The Y of hYperactivitY in *Anorexia Nervosa*

Role of neuropeptide Y in hyperactivity associated with Anorexia Nervosa

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Abstract

Anorexia Nervosa is a dramatic neuro-psychiatric disorder characterized by severe selective restriction of food intake, hypophagia and hypothermia but also behavioral hyperactivity. Behavioral hyperactivity aggravates the already serious food-deprived state of anorexia patients, hinders body-weight gain and increases the probability of relapse; therefore, it is an important target in the treatment of anorexia nervosa. The activity-based-anorexia (ABA) model of anorexia and is extensively used to investigate the underlying neurobiological systems involved in hyperactivity. The hypothalamus is considered the major brain site for regulation energy metabolism, because it can assess the immediate energy state of the organism and consists of several nuclei. These different nuclei communicate with each other by using neurotransmitters and neuropeptides. One of these neuropeptides is neuropeptide Y (NPY); it is considered to most potent orexigenic neuropeptide known and it heavily innervates the hypothalamic nuclei. This neuropeptide increases feeding behavior, is upregulated in response to food-deprivation and acts via its abundant, centrally spread Y1, Y2 and Y5 receptors. In anorexia patients, high levels of NPY have been found, however, these patients do not eat. This phenomenon is termed the “NPY-paradox”. In addition, most of these anorexia patients display high levels of physical activity, despite their severe state of emaciation.

The aim of this thesis was to investigate the potential role through which NPY contributes to hyperactivity associated with anorexia nervosa. First the possible underlying neurobiological systems involved in this behavior were described. Second, the existing hypotheses about function of hyperactivity in anorexia were described. These hypotheses include, the “foraging hypothesis”, the “hypothermia hypothesis” and the “reward hypothesis”. Furthermore, the possible role of NPY in these hypotheses was investigated with special attention to the NPY receptors. Based on the evidence provided by recent studies the most appropriate hypothesis seems to be a combination of the “foraging hypothesis” and the “reward hypothesis”. The role of NPY in this combined hypothesis is that of activating the neuro-endocrine systems involved in foraging behavior, to down regulate thermogenesis and to activate reward pathways to reinforce this behavior adjacent to direct pathways affecting these mechanisms.

Outline

Chapter 1: The brain, energy homeostasis, neuropeptide Y and *Anorexia Nervosa*

1.1 Anorexia Nervosa and hyperactivity

Anorexia Nervosa is a dramatic neuro-psychiatric disorder characterized by severe selective restriction of food intake, hypophagia and hypothermia. This disease has a high prevalence among young adolescent females (14-19 years), results in extreme body weight loss and has a mortality rate of up to 15%. Several other symptoms characterize anorexia patients, although these symptoms are not shown by the whole anorexia population (Kas et al., 2003). These symptoms include several types of anxiety disorders (Godart et al., 2002) and compulsion and obsession disorders (Halmi et al., 2003).

Behavioral hyperactivity is also considered an important characteristic of this disease (Hebebrand et al., 2003). Hyperactivity is observed in 31-80% of all anorexia patients, although not every anorexia patient displays hyperactivity (Beumont et al., 1994; Hebebrand et al., 2003). Hyperactivity has several forms and durations, ranging from excessive walking, fidgeting and motor restlessness to compulsive exercising (Casper, 2006). Mental alertness and continued normal to high activity levels in the presence of a negative energy balance and weight loss are considered relatively unique to AN patients as compared to individuals with semi-starvation due to other causes (Hebebrand et al., 2003). Many hospitalized anorexia nervosa patients with instruction to stop exercising require close supervision and will even expend energy even when they are confined to bed (Beumont 1994). As hyperactivity aggravates the already serious underweight of anorexia patients, hinders body-weight gain and increases the probability of relapse, it is an important target in the treatment of anorexia nervosa (Hillebrand et al., 2008).

1.2 How to study Anorexia Nervosa in laboratory animals: the ABA-model

Investigating the underlying mechanisms regarding anorexia nervosa in order to find possible treatments requires an animal model that shows specific symptoms of this disease. The activity-based-anorexia model (ABA-model) or semi-starvation induced hyperactivity (SIH) is an animal model of anorexia nervosa which mimics several of its symptoms, including voluntary caloric restriction (with time-restricted feeding) combined with excessive exercise resulting in extreme weight loss and decreased body fat and the significant risk of mortality if intervention does not take place (Epling et al., 1983; Routtenberg et al., 1967). In this model, rodents are food restricted (60-120 min/day) and have voluntary access to a running wheel. This paradigm leads to an increase in running-wheel activity up to 300-500% and a decrease in food-intake, which causes substantial body weight loss (> 20%) within a few days of caloric restriction. The animals also show hypothermia, loss of estrous cycle and stomach ulceration when body weight loss > 30% (Hillebrand et al., 2008). It has been found that mice with different genetic backgrounds display different amounts of running-wheel activity, suggesting that hyperactivity in the ABA-model has a genetic component as well (Kas et al., 2009).

In addition to an increased running-wheel activity in the ABA animals, also the distribution of activity throughout the day is disturbed. Often during the light fase, high levels of running-wheel activity are observed just before the animals were allowed to eat. This phenomenon is called food-anticipatory hyperactivity (Mistlberger 1994). In contrast to ABA animals, *ad libitum* fed animals with continuous access to running wheels show stable levels of running-wheel activity without food-anticipatory activity and eat more to compensate for the increased running. Control animals on the same restriction schedule as ABA animals, without a running-wheel, eat more in the time the food is present and weight loss is not so severe (Kas et al., 2003; Hillebrand et al., 2005).

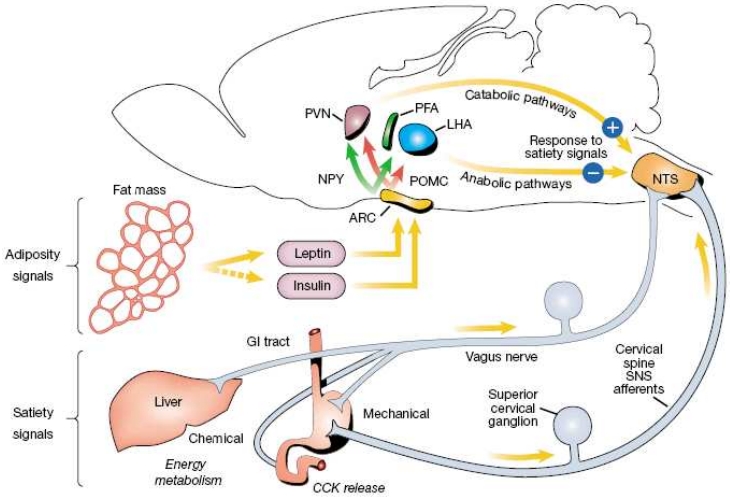
When food is *ad libitum* available to ABA animals, this will quickly reduce running-wheel activity and increase eating and body weight. So in contrast to humans, the anorexia in laboratory animals can be easily rescued (Dixon et al., 2003). This model is considered to have obvious face validity for anorexia nervosa as it reflects the negative relationship between exercise and feeding also found in the human condition (Casper, 2006). All changes in running and feeding are intrinsically motivated and not externally imposed by the experimenter, which is a large benefit of this animal model (Adams et

al., 2009).

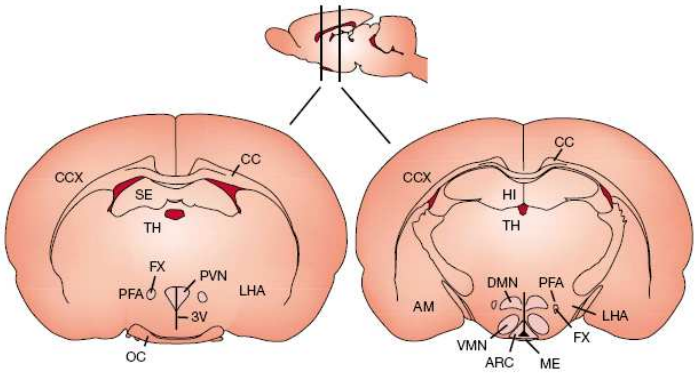
1.3 Energy homeostasis and neuropeptide Y

In order to understand the possible neurobiological mechanisms underlying AN, one first needs to know the complex systems involved in normal energy metabolism. Energy balance in mammals is a highly regulated physiological process and is maintained by a homeostatic system involving both the CNS and peripheral organs (Fig. 1a).

Figure 1: Neuroanatomical pathways in regulating feeding behavior (adapted from Schwartz et al., 2000)



1a. Peripheral and central pathways that regulate feeding behavior.



1b. Diagram showing the hypothalamic structures of the rat brain implicated in feeding behavior. The small picture on top is a longitudinal view of a rat brain (hindbrain on the right, olfactory bulb on the left). Cross-sections of the brain are made on two levels and indicated by vertical Lines. ARC: arcuate nucleus, PVN: paraventricular nucleus, PFA: perifornical area, FX: fornix, LHA: lateral hypothalamic area, VMN: ventromedial nucleus, DMN: dorsomedial nucleus. Other abbreviations: AM: amygdala, CC: corpus callosum, CCX: cerebral cortex, HI: hippocampus, ME: median eminence, OC: optic chiasm, SE: septum, TH: thalamus, 3V: third ventricle

1.3.1 Brain systems involved in energy homeostasis

There are two systems that regulate energy metabolism. The short-term system regulates the quantity of food intake at each meal by preventing overeating; the long term system regulates the maintenance of normal quantities of energy stores (fat) in the body (Havel, 2001). The hypothalamus and the Nucleus Tractus Solitarius (NTS) in the brain stem are sites in the CNS critically involved in the regulation of energy homeostasis. The NTS serves as gateway for neural signals from the gastrointestinal tract to the hypothalamic feeding centers. Also the circumventricular organs (in direct contact with circulating energy balance factors due to nerve endings outside blood-brain-barrier); nucleus accumbens and ventral tegmental area (influence motivation and reward-mediated feeding behavior); amygdala (processes emotional and rewarding experiences associated with food); and various regions of the neocortex (process higher sensory information that affects food-intake) are important in this regulation (Grill, 2006; Fry et al. 2007; Morrison et al. 2007).

The hypothalamus is considered the main feeding center in the brain, because it can assess the immediate energy state of the organism and restores energy balance homeostasis (Fig 1b). It consists of the ventromedial nucleus (VMN), lateral hypothalamic area (LHA), arcuate nucleus (ARC), dorsomedial medial nucleus (DMN), perifornical area (PFA) and the paraventricular nucleus (PVN). Neuronal signaling between these feeding circuits is mediated via several neurotransmitters and neuropeptides. Neuropeptides are traditionally considered to be either orexigenic (stimulating food-intake and related behaviors) or anorexigenic (inhibiting food-intake and related behaviors).

1.3.2 Neuropeptide Y

One of the neuropeptides involved in energy homeostasis is neuropeptide Y (NPY). Neuropeptide Y is 36-amino acid neuropeptide belonging to the pancreatic polypeptide family that also includes peptide YY, secreted by the gut as a long distance signal of energy state (Stanley et al., 2004) and pancreatic polypeptide PP, released from the pancreatic islets in response to food-ingestion (Batterham et al., 2003). NPY occurs abundantly in the mammalian central and peripheral nervous systems, and is involved in many physiological functions such as cardiovascular control, anxiety, sexual behavior, neuroendocrine function and feeding in mammals (Karl et al., 2007). Peripherally, NPY is abundant in the sympathetic nervous system, where it is co-stored and co-released with norepinephrine (Silva et al., 2002). NPY plays a crucial role in the control of food intake and body weight within the hypothalamus and is considered the most potent orexigenic agent known (Chee et al., 2008). Central intracerebroventricular administration of NPY or administration direct into hypothalamic sites such as the PFA, PVN and VMN stimulates robust feeding responses dose-dependently (Clark et al., 1984; Levine et al., 1984; Stanley et al., 1993), while chronic administration of NPY results in obesity through hyperphagia (Zarjevski et al., 1993). Food deprivation is one of the most important factors that increase the expression of NPY in the hypothalamus. Increased levels of NPY gene expression and peptide induce hyperphagia, furthermore, they are directly correlated with energy deprivation and demand (Sahu et al., 1997; Brady et al., 1990; Smith, 1993). Also NPY promotes white fat lipid storage and decreases brown adipose tissue thermogenesis (Billington et al., 1991).

The effects of NPY are mediated by G-protein coupled receptors. Today, there are 6 variants of the NPY receptor known (Kalra et al., 2004).The NPY receptor family includes the Y1 receptor, first characterized as a post-synaptic receptor, the Y2 receptor, known as a pre-synaptic receptor, the Y3 receptor is a NPY-preferring receptor, the Y4 receptor, first characterized as a PP receptor, the Y5 which is involved in feeding, and the y6 receptor, that has been cloned, although this receptor is not functional in humans. All these receptors, except for the Y3 receptor, have been cloned (Chee et al., 2008). However, with the use of KO-mice only Y1, Y2 and Y5 receptors seem to be implicated in feeding (Chee et al., 2008). While the Y1 and Y5 receptors stimulate NPY release, Y2 receptors suppress this release (Kalra et al., 2004). Mice lacking NPY have normal food-intake, adiposity and body weight and brain and organs are not altered (Thorsell et al., 2002).

Rats overexpressing NPY did not differ from their littermates in body weight and food intake (Thorsell et al., 2002). These results suggest that NPY is important in regulating energy homeostasis but it is not crucial, other molecules possibly provide a ‘back-up’ mechanism and take over the role of NPY in feeding behavior. Also receptor-knock outs for the NPY Y1, Y2 and Y5 receptor have been generated. Y1 receptor knock-out mice display moderate obesity, hyperinsulinemia and no hyperphagia (Kushi et al., 1998). Pedrazzini et al. (1998, 2000) showed that these mice had slightly diminished food intake and NPY-induced feeding; whereas fasting-induced feeding was greatly reduced. Energy homeostasis also includes thermogenesis and although Y1 receptor deficient mice displayed normal thermogenesis in ambient temperature and when exposed to cold, locomotor behavior (component of thermogenesis) was significantly reduced during the active dark phase. This inactivity of Y1 receptor deficient mice might be responsible for the increased fat deposition in observed in these animals (Thorsell et al., 2002). Y2 receptor deficient mice show a decrease response to orexigenic leptin treatment, but reacted normally to NPY-induced food-intake. These mice also showed normal regulation of re-feeding and body weight after food restriction (Naveilhan et al., 1999). Y5 receptor knock-out mice show normal fasting-induced re-feeding. These mice display late-onset obesity where they show increased food-intake, body weight and adiposity. Core body

temperature was unchanged suggesting no altered energy expenditure in these Y5 deficient mice (Marsh et al., 1998).

1.3.3 NPY in the hypothalamus

Within the hypothalamus, the ARC consists of two important neuronal populations involved in the regulation of energy balance. The NPY neurons in the ARC are partly located outside the blood-brain-barrier (Peruzzo et al., 2000) and can thereby serve as a sensor for the level of circulating factors important for energy homeostasis, such as leptin and insulin and ghrelin. Leptin is produced in adipocytes and gives information about adiposity and low leptin levels stimulate NPY-orexigenic pathways. Insulin is produced by the pancreas and serves as a satiety signal; high levels of insulin suppress NPY-orexigenic pathways. Ghrelin is released in response to the nutritional state and is thought to play a role in meal initiation; high ghrelin levels induce stimulation of NPY-orexigenic pathways. By integrating inputs from different circulating factors, the ARC mediates the central representation of metabolic state (Chee et al., 2008). The neuronal population located in the later ARC consists of ARC POMC neurons, because they express anorexigenic melanocortin peptides encoded by the pro-opiomelanocortin (POMC) preprohormone gene and by cocaine- and amphetamine-regulated transcript (CART). The neurons in the medial part of the ARC are called ARC NPY neurons, because they express orexigenic peptides such as NPY and agouti-related peptide (AgRP). The ARC NPY neurons project to adjacent structures in the hypothalamus including the VMN, DMN, PVN, PFA and LHA. Furthermore, these neurons innervate the ARC POMC neurons as well. The ARC NPY neurons suppress the anorexic tone supplied by the ARC POMC neurons by hyperpolarizing the membrane via Y1 and Y2 receptors causing an inhibition of the ARC POMC neurons (Acuna-Goycolea et al., 2006). The hypothalamic ARC POMC neurons release melanocortin peptides such as α-MSH (melanocyte stimulating hormone) and bind to melanocortin 3 and 4 receptors, which are only present in the brain including the PVN. Binding to these receptors activates them and reduces food intake, raises metabolic rate, increases energy expenditure and stimulates liplysis which can lead to anorexia. AgRP is a MC receptor antagonist, counteracting the anorexigenic effects of α-MSH (Ellacott et al., 2004).

The other hypothalamic structures innervated by ARC NPY neurons all play a role in energy homeostasis. Depletion of energy stores produced by fasting, promotes an increase of NPY synthesis in the ARC and release by the PVN, whereas when a normal eating pattern is resumed this involves the suppression of NPY secretion by the PVN and normalization of NPY synthesis by the ARC (Kalra et al., 2004). The VMN is directly implicated in feeding behavior, as injections of NPY in the VMN increase food-intake (Stanley et al., 1993). Electrolytic lesioning of the VMN induces obesity (Hetherington et al., 1942). Stimulation of the VMN suppresses food-intake, suggesting that an active VNM produces an anorexigenic tone (Brown et al., 1984). Anorexigenic signaling by α-MSH and CCK is partly mediated by inhibition of DMN NPY neurons (Chee et al., 2008). LHA activity stimulates food-intake, as was found that lesions of the LHA resulted in hypophagia (Brobeck et al., 1946 from Chee et al., 2008).

1.4 The anorexia-hyperactivity and the NPY paradox

NPY plays a crucial role in normal energy homeostasis and acts as an orexigenic signal promoting food-intake and decreasing energy expenditure. Energy expenditure consists of thermogenesis, food intake and physical activity. Normally, in a food deprived state, thermogenesis and physical activity is reduced as means to conserve energy, whereas during feeding the opposite effect is observed (Semjonous et al., 2009). Anorexia nervosa patients have depleted levels of peripheral fat stores and therefore low blood levels of leptin. As a consequence, these patients show elevated levels of orexigenic NPY in the brain. Suprisingly, these patients do not eat (Kaye et al., 1989). This situation, ”the NPY-paradox”, is inconsistent with the idea that NPY is an orexigen and with the conventional model of feedback regulation of body weight (Sodersten et al., 2008). Moreover, it questions the labels of neuropeptides as either orexigenic or anorexigenic (Ammar et al., 2000).

The pathological weight loss observed in anorexia patients does not lead to fatigue and motor

slowing often observed in natural semi-starvation (Eckert, 2001, Hebebrand et al., 2003). In contrast, emaciated anorexia patients display normal, occasionally high energy, activity levels, and mental alertness. From an evolutionary point of view, the display of hyperactivity by anorexia patients seems counterintuitive because hyperactivity in a severe underweight state will not only worsen the condition of the patient, but the patient would be better of saving energy as it is already suffering from hypothermia. Furthermore, essential for survival, the body seems to be "programmed" to promote orexigenic pathways rather than anorexic pathways. It is therefore interesting to investigate what actually causes this hyperactivity and what the role of NPY is in this phenomenon.

In this thesis, the potential role through which NPY contributes to hyperactivity associated with anorexia nervosa will be investigated. To this end, first the possible underlying neurobiological systems involved in this behavior will be described. Second, the existing hypotheses regarding hyperactivity in anorexia and the possible role of NPY in these hypotheses will be investigated with special attention to the NPY receptors.

Chapter 2: Brain systems possibly involved in hyperactivity in Anorexia Nervosa

Brain systems implicated in anorexia nervosa have been extensively investigated. The neurobiological mechanisms underlying the anorectic effects of exercise have received more and more attention. Several brain systems are thought to contribute to hyperactivity displayed by anorexia patients, as mentioned before, the detrimental effect of hyperactivity makes it a highly relevant target in the treatment of anorexia. Table 1 lists a set of drug treatments and the affected neurochemicals or structures resulting in reduced running wheel activity in the ABA model to illustrate the variety of systems possibly involved in this behavior. This list in not complete, several studies have found that brain systems such as the corticotropin releasing hormone system and the thyroid releasing hormone system are also involved in this behavior. This chapter describes the to date known most important brain mechanisms that might contribute the excessive exercising observed in anorexia patients and the uncontrolled wheel running activity observed in ABA rodents.

Table 1: List of drug treatments shown to reduce wheel-running activity in the ABA model (adapted from Young et al., 2007)

Treatment	**Neurochemicals / structures affected**	**References**
NPY	Y1, Y2, Y5 receptors	Nergardh et al., 2007
alpha-hCRH	CRH	Kawaguchi et al., 2005
$AgRP_{(83—132)}$	Melanocortin receptors	Hillebrand et al., 2006
Leptin	Leptin receptor	Exner et al., 2000
Chlorprozamine	Dopamine	Routtenberg et al., 1967
Olanzapine	Dopamine, serotonin, acetylcholine, catecholamines	Hillebrand et al., 2005
Clonidine guanfacine	Catecholamines	Wilckens et al., 1992
Fluoxetine	Serotonin	Altemus et al., 1996
5-HT1C agonist	Serotonin	Wilckens et al., 1992
Naloxone	Opioid receptor	Boer et al., 1990

2.1 Corticotropin releasing hormone system and the hypothalamic-pituitary-adrenal axis (HPA-axis)

Stress is a common feature of anorexia nervosa and involves the activation of the HPA-axis. Anorexia patients are known to have hyperactive secretion of corticotrophin releasing hormone (CRH, Klein et al., 2007). CRH neurons can be found the hypothalamic PVN, where they are activated by NPY (Rao et al., 2007). CRH is involved in the endocrine, autonomic, behavioral, and immune responses to stress and is thought to be an anorexigen. CRH triggers the release of ACTH in the pituitary gland which in turn triggers the release of adrenalin and cortisol in the adrenal glands. Cortisol is elevated in underweight patients with AN, although they have normal circulating levels of ACTH (Licinio et al., 1996). This is assumed to be a function of starvation, which is known to produce hypercortisolism. However, elevated cortisol may also be related to exercise behavior, which is also known to activate the HPA axis, independently of an eating disorder (Mastorakos et al., 2005). According to Mastorakos et al. (2005), exercise is a physical stressor which can lead to elevated plasma cortisol levels. According to Klein et al. (2007), anorexia patients that display excessive exercise show more increased levels of cortisol than less-active patients with the same degree of starvation. Effects of HPA axis over-activity could also include subjective restlessness and motor agitation in anorexia patients (Casper, 2006).

Evidence for this is provided by animal studies, in which administration of corticotropin releasing factor (CRF, which in turn stimulates release of corticosterone, the rodent equivalent of

cortisol) leads to behavioral activation with increased locomotor activity (Sutton et al., 1982). Both underweight patients with anorexia and ABA rats have elevated cortisol levels (Lo Sauro et al., 2008; Burden et al., (1993). Bergh et al. (1996) suggested that stressful situations like the ABA-model might increase CRH expression and release and ABA rats have been associated with elevated levels of corticosterone. When a comparison is made between semi-starved running rats and semistarved non-running rats has shown that starvation and hyperactivity have a synergistic effect on corticosterone (Broocks et al., 1990). Furthermore, treadmill running in rats is found to increase CRF mRNA in the PVN (Timofeeva et al., 2003), possibly via ARC NPY neurons as was found that chronic stress elevates NPY levels in rats (Thorsell, 2008). The CRH antagonist, alpha-hCRH attenuates or prevents exercise-induced anorexia (Kawaguchi et al., 2005; Rivest et al., 1990), suggesting that disturbance of the HPA-axis may play a causal role in behavioral hyperactivity (Klein et al., 2007). These findings suggest that not only a food deprived state but also activity itself might over-activate the HPA-axis. Duclos et al. (2005) found that rats of different strains differing in their HPA activity and reactivity showed varying vulnerability to the ABA-model. These authors found that a higher HPA-axis activation co-occured with higher wheel-running activity in food-resticted rats. These authors suggested that corticosterone in particular links food-restriction-induced weight loss to increased exercise (Duclos et al., 2005). This because elevated corticosterone levels promote the release of dopamine in the nucleus accumbens (Piazza et al., 1996), thereby increasing the behavioral activation induced by stress (Rivest et al., 1990) or increasing the compulsive nature of some activities. The pleasurable component of increased physical activity in both human anorexia nervosa and ABA rats could reinforce hyperactivity, and inhibit food intake via reward mechanisms (Exner et al., 2000; Berg et al., 1996). The authors proposed that “corticosterone may contribute to the physiopathology of excessive running in the face of reduced food intake, reinforcing the sensitization to running through its positive hedonic effects”. Corticosterone receptors have been found on dopaminergic neurons (Harfstrand et al., 1986) and dopamine can activate the HPA axis and has anorexigenic and rewarding effects (see 2.5.2).

In anorexia patients, studies are limited and contradicting. For example, Klein et al. (2007) found an association between locomotor activity and urinary cortisol, whereas Ehrlich et al. (2009) failed to find an association between hypercorisolism and excessive exercising. These differences could be attributed to the differences in methods and small sample sizes used.

2.2 Thyroid-releasing hormone system and the hypothalamic-pituitary-thyroid axis (HPT axis)

Another brain system thought to be implicated in hyperactivity and anorexia nervosa is the TRH system, which is essential for the regulation of energy expenditure (Semjonous et al., 2009). TRH is widely distributed in the CNS and is thought to be involved in the regulation of arousal, autonomic function (including thermogenesis), mood and spinal cord motor functions. TRH neurons in the hypothalamic PVN regulate the HPT axis. TRH triggers the release of TSH (thyroid stimulation hormone) in the pituitary which in turn triggers the release of T3 and T4 in the thyroid gland. Patients suffering from anorexia have a reduced thyroid volume (Stoving et al., 2001) and are in a hypothyroid state with decreased TSH and T3 levels and higher levels of the inactive rT3 (Lesem at al., 1994). By slowing down the HPT axis, thermoregulation is lowered to save energy, causing hypothermia in anorexia patients (Martin et al., 2006). Starvation specifically down-regulates TRH mRNA expression in the PVN where TRH neurons are located (Blake et al., 1991). This starvation-effect seems specific to the PVN, because it was found that TRH neurons in the lateral hypothalamus are unaffected by food-deprivation (Legardi et al., 1997). According to Casper (2006) “the hypothyroid state highlights the pathological aspects of the drive for movement and alertness, since the expectation would be a slowing of movement and lethargy”.

There is a very limited amount of animal studies that investigated the effect of the HPT axis on activity in anorexic animals. In healthy animals, it was found that the peripheral administration of TRH increased locomotor activity and decreased food intake in both rats and siberian hamsters (Schuhler et al., 2007). In the rat, TRH elicits locomotor activation when directly injected in the nucleus accumbens, the ventral tegmental area, the caudate and septal nuclei, and the ventromedial

hypothalamus (Nillni et al., 1999). Broocks et al. (1990) reported that T3 was reduced by semi-starvation and hyperactivity in a synergistic way. This suggests that TRH is important in thermoregulation and that both activity and starvation could attenuate the generation of heat by working on the HPT axis.

2.3 Leptin and the hypothalamus

Leptin is produced in white adipocytes and gives information about adiposity. Low leptin levels stimulate orexigenic pathways in the hypothalamus and in particular the ARC, where leptin inhibits the orexigenic NPY-AgRP neurons and stimulates anorexigenic POMC-CART neurons (Cowley et al., 2001). Furthermore, leptin regulates the neural controlling of feeding behavior, energy expenditure including thermogenesis, body weight and neuroendocrine function. Leptin receptors are abundantly expressed in several different sides in the within the hypothalamus, including the ARC, DMH, LHA en VMH (Dhillon et al., 2006). During starvation leptin levels decline more rapidly compared to body fat mass, and the body responds adaptively with a reduction in energy expenditure, suppression of the gonadal and thyroid axis and the activation of the adrenal axis (Hillebrand et al., 2008). Anorexia patient have depleted fat stores and as a consequence low leptin levels but, as mentioned above, these patients do not eat. Furthermore, these patients ranked their motor restlessness higher than when leptin levels were increased following treatment (Exner et al., 2000), suggesting that leptin is involved in hyperactivity observed in anorexia patients. Leptin is also proposed as a treatment for hyperactivity (Exner et al., 2000).

Leptin is also extensively studied in the ABA-model. Exner et al. (2000) found that running wheel activity is increased in ABA rats while plasma levels of leptin dropped. These authors, together with others also found that treatment with leptin is able to prevent the development of ABA (Hillebrand et al., 2005). Furthermore, leptin did not affect activity in *ad libitum* fed rats or food-restricted rats without access to a running wheel (Hillebrand et al., 2005). Also, when ABA was already developed, increasing leptin levels decreased running-wheel activity (Hillebrand et al., 2008). Obese mice lacking leptin show low activity, this can be reversed by leptin treatment (Pellymounter et al., 1995). Re-expressing the leptin receptor in mice lacking leptin specifically in the ARC increases locomotor activity, suggesting a role for the ARC in the effects of leptin on hyperactivity (Coppari et al., 2005). In *ad libitum* fed rats leptin reduces activity, while in ABA rats leptin reduces activity. These findings suggest that the effect of leptin on activity depends on the state of energy balance (Hillebrand et al., 2008). However, increased leptin levels also decrease food-intake and thermogenesis during the first four hours of the experiment which worsens the conditions of already underweight animals, suggesting that leptin treatment in anorexia patients should not be used in the acute fase of this disease (Hillebrand et al., 2008).

2.4 The melanocortin system

Another candidate system possibly underlying over-activity in anorexia is the melanocortin system. According to Hillebrand et al., (2006) there is limited information about the involvement of this system in anorexia nervosa. The symptoms displayed by anorexia patients, including hyperactivity, could indicate the activation of the melanocortin system (Walsh et al., 1998). It was found that plasma levels of leptin and central levels of the anorexigenic POMC are reduced in anorexia patients (Hebebrand et al., 1997) to promote an orexigenic drive.

In animal studies it was found that during early exposure to ABA, a brief upregulation of POMC in the ARC was found, followed by a significant down-regulation after one week (Kas et al., 2003; Hillebrand et al., 2006). These data indicated that the MC system is hyperactive in ABA rats, especially in the first period. Hillebrand et al., (2005c) and Kas et al., (2003) showed that physical activity in itself does not result in up-regulation of POMC gene expression. The mechanisms underlying this brief POMC up-regulation remain unclear. Up-regulation of the POMC system is in contrast with the widely described down-regulation of anorexigenic neuropeptides during food restriction to increase food intake and reduce energy expenditure (Schwartz et al., 1997). This upregulation is probably the result

of the unique situation of limited food access together with physical activity or might be a maladaptation of the melanocortin system during the ABA paradigm (Hillebrand et al., 2006).

It was also found that stimulation of melanocortin receptors in rats results in increased motor activity, reduced food intake, and activation of the HPA axis (Adan et al., 2003). The VMN has been found to be strongly associated with regulation of food intake. ABA rats show an increase in the density of MC receptors, specifically in the VMN (Kas et al., 2003). It was demonstrated that food restriction increased density of MC4 receptors. However, an increase in MC receptors will increase the sensitivity of the anorexigenic effect of α-MSH, which is paradoxal as well and also support the notion that the melanocortin system is maladaptive in the ABA model (Hillebrand et al., 2006).

Hillebrand et al., (2006) furthermore found that chronic SHU9119 (melanocortin receptor antagonist) treatment did not influence ABA but that chronic $AgRP_{(83—132)}$ treatment increased food intake and body temperature in the ABA model. The authors suggested that these results show that the increased agonism of the melanocortin receptors by endogenous α-MSH does not play a major role in ABA but that the antagonism of the melanocortin receptors by AgRP may play role in the decreased food ingestion and the increased running wheel activity.

α-MSH treatment was also found to influence HPA axis activity by increasing adrenocorticotropin (ACTH) and corticosterone release in rats (Cheung et al., 1997), which were found to be involved in hyperactivity in food deprived rats.

Melanin Concentrating Hormone (MCH) is also part of the melanocortin system. In mammals, high concentrations of MCH are found in neurons of the LHA with projections all over the brain (Nahon et al., 2006, Ludwig et al., 1998). The role of MCH in feeding behavior was based on the observations that obese mice overexpress MCH mRNA compared to non-obese littermates and fasting increases hypothalamic MCH mRNA expression in both obese and wild-type mice. Intracerebroventricular MCH administration stimulates food intake in the rat by antagonizing α-MSH (Qu et al., 1996, Rossi et al., 1997). Studies have also suggested an interaction between MCH and the hypothalamic-pituitary-adrenal (HPA) axis (Bluet Pajot et al., 1995), which may be a route through which this molecule may contribute to hyperactivity. In the ABA model, MCH mRNA expression was upregulated 2-fold in the LHA of food-restricted running rats, but not in food-restricted sedentary controls (De Rijke et al., 2005) supporting the notion that MCH expression is increased in situations of severe negative energy balance and might possibly be involved in hyperactivity.

2.5 The monoaminergic neurotransmitter systems

2.5.1 Noradrenalin

Central and peripheral noradrenergic and adrenergic pathways play an important role in neural and endocrine activation and are significantly down-regulated in extended fasting and acute anorexia (Halmi et al., 1978). NPY seems to be co-expressed in a subgroup of hindbrain noradrenalin neurons that project within the hindbrain and that greatly innervate the arcuate and paraventricular nuclei of the hypothalamus, but also other forebrain sites (Taylor et al., 2007). From the brain-stem to the hypothalamic PVN, NPY is also co-expressed with noradrenalin, suggesting that this cathecholamine is possibly involved in eating behavior and hyperactivity displayed by anorexia nervosa patients (Sawchenko et al., 1985).

In animal studies it was found that noradrenalin can induce feeding behavior. Furthermore noradrenergic activation of α_1- and β_2-adrenoceptors decreases food intake, and stimulation of the α_2-adrenoceptor increases food intake (Broocks et al., 1990). NPY-norandrenaline neurons have been found to increase food intake in rats when noradrenaline is injected in the fourth ventricle (Taylor et al., 2007). Also, injections of NPY or noradrenalin in the PVN induce feeding behavior (Leibowitz et al., 1988). Furthermore, injections of noradrenalin in the PVN increase corticosterone levels (Leibowitz et al., 1988), which was also found to add to hyperactivity in the ABA model. In the ABA model, it was shown that noradrenaline turn-over was elevated in food-restricted rats and Pirke and colleagues (1993) suggested that the hyperactivity displayed by these animals reverses the effect of starvation on noradrenaline. It was also found that running-wheel activity could be suppressed when α_2-antagonists were injected, whereas α_1- and β_2 antagonists did not suppress wheel running activity (Pirke et al.,

1993), suggesting that the activation of the α_2-adrenoceptor is involved in the induction of wheel running the ABA model. Agnati et al. (1983) found that NPY can affect catecholaminergic neurons by increasing the number of the presynaptic α_2-receptors, which may contribute to the increased activity. Cador et al., (1993) showed that amphetamine could stimulate the noradrenergic system dose-dependently and specifically in the nucleus accumbens, thereby increasing locomotor activity, also suggesting that noradrenaline may contribute to increased activity in anorexia patients.

2.5.2 Dopamine

Evidence that the DA system is involved in anorexia includes reduced cerebrospinal fluid levels of dopamine metabolites in both ill individuals and patients having recovered from anorexia and functional dopamine D2 receptor (DRD2) gene polymorphisms in subjects with anorexia (Berthoud et al., 2007). People with anorexia often exercise compulsively, are anhedonic and ascetic, and cannot find anything that is rewarding to them except from weight loss (Kaye et al., 2009). Dopamine dysfunction, in particular in striatal circuits, might contribute to an altered perception of reward and affect, decision-making and executive control, as well as stereotypic motor movements and decreased food ingestion in patients with anorexia nervosa (Frank et al., 2005). Dopamine signaling in the hypothalamus via neurons in the dorsomedial and arcuate nuclei seem to inhibit food intake (Schwartz et al., 2000). Movements seem to be regulated by dopaminergic pathways (Casper, 2006). Hebebrand et al. (2003) reported that dopamine levels increase following exercise in non-anorexic humans, which suggests that this system could also affected by exercise in anorexia patients. Many single- and multi-synaptic pathways connect the hypothalamus to the mesolimbic dopamine pathways were motivational aspects of specific stimuli are converted to motor responses (Berthoud et al., 2004, 2007), also suggesting that the dopamine system is possibly involved in hyperactivity displayed by anorexia patients. The dopaminergic system has been targeted therapeutically in anorexia nervosa and antipsychotic drugs were one of the first treatments studied for the treatment of anorexia.

In animals studies it was found that stimulation of either D1 or D2 dopaminergic receptors activate CRH neurons in the PVN (Eaton et al., 1996), which were also involved in hyperactivity in anorexia patients. Gelegen et al. (2008) showed that mice with a genetic deletion in the dopamine transporter gene (terminates the dopamine signal in synapses) do not develop behavioral hyperactivity in the ABA paradigm. A study by Verhagen et al. (2009) showed that treatment with the non-selective dopaminergic antagonist cis-flupenthixol increased food-intake and in ABA rats but not in *ad libitum* fed rats. In addition, these authors found that treatment with this antagonist reduced activity levels in both *ad libitum* fed and ABA rats. This study also showed that rats still displayed food-anticipatory activity prior to feeding in the ABA paradigm. Together these findings show that dopamine is possibly involved in excessive exercising in the ABA model. In addition, they suggest that other molecules are involved in activity in the ABA model as well.

2.5.3 Serotonin

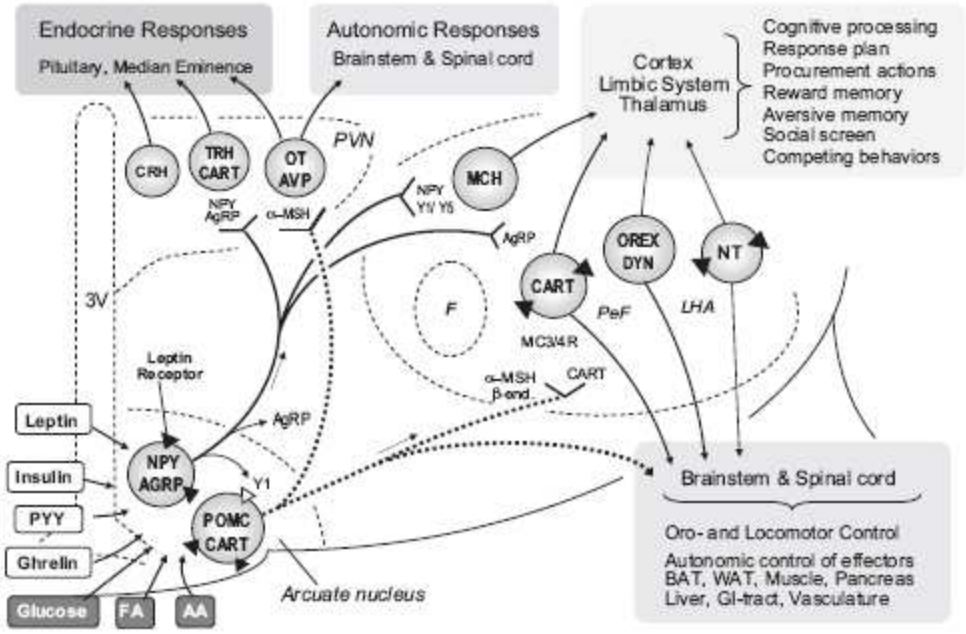
The complex serotonin-system is implicated in several functions in the body, including appetite, energy balance, mood and motor output (including compulsive behavior) (Casper, 2006). Serotonergic neurotransmission is changed in anorexia patients and also after recovery changes were observed (Frank et al., 2002). Serotonin is considered an anorexigenic molecule because it inhibits food intake in both anorexia patients and rats (Haleem et al., 2009). Serotonergic neurons innervate the hypothalamic ARC and PVN (Sawchenko et al., 1983). In the ARC, serotonin-containing nerve endings terminate on NPY-synthesizing cell bodies (Guy et al., 1988). The closeness of NPY- and serotonin-containing pathways in the hypothalamus, and their opposite effects on feeding suggest that the two may interact in the control of energy homeostasis. Antagonizing serotonin stimulates the ARC-PVN projection. In this way serotonin may modulate feeding by inhibiting NPY release, and the NPY- and serotonergic systems may therefore interact to regulate feeding behavior (Dryden et al., 1999). Levels of central serotonin are increased following exercise in non-anorexic humans (Hebebrand et al., 2003), suggesting that hyperactivity observed in anorexia patients may override the down-regulated anorexigenic serotonergic drive induced by food-deprivation.

In an animal study by Haider et al. (2000), it was found that 5-week food restriction decreased serotonin metabolism and synthesis in the hypothalamus. Brain levels of the precursor of serotonin, tryptophan, were also found to be decreased, especially in female rats. Reducing serotonin activity by treating ABA rats with 8-OH-DPAT, an agonist of the 5HT1A autoreceptor, does not influence food intake but reduces running wheel activity (both in the dark and light phase), resulting in attenuated body-weight loss (Atchley et al., 2006). In mice, it was found that 5-HT2C receptor agonists reduced locomotor activity and food intake (Fletcher et al., 2009). Serotonin mimicking drugs have been found to decrease feeding and reduced NPY levels in the PVN (Dube et al., 1992). The 5-HT1A receptor agonist flesinoxan (which increases feeding) increases NPY levels in the PVN and ARC when injected acutely (Dryden et al., 1995). A way in which serotonin could contribute to hyperactivity is that serotonin is known to inhibit dopamine neurotransmission (Haleem et al., 2009). By the reduction of serotonin levels through food-restriction, the inhibitory effect of serotonin on dopamine is decreased and could in this way promote hyperactivity.

2.6 Summary

Several brain systems upstream (leptin), adjecent to (Melanocortins) and downstream the NPY system have been described in relation to food deprivation and hyperactivity observed in most patients with anorexia nervosa. Still unclear is whether the described effects are a cause of this behavior, or a result that in addition helps to maintain this behavior. What is clear is that the HPA axis is upregulated and thermoregulation is downregulated via different pathways. Furthermore, in addition to the NPY-paradox, i.e. elevated levels of NPY but no food-ingestion, there also seems to be a POMC-paradox, where the anorexigenic melanocortin system is upregulated in the ABA model. The monoaminergic neurotransmitter systems might be implicated in the rewarding components of both food deprivation and excessive exercising, but also in the compulsive components. Figure 2 depicts the highly inter-related brain mechanisms and molecules associated with regulating energy metabolism.

Figure 2: Brain mechanisms and associated molecules implicated in regulating energy metabolism (adapted from Morrison et al., 2007)



Chapter 3: Hypotheses explaining hyperactivity in Anorexia Nervosa

Several hypotheses have been posited to explain the paradoxal display of hyperactivity in Anorexia Nervosa patients and ABA animals. Traditionally, hyperactivity in anorexia patients has been regarded as a conscious attempt to loose body weight, i.e. the drive for thinness reinforces hyperactivity (Gutierrez et al., 2009). However, this view has been replaced by others who acknowledge the neurobiological driven nature of these behaviors (Casper, 2006). Davis et al., 1997 suggested that in some patients the urge to exercise is out of the patient's control suggesting that an underlying biological system is involved in this hyperactivity and that this could be triggered by an unconscious biological drive that may involve parts of several behaviors and an altered neurobiological profile.

From an evolutionary perspective, hyperactivity is possibly an expression of foraging behavior, which is rewarding in itself (Kas et al., 2004). Another explanation for this excessive exercise is to increase body temperature, as both anorexia patients and ABA animals suffer from hypothermia (Gutierrez et al., 2002). It has also been proposed that anorexia patients and ABA animals show hyperactive behavior because this behavior is rewarding to them (Sherwin, 1998).

In this chapter the different hypotheses proposed to underlie hyperactivity will be described. Furthermore, the potential role of NPY in relation to the different brain mechanisms thought to underlie hyperactivity in anorexia, discussed in chapter 2, will be highlighted with special attention to the NPY receptors.

3.1 Hypothesis 1: Hyperactivity displayed by Anorexia Nervosa patients is an expression of appetitive ingestive behavior.

It has been suggested that humans and animals have evolved to adapt to fluctuations in the amount of available food. Decrease in body weight is a natural occurring phenomenon in response to food-shortage and the body is "programmed" to react to fluctuations in body weight by triggering behaviors to restore this imbalance such as ingestive behavior. Ingestive behavior has two phases: appetitive behavior, which is the search, locating and acquisition for food, and consummatory behavior, which consists of chewing and swallowing (Craig, 1918 from Sodersten et al., 2008). Berridge et al. (1998) introduced the term 'wanting' to label the process whereby stimuli gain motivational salience. In a food-deprived state, food becomes more attractive to both humans and animals, i.e. the incentive salience or 'wanting' increases, and this triggers goal-directed behavior to acquire food, appetitive ingestive behavior.

Hyperactivity of ABA-animals have been suggested to be a form of appetitive ingestive behavior, such as food foraging or hoarding (Epling et al. 1988; Ammar et al., 2000; Exner et al., 2000; Drazen et al., 2005; Day et al., 2005; Duclos et al., 2005; Sodersten et al., 2006, 2008; Dailey et al., 2009). The survival value of the relationship between eating and hyperactivity displayed by ABA-animals has been pointed out by (Epling et al. 1988). They suggested that during times of food scarcity, animals either hibernate and conserve energy or become mobile and migrate. Therefore, running in response to ongoing food-shortage may increase the survival-value of a free-living rat. Brain mechanisms that allow the effective search for food have survival value as well.

In human anorexia patients the drive for activity is a function of the starvation state of the patient (Hebebrand et al., 2003). The foraging hypothesis is also reinforced by reports of starved people in the Hungerwinter (1944-1945) in the Netherlands during the WWII. In this period it was often observed that people, despite their severe under-nutrition, made long bike rides to forage for food. This urge to collect food resulted in people even stealing, fighting and attacking each other for food (Van der Zee, 1998 from Sodersten et al., 2008). Furthermore, people could not stop thinking of food, which is also observed in anorexia patients who also dream about food (Casper et al., 1977). These examples together with the uncontrolled display of hyperactivity suggest that also anorexia patients show food foraging behavior.

3.1.2 NPY and the foraging hypothesis of hyperactivity in anorexia nervosa

Since long, it has been accepted that NPY functions to stimulate food intake. NPY has also effects in addition to affecting food intake. For example, NPY can both suppress and stimulate reproductive neuroendocrine function (Sahu et al., 1998) and behavior (Clark et al., 1985) and, in high doses, cause taste aversion (Woods et al., 1998). However, several studies have shown that NPY stimulates behavior that leads to food ingestion, i.e appetitive ingestive behavior, and these studies will be described below.

From the previous chapter and the description of the foraging hypothesis above, one can infer that food-deprivation induced appetitive ingestive behavior involves the HPA stress-system because under-nutrition and the accompanying fat depletion is a deviation of the homeostatic equilibrium of the body (Bernard, 1957 from Sodersten et al., 2008). Any deviation of such equilibrium is considered stress, which causes the body to react appropriately to regain its equilibrium. The brain reward system also seems to be involved because of the motivational aspects of food and foraging in itself and the rewarding properties of exercise.

Several authors have investigated the difference between consummatory and appetitive ingestive behavior. A technique to separate these two phases of ingestive behavior is the intra-oral intake paradigm. With this paradigm a direct estimation of sucrose intake can be made without confounds of general activity or differences in appetitive approach behaviors. In this procedure, rats have an implanted intra-oral catheter through which small amounts of solutions (e.g. sucrose) can be infused directly into the oral cavity. In this way, appetitive-ingestive behaviors such as the approach to a bottle are bypassed (Grill et al., 1987).

One of the first authors to suggest that excessive exercise, displayed during severe under-nutrition, is a form of appetitive ingestive behavior was Epling et al. (1988). He found in the ABA paradigm that excessive wheel running reduced the value of food reinforcement, which in turn decreased food intake. These authors also found that reduction of food intake increased the motivational value of physical exercise which produced an escalation in activity that further suppressed food ingestion. They furthermore suggested that in humans, cultural practices of diet and exercise are involved in the initiation of this anorexic cycle, and that once it is started the process is resistant to change (Epling et al., 1988). Experiments done by Seeley et al., (1995) showed that NPY did not increase food intake but instead increased appetitive ingestive behaviors. In their experiments they administered NPY centrally which caused an increase in sucrose-solution ingestion from a bottle on the home-cage. These authors also equipped animals with intra-oral catheters and found that centrally administered NPY did not increase sucrose ingestion when sucrose was directly deposited in the oral cavity. Seeley et al., (1995) therefore suggested that NPY was important for appetitive but not for consummatory ingestive behaviors. Ammar et al., (2000) did not only confirm the results found by Seeley et al., (1995), in addition they found that during intra-oral infusion of sucrose of NPY-treated rats that these animals visited an empty bottle, from which they had been trained to expect a sucrose solution, more often compared to control rats. These authors also found that NPY treatment increased intake from a bottle, but the treatment inhibited intra-oral intake when a sucrose solution was infused with 0,5 ml/min. When the animals received this rate of intra-oral infusion and when a bottle was present in the cage, NPY caused a behavioral shift. The frequency of appetitive behavior increased while the frequency consummatory ingestive behavior decreased. This caused that the total amount of ingested solution decreased. Ammar et al. (2000) suggested that these finding confirm the hypothesis that NPY only stimulates food-intake when rat have to search for their food, thus, NPY promotes appetitive ingestive behavior and decreases consummatory ingestive behavior.

However, Benoit et al. (2005) could not repeat these findings as they found that administration of NPY and also MCH could increase ingestion of intraoral administration of sucrose. The animals of in the experiments of Seeley et al., (1995) were trained with intraoral infusion seven days prior to testing, whereas the animals of Benoit et al., (2005) received their first intraoral infusion on the day of testing. This might be a cause for the differences found.

Using the same intra-oral catheter paradigm, Kaplan et al. (1992) found that the search for one goal and the subsequent consumption of that goal prevents the search for a competing goal. These authors found that consummatory sexual and ingestive behavior could be displayed simultaneously

when a male rat was offered a receptive female and was infused with sucrose at the same time. However, when the rat had to search for a bottle with the sucrose solution, i.e. show appetitive ingestive behavior, the presence of a receptive female greatly reduced intake of sucrose. In case of severe starvation the priority changed from sexual behavior to appetitive ingestive behavior. Ammar et al. (2000) found that when rats were treated with 10 µg NPY intracerebroventricularly, male rats moved away from the receptive female and to a bottle with a sucrose solution when offered the choice. Furthermore, the number of ejaculations was significantly reduced. According to Sodersten et al., (2008), these findings suggest that in state of chronic food-deprivation, where NPY levels are elevated, NPY possibly shifts attention to behaviors that are relevant for acquiring food. NPY has indeed been found in brain systems involved in directing attention, such as the locus coeruleus in the brain stem (Sawchenko et al., 1985), where NPY is co-localized with noradrenalin. Noradrenalin is also found in the NTS, the gateway for neural signals from the gastrointestinal tract to the hypothalamic feeding centers. Lesioning of noradrenergic input to the NTS was found to attenuate the inhibitory effect of NPY on intra-oral intake in rats and Ammar et al. (2005) proposed that noradrenergic innervation of the NTS is a way in which NPY can exert its inhibitory effect on consummatory ingestive behavior. However, this hypothesis still needs to be tested.

In addition to the well known laboratory rats and mice, the Siberian hamster (*Phodopus sungorus*) is also studied to investigate the role of NPY in appetitive and consummatory ingestive behaviors. The authors of these studies argue that the Siberian hamster reacts to food deprivation by increasing food foraging and hoarding (the storage of food for later ingestion), while the increase in food intake is minimal (Day et al., 2003) which makes it a suitable species to investigate appetitive ingestive behavior. Day et al., (2005) found that NPY stimulated food hoarding and foraging in these Siberian hamsters. Food foraging was increased especially in the first hour after NPY injection. Food hoarding was increased until the last measured time-point (24 hours after injection) and food hoarding occurred to a greater extent than food ingestion. Day et al., (2005) found that the administration of the Y1-receptor agonist [Pro34]NPY into the 3rd ventricle stimulated food foraging and in particular food hoarding more compared to food intake. These authors also found that administration of the Y5-receptor agonist [D-Trp34]NPY stimulated food ingestion more compared to food foraging and hoarding. Keen-Rhinehart et al. (2007) demonstrated that the Y1-receptor antagonist 1229U91 blocked fasting-induced increases in food hoarding and foraging. These authors also demonstrated that the effect of Y1-R antagonism only inhibited fasting induced increases in foraging and food hoarding and did not have an effect on normal locomotion (Keen-Rhinehart et al., 2007). The authors argue that because appetitive ingestive behavior is an important behavior, it seems unlikely to be regulated by a single receptor. In the same species, Dailey et al. (2009) confirmed the findings of Day et al., (2005) and extended their findings by looking at specific brain-sites. They showed that micro-injections of NPY in the PVN and the PFA stimulated food hoarding more than food foraging and food-intake. Y1-receptor antagonism was found to inhibit food-deprivation increased hoarding when injected into the PVN and the PFA. Y1-receptor antagonism in the PFA specifically inhibited food-deprivation increased foraging behavior. The authors suggested that NPY in different brain sites may elicit different responses in appetitive ingestive behaviors (Dailey et al., 2009). In the PVN, TRH and CRH neurons are found to have Y1-receptors (Broberger et al., 1999; Li et al., 2000) and could, through stimulation of NPY in the ARC by food deprivation, be involved in appetitive ingestive behaviors. CRH could in this way activate the HPA axis, which in turn triggers other molecules involved in food foraging. TRH in addition, reduces energy expenditure and by saving energy could help the food-deprived animal in his search for food to replenish energy reserves. A word of caution is needed here. In these studies the authors differentiate between food foraging and hoarding. However, food hoarding is rarely tested in rats or mice (Day et al., 2003) and the abovementioned findings are mainly about food hoarding instead of food foraging, in which the authors found less convincing results. Nonetheless, both behaviors are appetitive ingestive behaviors, which ultimately leads to food ingestion and appetitive ingestive behavior is what is being investigated in this hypothesis.

In the previously described ABA model, NPY synthesis was found to be increased in female rats and also was found that infusion of NPY increased wheel-running and decreased food intake and body weight (Nergardh et al., 2007). In ABA rats who had access to food all the time, a statistically

insignificant increase in wheel-running, an increase in food-intake and an insignificant increase in body-weight was found. The authors suggested that the effects of NPY depend on the physiological state of the organism. NPY-treated rats eat more food when food is present *ad libitum*. In times of food shortage, NPY-treated rats eat less food and in addition start searching for food (Nergardh et al., 2007).

In summary, several authors showed, with an intra-oral catheter paradigm, that NPY increases behavior that directs attention to acquiring food, rather than ingesting it. Increased foraging behavior in reaction to NPY treatment and food-deprivation was not only found in rats and mice but also in Siberian hamsters. Furthermore, NPY was found to increase wheel-running activity in the ABA paradigm.

3.2 Hypothesis 2: Hyperactivity observed in Anorexia Nervosa is caused by hypothermia

Anorexia patients suffer, in addition to emaciation and hyperactivity, from hypothermia. People with anorexia nervosa often complain of feeling cold because their body temperature drops. As a consequence of prolonged hypothermia, these patients may even develop Hypertrichosis lanuginosa acquisita (HLA), characterized by the progressive development of multiple, long, thin, unmedullated hairs (laguno hair) that grows all over their body as a natural physiological reaction to severe starvation that serves to keep the body warm in the absence of fat (Schulze et al., 1999).

Already in the 18th century, the beneficial effect of external heat supply in the treatment of hyperactivity in anorexia patients was recognized by C. Chossat (Swiss doctor who observed the consequences of starvation in different species). Gull (1874), based on the results of C. Chossat, proposed a treatment with external heat for anorexia patients (Gutierrez et al., 2002). Gutierrez et al. (2001) found that the supply of external heat not only reduced excessive exercising in anorexia patients, these patients also reported that they did not feel anxious, depressive, or had other unpleasant experiences with the decrease in activity, symptoms also involved in anorexia. In contrast, the patients experienced the heat as calming and relaxing and these psychological changes were followed by a normalization of the eating pattern. These changes continued during follow-ups even after the heat treatment was stopped. One possible way to supply external heat to anorexia patients is a sauna bath (Gutierrez et al., 2002). In healthy people, sauna baths affect the HPA axis. It was found the sauna increased the activity of the HPA axis (Gutierrez et al., 2002). Furthermore, changes in hormone secretion levels produced by sauna are found to be similar to those produced by exercise, but without the energy cost of exercise. It was found that sauna increased cortisol, ACTH and endogenous opioid levels (Kukkonen-Harjula et al., 1986). The fact that treatments with sauna baths and exercise have similarities in hormone secretion is proposed to cause the decrease in hyperactivity observed in anorexia patients (Gutierrez et al., 2002).

In animal models of anorexia, Stevenson et al. (1957) were one of the first to propose that increased locomotor activity observed in a food-deprived state could be associated with changes in thermoregulation during a food-deprived state. These authors found an inverse relationship between the amount of activity displayed by food-deprived male rats and environmental temperature. They also found that body temperature seems to decrease when rats were food-deprived and activity is restricted (animals were held in canvas slings), whereas when the food-deprived rats were not activity-restricted this fall in body temperature was not observed. Campbell et al., (1967) found that rats have increased motivation to increase body temperature under food-deprivation. Rats pressed a bar to obtain a short amount of exposure to a heat lamp and this bar pressing increased when food-deprivation progressed. Wheel running in the ABA model was found to increase body temperature during the active, dark period and during the rest, light period as well (Kent et al., 1991; Weinert et al., 1998). Also food-anticipatory activity before a feeding period in food-deprived rats was found to increase body-temperature (Bolles et al., 1969). According to Hebebrand et al. (2003), if hyperactivity is a form of thermoregulatory behavior, this behavior should attenuate when rats are exposed to a thermo-neutral environment. Indeed, Hillebrand et al., (2005d) found in the ABA model that voluntary access to a warm plate reduced hyperactivity. Moreover, Gutierrez et al. (2006) found that that the ABA procedure in a thermo-neutral (29° C) environment prevented excessive exercise, whereas when

the animals were held at 21° C excessive exercising was still observed. In addition, Gutierrez et al. (2006) have also found that when running in the ABA model had become excessive and body weight loss was above 20%, an increase environmental temperature decreased the excessive exercise and prevented the animals from further weight loss. This caused a recovery of 100% in rats which, according to the authors, has never been observed before (Gutierrez et al., 2009). For anorexia patients the outcomes of the abovementioned studies are important because they show that the supply of external heat not only prevents the development of ABA but also is able to stop it when it already is expressed, which may help in developing effective treatments for anorexia patients as well (Gutierrez et al., 2009).

Based on the abovementioned results, the "hypothermia hypothesis" of excessive exercise in anorexia patients states that this hyperactivity is caused by hypothermia to alleviate the effects of this condition (Hebebrand et al., 2003).

3.2.1 NPY and the hypothermia hypothesis

When centrally applied, NPY stimulates food intake and suppresses the sympathetic nerve activity to brown adipose tissue (BAT), which suppresses thermogenesis (Egawa et al., 1991; Bouali et al., 1995, Currie et al., 1995; Pedrazzini et al., 1998). NPY has also been found to be important for the regulation of the HPT axis which, as mentioned before, is important for the regulation of energy expenditure (Semjonous et al., 2009). NPY neurons from the ARC are found to project to the PVN and Y1R's have been found on the TRH neurons in the PVN (Broberger et al., 1999). An intracerebroverntricular injection of NPY has been shown to suppress proTRH mRNA in the PVN and circulating thyroid hormone levels (Fekete et al., 2001). NPY could cause hypothermia by inhibiting the HPT axis (Silva, 2001). Adjacent to NPY, AgRP also has an inhibitory effect on HPT axis, and NPY is co-localized with AgRP in the ARC. It has been shown in the ABA paradigm, expression of AgRP is upregulated, whereas that of POMC is downregulated (Kas et al., 2003; De Rijke et al., 2005). AgRP inhibits the melanocortin-system that has a stimulatory influence on the HPT axis. POMC neurons have been found to project from the ARC to the PVN TRH neurons as well.

The Y5R is thought to play an important role in causing hypothermia in anorexia patients. The intracerebroventricular infusion of the Y4/Y5 agonist hPP, but not the selective Y4 agonist rPP, has been found to stimulate feeding and causes hypothermia in rats. This suggests that the Y5R is the receptor responsible for the effects on feeding and energy expenditure. Also it was shown that the Y2/Y5 agonists NPY-(2-36) and PYY-(3-36) increased food intake and decreased BAT temperature. Because it was also found that the Y2 selective agonist C2-NPY had no effect on food intake or BAT temperature, the activity of Y2/Y5 agonists must, according to Hwa et al. (1999), be due to activation of the Y5R. In addition, the Y5 selective agonist D-[Trp32]-NPY was found to induce feeding and significantly reduced BAT temperature in satiated rats, further supporting the role of Y5R in thermoregulation. The Y2/Y5 agonist PYY-(3—36), the Y4/Y5 agonist hPP, and the Y5 agonist D-[Trp32]-NPY were all found to be inactive at the Y1 receptor, which suggests according to Hwa et al. (1999), that Y1 receptors are not essential for eliciting feeding and BAT hypothermic effects.

To summarize the evidence found for the "hypothermia hypothesis", one can say that heat-treatment is indeed effective in both humans and animals in reducing hyperactivity associated with anorexia. Furthermore, NPY is found by several authors to cause hypothermia by down-regulation of the HPT-axis. However, no literature was found that directly linked the effect of NPY to hyperactivity via hypothermia. As already pointed out by Campbell et al. (1967), these experiments suggest a thermoregulatory explanation for hyperactivity shown in anorexia patients; they do not necessarily demonstrate a causal link between hypothermia and locomotor activity.

3.3 Hypothesis 3: Hyperactivity displayed by Anorexia Nervosa patients is rewarding

Another hypothesis to explain the counterintuitive hyperactive behavior displayed by anorexia patients is that this behavior is rewarding to them. In healthy subject's it was already found that running long distances is rewarding, also referred to as the "runners high", and can even be addictive (Martin et al., 2006).

Anorexia has been associated with anhedonia, the reduced capacity to experience reward or pleasure, and patients who displayed excessive exercise have been found to show a more severe form of anhedonia (Davis et al., 2002). These authors also suggested that the hyperactivity may be used to alleviate this disposition because exercise induces activation of dopaminergic reward pathways via the activation of the HPA axis and the endogenous opioid system (Hebebrand et al., 2003; Brene et al. 2007). Davis et al. (2002) proposed an answer to the question whether differences in reward sensitivity are a cause or effect of starvation. They suggested it is both; anhedonia probably facilitates food restriction in people who want to lose weight because they find food relatively less rewarding. As dieting becomes chronic and extreme, the stress of this behavior may worsen the anhedonic state, by making eating even less rewarding compared to the onset of the disorder. Excessive exercising is then increased to counteract this hedonic state.

Another view on this hypothesis is proposed by Bergh et al. (1996), who proposed that anorexia develops because it is initially rewarding to the patients to eat less food and to exercise, as anorexia is associated with the fear of gaining weight (Keating, 2009). Anorexia is then maintained through the conditions that provide this reward, no eating and excessive exercise, which explains why patients still display these behaviors even when these behaviors are devastating for the body itself. At a biological level there seem to be similarities between eating disorders and addiction, as they activate the reward pathways and it has been suggested that anorexia is a form of auto-addiction to endogenous opiates (Marzzini et al., 1986; Aravich et al., 1996).

Recently, even another view on this hypothesis has been proposed by Keating (2009), who suggests that anorexia patients have a disfunction in the processing of reward and punishment which causes that the patients are unable to discriminate between the two. This view suggests that excessive exercising is perceived as rewarding instead of a punishment to the body in anorexia patients. Neuro-anatomical abnormalities have been linked to anorexia in brain area's involved in reward such as the amygdala, the anterior cingulate cortes, the thalamus and the medial prefrontal cortex (Takano et al., 2001). Anorexia has also been associated with abnormalities in the serotonergic and dopaminergic systems (e.g. Kaye, 2008; Frank et al., 2005).

3.3.1 NPY and the reward hypothesis

Excessive exercising in anorexia patients and drugs of abuse activate overlapping pathways (for a review, see Kanarek et al., 2009). The rewarding effects of exercise may be due to the direct activation of the reward dopamine pathways or indirect via the endogenous opiate system.

Increases in extracellular dopamine levels in the shell region of the nucleus accumbens seems to be involved in mediating reinforcement of addictive drugs. NPY and its "brain" receptors (Y1, Y2, Y5) are found in the nucleus accumbens as well (Di Chiara et al., 2004). Several studies suggest a role for NPY in brain mechanisms of addiction. NPY-deficient mice consumed more ethanol than control mice, whereas mice that over-express NPY consumed less ethanol (Thiele et al., 1998). In addition, NPY injections into the nucleus accumbens caused place preference, a widely accepted animal model of drug reinforcement, while the dopamine antagonist, cis-flupenthixol, blocked this effect (Josselyn et al., 1993). A study by Sorensen et al. (2009) showed that NPY directly infused into the nucleus accumbens shell of the rat dose-dependently increased dopamine levels. NPY was also found to modulate dopamine levels in several other brain regions, including the hypothalamus (Matos et al., 1996). NPY is thought to act by increasing dopamine release, possibly via the Y2 and Y5 receptors. In slices of the nucleus accumbens, the Y2 antagonist, BIIE0246, blocked KCl-activated release of newly synthesized dopamine after local NPY infusion (Adewale et al., 2007), whereas this effect was not seen in the hippocampus (Meurs et al., 2007). In the hypothalamus, the Y5R agonist [D-Trp^{32}]-NPY increased dopamine levels in microdialysates from the hypothalamus after intracerebroventricular injection, the same effect that was found with NPY (Matos et al., 1996). In this way the high NPY levels under food-deprived conditions may stimulate excessive exercising, while this hyperactivity in itself also stimulates more exercising via its addictive-like properties. Food-restriction or deprivation has been shown to enhance drug-related behavior (Maric et al., 2008). NPY has also been shown to increase food intake under a progressive ratio schedule of food

reinforcement, suggesting an increase in the rewarding value of food in a food-deprived state (Jewett et al., 1995).

As mentioned before, exercise triggers endocannabinoid (2-arachidonoylglycerol and anandamide) release. The endocannabinoid receptor CB1, has been found in hypothalamic brain-sites, including the ARC, PVN, VMN and DMN. Endocannabionoids have been found in highly increased levels under food-deprived conditions and strengthen NPY release in the hypothalamus (Kirkham et al., 2002; Gamber et al., 2005). The endocannabinoids are found to increase dopamine release in the VTA (Cheer et al., 2004), which in turn is thought to triggers locomotor behavior.

Another way in which the reward pathways could be activated by NPY is via the LH. The LH has direct connections with the brains reward pathway; it projects to the nucleus accumbens via the ventral tegmental area (Fadel et al., 2002; Kelley et al., 2005). Lesions of the LH have been found to suppress food intake in mice, the same effect that is seen in dopamine deficient mice (Szczypka et al., 1999). In the LH, two types of neurons have been identified, MCH and orexin (hypocretin) neurons. Both have been found to stimulate food intake and arousal (Gao et al., 2008). These neurons are innervated by both POMC and NPY via the ARC. Both MCH and orexin neurons project widely into the brain and are involved in a variety of behaviors such as learning, memory, emotion, motivation and motor responses in association with changes in the energy state (see Gao et al., 2008). Orexin neurons project to the dopamine neurons in the VTA, whereas MCH neurons project to the nucleus accumbens (Fulton et al., 2009).In the LH, MCH and orexin neurons have connections with each other, and with nearby neurons (Guan et al., 2002). Orexin neurons also project back to the hypothalamus, all hypothalamic neurons that are activated by fasting are innervated by orexin neurons including the ARC and in particular NPY neurons (Horvath et al., 1999). Orexin neurons are found to be activated by NPY signaling via the Y1 and Y5 receptors (Jain et al., 1999; Yamanaka et al., 2000; Dube et al.,2000). Jain et al., (1999) found that the Y1R antagonist 1229U91 blocked feeding behavior which was stimulated by infusion of orexin A. Yamanaka et al. (2000) found that the Y1R antagonist BIBO3304 inhibited orexin A-induced feeding. Dube et al., (2005) showed that the Y5R antagonist CGP71683A, inhibits NPY-induced feeding and suppressed orexin A-induced feeding dose-dependently. Also a synergistic action between NPY and orexin was found to induce feeding behavior, NPY neurons activate orexin neurons in the LH and orexin neurons activate NPY neurons in the ARC (Sahu et al., 2002). The hypothalamic peptides NPY, a-MSH, AgRP, orexin and MCH, have been found to affect the activity of dopaminergic neurons that innervate the nucleus accumbens (Berthoud, 2004). The ARC is thought to convey metabolic information from signals such as leptin via NPY to regulate the activity of the mesolimbic dopaminergic system via direct projections to the nucleus accumbens, or indirectly through the activation of orexin or MCH neurons that also project to both the VTA and nucleus accumbens (Berthoud, 2004). In addition, the VTA is also found to be sensitive to leptin, insulin and ghrelin, and it is found that the activity of dopaminergic neurons in the VTA can also be directly regulated by these molecules (Guan et al., 1997; Figlewitz et al., 2003). For example, Hillebrand et al. (2008) suggested that the found suppression of running-wheel acitivity in the ABA model by increasing leptin levels was mediated by the binding of leptin on dopamine neurons in the VTA, which caused the silencing of these neurons an probably decreased the motivation to run. So, there may be two mechanisms working together by which reward pathways are activated in response to under-nutrition, directly via leptin, insulin and ghrelin or indirectly via NPY in the hypothalamus (Fulton et al., 2000; Figlewitz et al., 2003).

The increased NPY caused by starvation and physical activity activates the HPA axis and causes elevated cortisol levels. Cortisol could activate dopamine and noradrenalin neurons in the limbic system which could reinforce self-starvation and excessive running via reward mechanisms, thereby increasing the behaviorally reinforcing properties of stress or increasing the compulsiveness of some activities (Bergh et al., 1996). Challet et al., (1999) found that in rats, in the ABA model, locomotor activity was suppressed in food-deprived adrenalectomised rats and this activity was restored by corticosterone replacement.

In contrast to the literature showing that dopamine is involved in hyperactivity associated with anorexia, a recent study questions this concept. The study of Verhagen et al. (2009b) determined the release of DA, serotonin and their metabolites in the nucleus accumbens in the ABA paradigm. They

found that in ABA rats, dopamine release and its metabolites were increased during food intake, but not during food-anticipatory activity (period preceding food delivery) in the nucleus accumbens. They also found that as running-wheel activity increased over days, dopamine release was still lower compared to *ad libitum* fed rats. Furthermore, the authors found that ABA rats treated with a dopamine antagonist still showed food-anticipatory activity (Verhagen et al., *in press* from Verhagen et al., 2009b). The authors suggested that dopamine is not involved in the initiation of food-anticipatory activity

To summarize, the role of NPY in the "reward" hypothesis is to activate dopaminergic neurons in the nucleus accumbens and the VTA, thereby reinforcing the rewarding effects of exercise and the rejection of food. This effect seems to be mediated via the Y2 and Y5 receptors. Endocannabinoids, released in response to exercise possibly initiate and reinforce the effects of NPY by activation of the NPY neurons. Furthermore, NPY activates orexins via Y1 and Y5 receptors and MCH in the LH, orexins in turn activate NPY neurons. Orexin and MCH activate dopaminergic neurons in the nucleus accumbens and the VTA, thereby also possibly reinforcing hyperactivity. NPY also activates the HPA axis, which in turn increased dopamine levels. However, some authors question the involvement of dopamine in hyperactivity. The abovementioned studies provide convincing evidence that once exercising is started it can be maintained via dopamine pathways (Gelegen et al., 2007). However, the studies do not provide convincing evidence for the fact if dopamine is essential to initiate hyperactivity.

3.4 Brain imaging study to give an indication of brain areas involved in ABA rats

In a brain imaging study, brain metabolism of rats in the ABA model and controls was studied using voxel-based analysis (Van Kuyck et al., 2007). The study suggests a complex interaction of brain circuits involved in locomotor activity, food-related behavior and somatosensation. They found metabolic changes in brain areas involved in reward and locomotion and especially in selecting the appropriate motor response, such as the nucleus accumbens core and the superior colliculus (King, 2004; Zahm, 2002). In chapter 2, it was already mentioned that dopamine stimulates locomotor behavior. Berridge et al. (1998) proposed that appetitive responses in general are thought to be dependent on dopamine innervation of the nucleus accumbens, whereas consummatory responses are not. Appetitive ingestive behavior was found to be stimulated by infusion of NPY in to the perifornical hypothalamus and could be blocked by dopamine receptor blockade of the nucleus accumbens (Brown et al., 2000). The study by Van Kuyck et al. (2007) could not find any differences in metabolism in the hypothalamus but the authors suggested that this was because of the small size of the hypothalamus. What they also found was the involvement of a higher brain circuitry, the insular cortex. This brain region mediates gustatory and viscerosensory stimuli and projects to the cingulate cortex which in turn projects to the nucleus accumbens (Kelley et al., 2005). The nucleus accumbens projects via the ventral pallidum to the mediodorsal thalamic nucleus which in turn project to the cingulate cortex (Mega et al., 2001). According to Van Kuyck et al. (2007), this cortico-striatal-thalamic-cortical circuity has been linked to obsessive compulsive disorder. Hyperactivity displayed by food deprived rats may have a compulsive component.

3.5 NPY receptor distribution in the brain

The distribution of NPY receptors in different brain sites might indicate which pathways are involved in appetitive ingestive behaviors shown in ABA animals. Y1, Y2, Y4 and Y5 receptors are all abundantly found in the central nervous system, only the Y1, Y2 and Y5 receptors are thought to be implicated in feeding behavior and these receptors also have the highest affinity for NPY (Parker et al., 2000). However, the only studies that investigated the role of NPY receptors in appetitive ingestive behavior are mentioned above (Day et al., 2005; Keen-Rhinehart et al., 2007; Dailey et al., 2009).

Another way of investigating if NPY receptors are possibly involved in appetitive ingestive behavior is by looking at the distribution of these receptors in brain areas associated with this specific behavior such as the motor initiation areas (nucleus accumbens) and brain areas involved in incentive salience (limbic system) and stress. However, it should be taken into account that the presence of a

particular receptor subtype in a particular area involved in appetitive ingestive behavior is not direct evidence that this particular behavior is mediated by this receptor. It only gives an indication for experiments that could be done with specific receptor agonists of antagonists to investigate the effects on this specific behavior.

In the rat brain the Y1 receptor is abundantly spread. Y1 receptor mRNA was found in the cortex, hippocampus, thalamus, amygdala and specific nuclei of the hypothalamus, especially in the ARC (Kamji et al., 2007). Mice lacking NPY 1 receptor become obese and show a reduction in locomotor activity (Segal-Lieberman et al., 2003), suggesting that these receptors might influence activity under food-deprived conditions. In the human brain, high Y1 receptor levels were found in the hypothalamus, in particular the ARC and the PVN (Jacques et al., 1996). It was also found that changes in feeding behavior and energy balance induced changes in Y1 receptor function and expression in certain regions of the hypothalamus and not in others (reviewed in Eva et al., 2006). Fasting increased NPY levels and decreased Y1 receptor expression in ARC and PVN but not in Y2 and Y5 receptors (Cheng et al., 1998; Xu et al., 1998).

Stanic et al. (2006) investigated the distribution of Y2 receptors (Y2R) in the mouse brain and found the following. Y2R's were found in the amygdala and the authors suggested that this receptor is possibly involved in stress responses. They also found this receptor in the locus coeruleus, a major brain site for the production of noradrenalin. Y2R's were also expressed in the hypothalamus and in particular in the ARC, DMN, PFA and the PVN (Kamji et al., 2007). Sainsbury et al., (2002) found that deletion of the Y2R causes a reduction in body weight and an increase in food intake because this increases NPY expression in the ARC. NPY gene expression in DMN was found to be increased in response to chronic food restriction and long term exercise (Bi, 2007), but not to acute food deprivation (Bi et al., 2003), it was suggested by the authors that especially DMN NPY, instead of NPY in the ARC, might play an important role in maintaining energy homestasis only in response to long-term changes in energy intake or expenditure (Bi et al., 2003). The striatum, involved in the regulation of movement, behavior, emotions and reward processing, also contained Y2R's (Stanic et al., 2006). These receptors were found as well in the lateral septum, a brain site implicated in fear responses and motivation (Sheehan et al., 2004). The inactivation of theY2 receptor produces Y2 null mutant mice and these animals developed mild obesity with increased fat deposition which was caused by hyperphagia and reduced energy expenditure due to reduced activity during the light and dark phases (Naveilhan et al., 1999). Tasan et al. (2009) found that the Y2/Y4 double knockout mouse also showed reduced locomotion and food intake. These findings suggest that this receptor is possibly involved in the hyperactivity associated with anorexia, probably influencing incentive salience of food, motivation and motor responses.

The Y5R has been found in hypothalamus but also in the thalamus, hippocampus, NTS and the cortex of the rat (Campbell et al., 2001). Kamji et al., (2007) found that Y5R's were less abundant in the CNS compared to Y1 and Y2 and this receptor was predominantly found in hippocampal and hypothalamic regions.

As can be extracted from the abovementioned studies, more that one receptor is present in most of the brain systems possibly implicated in appetitive ingestive behaviors and reward processing. Kamji et al. (2007) found co-expression of NPY mRNA with Y2R's but not Y1R's in the cerebral cortex, hippocampus, amygdala, striatum and nucleus accumbens. However, Fettisov et al. (2004) did find co-expression of Y1R's and Y2R's mRNA in the cerebral cortex and the amygdala. These differences may be due to differences in techniques used. In the mouse brain all area's that expressed Y5R's also expressed Y1R's but not the other way around (Naveilhan et al., 1998). In the ARC Y1,Y2,Y5 receptors have been found, whereas in the PVN the Y1,Y2,Y4,Y5 receptors have been shown (Durkin et al., 2000). Co-localization Y1 and Y5 was found in the cortex, hippocampus, hypothalamus, amygdala and brainstem (Wolak et al., 2003). Co-expression and localization possibly suggest differences in function of the different receptors. For example, Henry et al., (2005) found in mice that activation of the Y5R's caused an increase in feeding and adiposity through a combination of hyperphagia and nutrient partitioning, without changes in total energy expenditure. This resulted in a significant reduction of calories metabolized per calories ingested (Henry et al., 2005). Chronic activation of central Y1R's was found to cause an increase in body-weight by changing nutrient

partitioning alone. Central activation of either Y1 or Y5 receptors could modify nutrient partitioning by reducing energy substrates derived from lipid oxidation and/or increasing lipogenesis (Hwa et al., 1999). Chronic activation of central Y2R's was found to cause a transient decrease in weight, mainly via hypophagia and has only little effect on energy expenditure (Henry et al., 2005). Different actions of the receptors could indicate a possible mechanism by which NPY could regulate both food intake and locomotor activity in both ABA rats and anorexia patients.

As mentioned before, different strains of mice display differences in locomotor behavior in response to the ABA paradigm (Kas et al., 2009). There also seem to be differences in genetic background and expression of certain NPY receptors. Gelegen et al. (2006) found a difference in Y1R expression in three different strains of mice. Y1R expression was investigated under food-restriction in C57BL/J6, A/J and DBA/2J mice. Only DBA/2J had an upregulation of the hypothalamic Y1 receptor under these conditions and this strain also showed increased locomotor activity and a decrease in body temperature and adipose tissue (Gelegen et al., 2006).

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Chapter 4: Discussion: The role of NPY in hyperactivity in *Anorexia Nervosa*

In this thesis an attempt was made to shed some light on the possible role of NPY in the paradoxal behavioral hyperactivity often observed in patients with Anorexia Nervosa. A number of interrelated brain systems seem to be involved in this behavior of which most are discussed in this thesis (Chapter 2, except ghrelin, insulin and brain-derived neurotrophic factor). Furthermore, three hypotheses have been proposed by the literature to explain this counterintuitive and devastating behavior; the foraging hypothesis, the hypothermia hypothesis and the reward hypothesis. In addition, the involvement of the different NPY receptors has been highlighted.

In order to answer the question which role NPY serves in hyperactivity associated with anorexia, first the question of function of this behavior needs to be answered. From the three hypotheses discussed, the foraging hypothesis seems the most convincing. This hypothesis not only provides an answer to the question of how this behavior is initiated (chronic food-deprivation) and how it is maintained (redirecting attention to relevant stimuli, increasing "wanting" for food), it also is the only hypothesis, to my knowledge, providing an answer to the question what purpose this behavior serves in evolutionary terms. In this hypothesis, the role of NPY is that of an orexigenic gateway that adjacent to other molecules, receives input from peripheral (adiposity) signals and the HPA-axis to activate brain systems that initiate the search for food, which in turn activate other pathways that maintain and enhance the orexigenic drive. This is done by activation TRH and CRH to shut down thermogenesis and activate the HPA-axis and the monoaminergic pathways to redirect attention to stimuli (noradrenalin) and increase the rewarding value of certain behaviors that eventually lead to food ingestion (dopamine and serotonin). These neuroendocrine changes allow the individual to adopt behavioral strategies that are needed rather than only maintaining body weight homeostasis (Sodersten et al., 2006). The view that NPY is an orexigen was questioned by several authors (e.g. Ammar et al., 2000) but evidence shows that NPY can still be considered an orexigen although it initially helps to show paradoxal behavior.

The least convincing hypothesis is the hypothermia hypothesis because it lacks an evolutionary plausible explanation for displaying hyperactivity. The hypothesis states that activity is increased to compensate for the decreased thermoregulation of the body (Gutierrez et al., 2002, 2006, 2009). It was found that activity increases as starvation progresses, but body temperature decreases and is kept on a stable lower level in a food deprived state in a constant environmental temperature (Sakurada et al., 2000). It seems unlikely that in a more severe food-deprived state it is needed to be more hyperactive to generate enough heat to maintain that same body temperature. Furthermore, it also seems counterintuitive to down-regulate thermogenesis to save energy in a food-deprived state and to compensate that with behavior that even costs more energy and serves no other purpose. The role of NPY in this hypothesis is to down regulate thermogenesis by decreasing activity of the HPT-axis in a severe food-deprived state (Semjonous et al., 2009). In my opinion hyperactivity serves another purpose and the fact that it generates heat which alleviates the symptoms of hypothermia is just a (welcome) side-effect. Case reports of anorexia patients have also found that some patients open windows and shut down heaters in order to loose weight. However, case reports also show that these patients also exercise to increase body temperature (Gutierrez et al., 2009) and to loose weight at the same time.

The evidence for the reward hypothesis is quite convincing is well. However, it also lacks an evolutionary explanation for its function. In contrast to the foraging hypothesis, this hypothesis also takes into account the conscious act of the anorexia patient to exercise and to refuse to eat into consideration. The role of NPY in this hypothesis is to activate the reward-pathways in the brain to reinforce hyperactivity and self-starvation. However, NPY seems not essential for this task as endocannabinoids and adiposity signals, such as leptin, can also directly affect reward pathways in the VTA and the nucleus accumbens (Fulton et al., 2000; Figlewitz et al., 2003). In addition, recent evidence in the ABA model questions the involvement of dopamine in initiation of hyperactivity.

In my opinion, the studies discussed in chapter 3 show that the reward hypothesis and the hypothermia hypothesis are part of the foraging hypothesis. By combining the foraging hypothesis and

the reward hypothesis, this explains how hyperactivity displayed by anorexia patients is initiated (conscious attempt to loose weight via exercising and self-starvation, which both are rewarding), how it is maintained (rewarding to exercise, “wanting” behavior increased by the rewarding effect of behaviors that lead to food-ingestion, attention directed away from food-ingestion to food-acquiring behavior), why it escalates (rewarding pathways that reinforce the search for food, make these behaviors addictive (Marzzini et al., 1986; Aravich et al., 1996)) and how this, at first sight, counterintuitive behavior is explained in evolutionary terms (individuals that react to food-shortage with searching for food have greater survival value, so brain-systems involved in this behavior have survival value as well). The main purpose of hyperthermia is then to save energy that is needed for food-searching behavior. The role of NPY in this combined hypothesis is to activate the neuroendocrine systems involved in foraging behavior, the down regulate thermogenesis and to activate reward pathways to reinforce this behavior. The localization of the NPY receptors, discussed in chapter 3 and the metabolic activity seen in de study of Van Kuyck et al. (2007), show that both reward pathways and brain-systems involved in inducing foraging behavior have a lot of NPY receptors, especially Y1 and Y5 and less abundant Y2 and have increased metabolic rate in these areas. However, the precise roles of the NPY receptors in these hypotheses still have to be elucidated.

The animal model for anorexia nervosa, the ABA model is extensively used to investigate the neurobiological mechanisms underlying the behavior. Although it is mimics most of the symptoms associated with this disease, it also has a flaw. When animals subjected to this paradigm eating can reverse the excessive exercising together with the reduced food-intake displayed (Dixon et al., 2003). In humans, consciousness also plays a big role in anorexia as these patients consciously refuse to eat and engage in excessive exercising because of obsessive fear of gaining weight and a distorted body image (Gutierrez et al., 2009, Casper et al., 2006). Therefore, results obtained via this model should be treated with great care.

In conclusion, of the three discussed hypotheses the foraging and reward hypothesis seem the most convincing. A combination of these two hypotheses could explain the initiation, maintenance, escalation and the evolutionairy perspective of this behavior even better. The role of NPY in this combined hypothesis is to activate the neuro-endocrine systems involved in foraging behavior, the down regulate thermogenesis and to activate reward pathways to reinforce this behavior adjacent to direct pathways affecting these mechanisms. Studies investigating the involvement of the NPY receptors provide extra evidence that brain mechanisms involved in foraging behavior and reward are implicated in this behavior. However, specific experiments testing these hypotheses are lacking. Also a brain imaging study in the animal model of anorexia, the ABA paradigm, showed increased activiation of brain regions involved in both mechanisms. Although the ABA model seems a good animal model for anorexia nervosa, it lacks the consciousness choices made by anorexia patients.

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