medicine and Science in Sports and Exercise* 29:1076-1089.

Larsen, L.H., S.M. Echwald, T.I. Sørensen, T. Andersen, B.S. Wulff, and O. Pedersen. 2005. Prevalence of mutations and functional analyses of melanocortin 4 receptor variants identified among 750 men with juvenile-onset obesity. *Journal of Clinical Endocrinology and Metabolism* 90:219-224.

Le Moal, M, and H. Simon. 1991. Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiological Reviews* 71:155-234.

Le Moine, C., E. Normand, A.F. Guitteny, B. Fouque, R. Teoule, and B. Bloch. 1990. Dopamine receptor gene expression by enkephalin neurons in rat forebrain. *Proceedings of the National Academy of Sciences of the United States of America* 87:230-234.

Le Moine, C., P. Svenningsson, B.B. Fredholm, and B. Bloch. 1997. Dopamine-adenosine interactions in the striatum and the globus pallidus: inhibition of striatopallidal neurons through either D2 or A2A receptors enhances D1 receptor-mediated effects on c-fos expression. *Journal of Neuroscience* 17:8038-8048.

Leibowitz, S.F, and L.L. Brown. 1980. Histochemical and pharmacological analysis of noradrenergic projections to the paraventricular hypothalamus in relation to feeding stimulation. *Brain Research* 201:289-314.

Lewis, D.E., L. Shellard, D.G. Koeslag, D.E. Boer, H.D. McCarthy, P.E. McKibbin, J.C. Russell, and G. Williams. 1993. Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *American Journal of Physiology* 264:E279-E284.

Li, Y., X.B. Gao, T. Sakurai, and A.N. van den Pol. 2002. Hypocretin/Orexin excites hypocretin neurons via a local glutamate neuron-A potential mechanism for orchestrating the hypothalamic arousal system. *Neuron* 36:1169-1181.

Lin, L., J. Faraco, R. Li, H. Kadotani, W. Rogers, X. Lin, X. Qiu, P.J. de Jong, S. Nishino, and E. Mignot. 1999. The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* 98:365-376.

Lin, S., D. Boey, and H. Herzog. 2004. NPY and Y receptors: lessons from transgenic and knockout models. *Neuropeptides* 38:189-200.

Lindén, A, and P. Södersten. 1990. Relationship between the concentration of cholecystokinin-like immunoreactivity in plasma and food intake in male rats. *Physiology & Behavior* 48:859-863.

López, M., S. Tovar, M.J. Vázquez, L.M. Williams, and C. Diéguez. 2007. Peripheral tissue-brain interactions in the regulation of food intake. *Proceedings of the Nutrition Society* 66:131-155.

Louis, G.W, and M.G. Myers. 2007. The role of leptin in the regulation of neuroendocrine function and CNS development. *Reviews in Endocrine & Metabolic Disorders* 8:85-94.

Luckman, S.M., R.E. Dyball, and G. Leng. 1994. Induction of c-fos expression in hypothalamic magnocellular neurons requires synaptic activation and not simply increased spike activity. *Journal of Neuroscience* 14:4825-4830.

Luedtke, R.R., S.A. Griffin, S.S. Conroy, X. Jin, A. Pinto, and S.R. Sesack. 1999. Immunoblot and immunohistochemical comparison of murine monoclonal antibodies specific for the rat D1a and D1b dopamine receptor subtypes. *Journal of Neuroimmunology* 101:170-187.

Lundberg, J.M., L. Terenius, T. Hökfelt, C.R. Martling, K. Tatemoto, V. Mutt, J. Polak, S. Bloom, and M. Goldstein. 1982. Neuropeptide Y (NPY)-like immunoreactivity in peripheral noradrenergic neurons and effects of NPY on sympathetic function. *Acta Physiologica Scandinavica* 116:477-480.

Lundberg, J.M., L. Terenius, T. Hökfelt, and K. Tatemoto. 1984. Comparative immunohistochemical and biochemical analysis of pancreatic polypeptide-like peptides with special reference to presence of neuropeptide Y in central and peripheral neurons. *Journal of Neuroscience* 4:2376-2386.

Makihara, Y., H. Yamamoto, M. Inoue, K. Tomiyama, N. Koshikawa, and J.L. Waddington. 2004. Topographical effects of D1-like dopamine receptor agonists on orofacial movements in mice and their differential regulation via oppositional versus synergistic D1-like: D2-like interactions: cautionary observations on SK&F 82958 as an anomalous agent. *Journal of Psychopharmacology* 18:484-495.

Mamoun A.H., Anderstam B., Bergström J., Qureshi G.A. and Södersten P. (1995) Diet-independent suppression of ingestive behavior by cholecystokinin octapeptide and amino acids. *Am J Physiol* 268, R520-R527.

Mamoun, A.H., J. Bergström, and P. Södersten. 1997. Cholecystokinin octapeptide inhibits carbohydrate but not protein intake. *American Journal of Physiology* 273:R972-R980.

Manne, J., A.C. Argeson, and L.D. Siracusa. 1995. Mechanisms for the pleiotropic effects of the agouti gene. *Proceedings of the National Academy of Sciences of the United States of America* 92:4721-4724.

Mistlberger, R.E. 1994. Circadian food-anticipatory activity: formal models and physiological mechanisms. *Neuroscience and Biobehavioral Reviews* 18:171-195.

Moran T.H., Ameglio P.J., Schwartz G.J. and McHugh P.R. (1992) Blockade of type A, not type B, CCK receptors attenuates satiety actions of exogenous and endogenous CCK. *Am J Physiol* 262, R46-R50.

Moran T.H., Robinson P.H., Goldrich M.S. and McHugh P.R. (1986) Two brain cholecystokinin receptors: implications for behavioral actions. *Brain Research* 362, 175-179.

Morgan, J.I, and T. Curran. 1991. Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annual Review of Neuroscience* 14:421-451.
Morgan, J.I, and T. Curran. 1995. Immediate-early genes: ten years on. *Trends in Neurosciences* 18:66-67.
Mutt, V, and E. Jorpes. 1971. Hormonal polypeptides of the upper intestine. *Biochemical Journal* 125:57P-58P.
Nergårdh, R., S. Oerther, and B.B. Fredholm. 2005. Differences between A 68930 and SKF 82958 could suggest synergistic roles of D(1) and D(5) receptors. *Pharmacology, Biochemistry, and Behavior* 82:495-505.
Nicola, S.M., J. Surmeier, and R.C. Malenka. 2000. Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annual Review of Neuroscience* 23:185-215.
Nillius, S.J., H. Fries, and L. Wide. 1975. Successful induction of follicular maturation and ovulation by prolonged treatment with LH-releasing hormone in women with anorexia nervosa. *American Journal of Obstetrics and Gynecology* 122:921-928.
Nixon, J.P, and L. Smale. 2004. Individual differences in wheel-running rhythms are related to temporal and spatial patterns of activation of orexin A and B cells in a diurnal rodent (Arvicanthis niloticus). *Neuroscience* 127:25-34.
O'Dea, K., N.G. White, and A.J. Sinclair. 1988. An investigation of nutrition-related risk factors in an isolated Aboriginal community in northern Australia: advantages of a traditionally-orientated life-style. *Medical Journal of Australia* 148:177-180.
Ohno, K, and T. Sakurai. 2008. Orexin neuronal circuitry: role in the regulation of sleep and wakefulness. *Frontiers in Neuroendocrinology* 29:70-87.
Ollmann, M.M., B.D. Wilson, Y.K. Yang, J.A. Kerns, Y. Chen, I. Gantz, and G.S. Barsh. 1997. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 278:135-138.
Paxinos, G, and C. Watson. 1998. *North Ryde, Australia: Academic Press; 1998*
Periwal, V, and C.C. Chow. 2006. Patterns in food intake correlate with body mass index. *American Journal of Physiology. Endocrinology and Metabolism* 291:E929-E936.
Peyron, C., J. Faraco, W. Rogers, B. Ripley, S. Overeem, Y. Charnay, S. Nevsimalova, M. Aldrich, D. Reynolds, R. Albin, R. Li, M. Hungs, M. Pedrazzoli, M. Padigaru, M. Kucherlapati, J. Fan, R. Maki, G.J. Lammers, C. Bouras, R. Kucherlapati, S. Nishino, and E. Mignot. 2000. A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nature Medicine* 6:991-997.
Qian, M., G.S. Wu, A. Adem, A.E. Johnson, and P. Södersten. 1999. CCK-8 can inhibit ingestive behavior by acting on the liver. *Neuroreport* 10:359-362.
Ralph, M.R., R.G. Foster, F.C. Davis, and M. Menaker. 1990. Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247:975-978.
Reppert, S.M, and D.R. Weaver. 2002. Coordination of circadian timing in mammals. *Nature* 418:935-941.
Rosenbaum, M., M. Nicolson, J. Hirsch, S.B. Heymsfield, D. Gallagher, F. Chu, and R.L. Leibel. 1996. Effects of gender, body composition, and menopause

on plasma concentrations of leptin. *Journal of Clinical Endocrinology and Metabolism* 81:3424-3427.

Routtenberg, A, and A.W. Kuznesof. 1967. Self-starvation of rats living in activity wheels on a restricted feeding schedule. *Journal of Comparative and Physiological Psychology* 64:414-421.

Sakurai, T., A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S.C. Williams, J.A. Richardson, G.P. Kozlowski, S. Wilson, J.R. Arch, R.E. Buckingham, A.C. Haynes, S.A. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J.A. Terrett, N.A. Elshourbagy, D.J. Bergsma, and M. Yanagisawa. 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92:573-585.

Salmi, P, and S. Ahlenius. 2000. Sedative effects of dopamine D1 receptor agonist A 68930 on open-field behavior. *Neuroreport* 11:1269-1272.

Salmi, P., P. Jimernez, and S. Ahlenius. 1993. Evidence for specific involvement of dopamine D1 and D2 receptors in the regulation of body temperature in the rat.. *European Journal of Pharmacology* 236:395-400.

Salmi, P., K. Malmgren, T.H. Svensson, and S. Ahlenius. 1998. Stimulation of forward locomotion by SCH-23390 and raclopride in d-amphetamine-treated rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 357:593-599.

Saper, C.B., T.E. Scammell, and J. Lu. 2005. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437:1257-1263.

Scheurink, A.J., A.A. Ammar, B. Benthem, G. van Dijk, and P.A. Södersten. 1999. Exercise and the regulation of energy intake. *International Journal of Obesity* 23 Suppl 3:S1-S6.

Schulkin, J. 2007. Curt Richter: psychobiology and the concept of instinct. *History of Psychology* 10:325-343.

Schwartz, M.W., R.J. Seeley, L.A. Campfield, P. Burn, and D.G. Baskin. 1996. Identification of targets of leptin action in rat hypothalamus. *Journal of Clinical Investigation* 98:1101-1106.

Sederholm, F., A.A. Ammar, and P. Södersten. 2002. Intake inhibition by NPY: role of appetitive ingestive behavior and aversion. *Physiology & Behavior* 75:567-575.

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

Selbach, O, and H.L. Haas. 2006. Hypocretins: the timing of sleep and waking. *Chronobiology International* 23:63-70.

Shutter, J.R., M. Graham, A.C. Kinsey, S. Scully, R. Lüthy, and K.L. Stark. 1997. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes & Development* 11:593-602.

Sibley, D.R. 1999. New insights into dopaminergic receptor function using antisense and genetically altered animals. *Annual Review of Pharmacology and Toxicology* 39:313-341.

Spear, N.N, and W.F. Hill. 1962. Methodological note: weight loss in rats living in running wheel cages. *Psychological Reports* 11:437-438.

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

Stellar, E. 1954. The physiology of motivation. *Psychological Review* 61:5-22.

Stubbs, R.J, and B.J. Tolkamp. 2006. Control of energy balance in relation to energy intake and energy expenditure in animals and man: an ecological perspective. *British Journal of Nutrition* 95:657-676.

Svenningsson, P., R. Nergårdh, and B.B. Fredholm. 1998. Regional differences in the ability of caffeine to affect haloperidol-induced striatal c-fos mRNA expression in the rat. *Neuropharmacology* 37:331-337.

Tartaglia, L.A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G.J. Richards, L.A. Campfield, F.T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarty, K.J. Moore, J.S. Smutko, G.G. Mays, E.A. Wool, C.A. Monroe, and R.I. Tepper. 1995. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83:1263-1271.

Tatemoto, K., M. Carlquist, and V. Mutt. 1982. Neuropeptide Y--a novel brain peptide with structural similarities to peptide YY and pancreatic polypeptide. *Nature* 296:659-660.

Teitelbaum, P, and A.N. Epstein. 1962. The lateral hypothalamic syndrome: recovery of feeding and drinking after lateral hypothalamic lesions. *Psychological Review* 69:74-90.

Terry, K.K., L.A. Chatman, G.L. Foley, E. Kadyszewski, T.L. Fleeman, M.E. Hurtt, and R.E. Chapin. 2005. Effects of feed restriction on fertility in female rats. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology* 74:431-441.

Ungerstedt, U. 1971. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum* 367:95-122.

Waddington, J.L., J.J. Clifford, F.N. McNamara, K. Tomiyama, N. Koshikawa, and D.T. Croke. 2001. The psychopharmacology-molecular biology interface: exploring the behavioural roles of dopamine receptor subtypes using targeted gene deletion ('knockout'). *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 25:925-964.

Waddington, J.L., S.A. Daly, R.P. Downes, A.M. Deveney, P.G. McCauley, and K.M. O'Boyle. 1995. Behavioural pharmacology of 'D-1-like' dopamine receptors: further subtyping, new pharmacological probes and interactions with 'D-2-like' receptors. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 19:811-831.

Wang, J., R. Liu, M. Hawkins, N. Barzilai, and L. Rossetti. 1998. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 393:684-688.

Wang, Q., C. Bing, K. Al-Barazanji, D.E. Mossakowaska, X.M. Wang, D.L. McBay, W.A. Neville, M. Taddayon, L. Pickavance, S. Dryden, M.E. Thomas, M.T. McHale, I.S. Gloyer, S. Wilson, R. Buckingham, J.R. Arch, P. Trayhurn, and G. Williams. 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.

Wang, T., C.C. Hung, and D.J. Randall. 2006a. The comparative physiology of food deprivation: from feast to famine. *Annual Review of Physiology* 68:223-251.

Wang, Tobias, Carrie C.Y. Hung, and David Y. Randall. 2006b. THE COMPARATIVE PHYSIOLOGY OF FOOD DEPRIVATION: From Feast to Famine. *Annual Review of Physiology* 68:223-251.

Watts, A.G., A.M. Khan, G. Sanchez-Watts, D. Salter, and C.M. Neuner. 2006. Activation in neural networks controlling ingestive behaviors: what does it mean, and how do we map and measure it? *Physiology & Behavior* 89:501-510.

Watts, A.G, and L.W. Swanson 2002. Anatomy of motivational systems. In Hal Pashler and Randy Gallistel, eds., *"Stevens" handbook of experimental psychology, learning, motivation and emotion*. : John Wiley and Sons., pp 563-632.

White, N.G. 1985. Sex differences in Australian Aboriginal subsistence: possible implications for the of hunter-gatherers. In F. Newcomb, J. Ghesquierre and R. Martin, eds., *Human sexual dimorphism*. London: Taylor & Francis., pp 148-153.

Wise, R.A., J. Spindler, H. deWit, and G.J. Gerberg. 1978. Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. *Science* 201:262-264.

Xu, B., P.S. Kalra, W.G. Farmerie, and S.P. Kalra. 1999. Daily changes in hypothalamic gene expression of neuropeptide Y, galanin, proopiomelanocortin, and adipocyte leptin gene expression and secretion: effects of food restriction. *Endocrinology* 140:2868-2875.

Yamanaka, A., C.T. Beuckmann, J.T. Willie, J. Hara, N. Tsujino, M. Mieda, M. Tominaga, K. Yagami, F. Sugiyama, K. Goto, M. Yanagisawa, and T. Sakurai. 2003. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38:701-713.

Yamanaka, A., K. Kunii, T. Nambu, N. Tsujino, A. Sakai, I. Matsuzaki, Y. Miwa, K. Goto, and T. Sakurai. 2000. Orexin-induced food intake involves neuropeptide Y pathway. *Brain Research* 859:404-409.

Yancey, A.K., C.M. Wold, W.J. McCarthy, M.D. Weber, B. Lee, P.A. Simon, and J.E. Fielding. 2004. Physical inactivity and overweight among Los Angeles County adults. *American Journal of Preventive Medicine* 27:146-152.

Yannielli, P.C, and M.E. Harrington. 2001. Neuropeptide Y in the mammalian circadian system: effects on light-induced circadian responses. *Peptides* 22:547-556.

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