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Brain circuitries in control of feeding behaviors

Focus on Neuropeptide Y

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Chapter I. Introduction

Partly adapted from: *The effect of obesogenic diets on brain Neuropeptide Y*
Gumbs et al. (2016). *Physiology and Behavior*, 162, 161-173

Obesity: a role for environmental factors

Obesity is defined as a body mass index exceeding 30 kg/m² and is characterized by excessive white fat mass accumulation due to an imbalance in energy uptake (i.e. hyperphagia) and energy expenditure (i.e. decreased metabolic rate and/or decreased voluntary energy expenditure). Excessive adiposity is a severe risk factor for several adverse diseases such as type II diabetes mellitus, cardiovascular diseases, and several types of cancer (Bianchini, Kaaks, & Vainio, 2002; Haslam & James, 2005; Meldrum, Morris, & Gambone, 2017; Wannamethee & Shaper, 1999). Worldwide prevalence of obesity has nearly tripled over the past 40 years, with 39% of adults being overweight and 13% obese in 2016, which has increased personal and societal disease burden immensely, and thus warrants immediate attention (Blucher, 2019; World Health Organization, 2019).

The rapid increase in obesity prevalence suggests that environmental factors, as opposed to, but likely in combination with, (epi)genetic susceptibility, play a predominant role in the recent increase in the prevalence of obesity (Pigeyre, Yazdi, Kaur, & Meyre, 2016; Xia & Grant, 2013). Recognized environmental factors include a sedentary lifestyle and, importantly, dietary changes such as the increased availability of highly palatable- and energy dense foods, containing a high percentage of fat and sugar, and the occurrence of larger portion sizes (Carden & Carr, 2013; Kant & Graubard, 2015; Malik et al., 2010; Piernas & Popkin, 2011; Wright & Aronne, 2012). Freely available palatable foods are generally overconsumed and can lead to altered feeding behaviors such as snacking and intake of larger portion sizes, which may further increase caloric intake. In addition, consumption of these palatable food items alter, and possibly disrupt, peripheral and central processes that regulate energy balance.

Treatment options for obesity include initial (preventive) dietary and exercise management strategies, and can be supplemented with (preventive) psychological, pharmacological or surgical treatment options when the former strategies fail to lead to the desired weight loss. However, most treatment options for obesity show limited (long-term) effectiveness and often present adverse side effects (Lupoli et al., 2017; Pilitsi et al., 2019). Research on the biological mechanisms regulating food intake as well as research into the physiological changes induced by diet and obesity, is therefore necessary to optimize existing treatments and to lead to new treatment options.

Neural circuitries related to food intake regulation

The regulation of energy homeostasis is highly complex and involves the integration of internal and external signals. These signals are sensed and processed in the periphery and in the brain to adjust energy metabolism and behavior via endocrine signals, the autonomic nervous system and motor output (Berthoud, 2002; Broberger, 2005). This thesis focusses on

the regulation of energy uptake at the level of the organism; i.e. food intake regulation, which, in its most basic form, requires sensing of the metabolic status, processing and integrating relevant information, and the mobilization of motivational and motor circuits, which may lead to adaptive changes in food intake behavior. Many brain areas and brain circuitries are involved in the regulation of feeding behavior. For clarity, and in accordance with previous literature, this thesis will conceptualize two systems that are highly interactive and interlinked as separate circuitries; a homeostatic system that pertains to the maintenance of energy balance, and a reward system that pertains to the hedonic and motivational aspects of feeding behavior (Berthoud, Munzberg, & Morrison, 2017; Broberger, 2005; Morton, Cummings, Baskin, Barsh, & Schwartz, 2006).

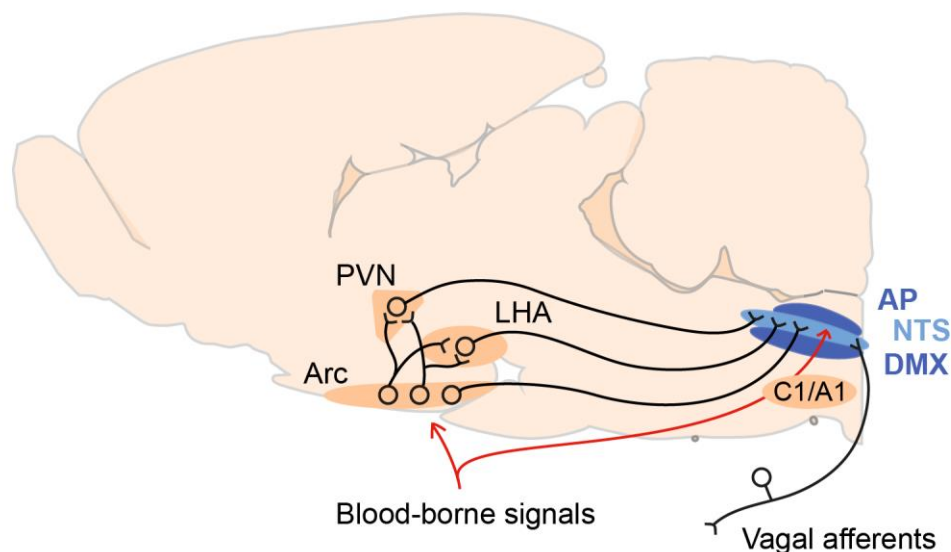


Figure 1. Schematic overview of the homeostatic system. Afferent input on energy status can reach the hypothalamus and hindbrain via blood-borne signals or the cranial nerves (example of vagal afferents in figure). Signals that reach the Arc are transferred to other hypothalamic regions, and to the hindbrain. Information reaching the hindbrain directly can also be transmitted to the hypothalamic regions (not drawn). For clarity, projections from the C1/A1 region and reciprocal connections between the hypothalamic and hindbrain regions are not drawn (see text for details). Arc = arcuate nucleus of the hypothalamus, AP = area postrema, C1/A1 = catecholaminergic cell groups in the ventrolateral medulla of the hindbrain, DMX = dorsal motor nucleus of the vagus, LHA = lateral hypothalamic area, NTS = nucleus of the solitary tract, PVN = paraventricular nucleus of the hypothalamus. Adapted with permission from (Morton, Cummings, Baskin, Barsh, & Schwartz, 2006).

Brain circuitries related to homeostatic regulation of food intake

Homeostatic circuitries of food intake regulation adaptively adjust feeding behavior to meet the biological needs of the animal. These circuitries originate in the hypothalamus and hindbrain (see Figure 1), which are historically recognized as key regulators of several

homeostatic processes. Information on the metabolic status of the organism can be sensed by several hypothalamic and hindbrain regions along the third and fourth ventricles that have a relatively penetrable blood-brain-barrier (Broadwell & Brightman, 1976; Ciofi, 2011; Rodriguez, Blazquez, & Guerra, 2010). Receptors and transporters relevant for detecting signals of hunger (i.e. the stomach-derived ghrelin), satiety (e.g. pancreas-derived insulin, or other gastrointestinal-derived signals, such as cholecystokinin), and adiposity (i.e. adipose tissue-derived leptin), but also changes in nutrients levels (i.e. free fatty acids, glucose, and amino acids) are highly expressed in these areas (Anthony & Gietzen, 2013; Elmquist, Bjorbaek, Ahima, Flier, & Saper, 1998; Havrankova, Roth, & Brownstein, 1978; Leloup et al., 1994; Sarruf et al., 2009; Zigman, Jones, Lee, Saper, & Elmquist, 2006). In addition, neural information can be relayed to the hindbrain from organs of the gastro-intestinal tract via primary visceral afferents in the cranial nerves (i.e. the trigeminal [V], facial [VII], glossopharyngeal [IX], and vagus [X] nerves). There are thus interacting descending (from the hypothalamus to the hindbrain) and ascending (from the hindbrain to the hypothalamus) information streams that process information that is important for the regulation of food intake.

Descending information stream: Hypothalamic circuitries involved in food intake regulation

The hypothalamus can be grossly categorized into periventricular, medial and lateral regions based on their function (Berthoud, 2002). The periventricular region, which includes the arcuate nucleus (Arc) and paraventricular nucleus (PVN) of the hypothalamus, mainly detects blood-borne signals and controls autonomic and endocrine responses. The Arc and PVN are connected with other hypothalamic and extrahypothalamic regions via efferent and afferent neural projections. The medial region includes several nuclei such as the dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei. These regions receive different kinds of sensory inputs, are extensively connected with other regions of the hypothalamus, and are more involved in regulating adaptive behaviors. The lateral region is less well defined; it is comprised of the lateral hypothalamic area (LHA) and several of its divisions. This region has extensive hypothalamic and extrahypothalamic connections and is therefore optimally located to process and relay information regarding energy homeostasis as well as reward (Berthoud & Munzberg, 2011). Although this broad organization of the hypothalamic regions captures the general functional organization, it is important to note that these divisions do not capture the complexity and nuances in the neuroanatomical connections of these regions. The Arc, PVN, and LHA are most relevant to the studies performed in this thesis and are therefore elaborated on.

The Arc resides closest to the partial blood-brain barrier and is the major hypothalamic hub for integrating energy-status relevant information, and relaying this to hypothalamic and extrahypothalamic regions (Chronwall, 1985; L. Zhang, Hernandez-Sanchez,

& Herzog, 2019). Two principal Arc neuronal populations regulate food intake in an opposite manner: anorexigenic pro-opiomelanocortin-cocaine-and-amphetamine-related transcript (POMC/CART) neurons and orexigenic Neuropeptide Y/Agouti-related protein (NPY/AgRP) neurons (Kageyama et al., 2012; Q. Wei et al., 2018). Both neuronal groups are sensitive to peripheral signals and project to hypothalamic and extrahypothalamic structures to influence second order neurons and ultimately influence behavior (R. D. Cone, 2005; Kohno & Yada, 2012; D. Wang et al., 2015). POMC and AgRP both regulate food intake via melanocortin receptors 3 and 4. These receptors are found abundantly in hypothalamic regions, but also throughout the brain, and exert tonic inhibition of feeding (Adan, Cone, Burbach, & Gispen, 1994; Fan, Boston, Kesterson, Hruby, & Cone, 1997; Mountjoy, Mortrud, Low, Simerly, & Cone, 1994). The POMC cleavage products α - and β -melanocyte stimulating hormone (MSH) lead to a long-lasting reduction in food intake and body weight, and an increase in energy expenditure via the melanocortin receptors 3 and 4, whereas AgRP reduces these effects by signaling as an inverse agonist at these receptors (Fan et al., 1997; Nijenhuis, Oosterom, & Adan, 2001; Ollmann et al., 1997; M. Rossi et al., 1998). Acute changes in food intake are regulated via the second output pathway, which signals via NPY receptors (NPYR). NPYR are also found within the hypothalamus and throughout the brain (R. M. Parker & Herzog, 1999). Arc NPY effector pathways include the Arc POMC neurons (Cowley et al., 2001; Ghamari-Langroudi, Colmers, & Cone, 2005; Roseberry, Liu, Jackson, Cai, & Friedman, 2004), and extensive projections to hypothalamic regions such as the PVN and LHA. However, they also include less extensive projections to the VMH and DMH, as well as to extrahypothalamic brain regions including areas in the forebrain, the midbrain and the hindbrain (Bai et al., 1985; Elias et al., 1998; D. Wang et al., 2015).

The PVN contains a variety of neurons that play a role in the regulation of energy balance, but the neuron types that regulate feeding are not completely characterized yet. Among the neuronal types are oxytocin-, vasopressin-, corticotrophin-releasing hormone- and thyrotropin-releasing hormone-expressing neurons (Sutton, Myers, & Olson, 2016). PVN neurons express melanocortin receptor subtypes 3 and 4, as well as several of the NPYR subtypes onto which the NPY/AgRP and POMC/CART neurons from the Arc synapse. Apart from the Arc projections, the PVN also receives information that is relevant for food intake regulation from the hindbrain (Guevara-Aguilar, Jimenez-Montufar, Garcia-Diaz, Wayner, & Armstrong, 1988). From the PVN, information is relayed as endocrine and autonomic output via the different hypothalamic pituitary axes (connecting the anterior pituitary gland with the thyroid gland [HPT-axis], the adrenal glands [HPA-axis] or the gonads [HPG-axis] via releasing factors), and via autonomic projections to regions in the hindbrain and spinal cord that are involved in the regulation of satiety (M. M. Li et al., 2019; Sawchenko & Swanson, 1982; Sutton et al., 2016). These regions include the nucleus of the solitary tract, the parabrachial nucleus and the dorsal motor nucleus of the vagus, as well as the spinal intermediolateral

nucleus. Of the different hypothalamic structures, the PVN is the major output structure to the hindbrain in the control of feeding (Sawchenko & Swanson, 1982).

The LHA is the most extensively connected area of the hypothalamus, having reciprocal connections with a plethora of brain regions involved in endocrine and autonomic processes, visceral sensory processes, and reward processes, as well as cognitive, emotive, and arousal processes (Berthoud, 2002; Berthoud & Munzberg, 2011). The LHA is thus connected with hypothalamic, midbrain, and hindbrain regions, but also with limbic and cortical regions. The LHA thus receives a wide variety of information and has access to the three major output systems of the brain. Several well-studied neuronal populations that affect feeding when activated reside in the LHA, including the orexin/hypocretin and melanin-concentrating hormone populations (Berthoud & Munzberg, 2011). These populations project to brain regions involved in the hedonic processes of food intake regulation such as the ventral tegmental area and the nucleus accumbens, and affect food-related motivation or hedonic processing (Phillipson, 1979; Qualls-Creekmore & Munzberg, 2018; Tyree & de Lecea, 2017).

Ascending information stream: Hindbrain circuitries involved in food intake regulation

The hindbrain also has specialized areas that can sense blood-borne factors related to energetic status, and receives neural information from other regions including many of the hypothalamic nuclei (Berthoud, 2002; Grill & Hayes, 2012; Schneeberger, Gomis, & Claret, 2014). In addition, the hindbrain receives direct feeding-related information from the alimentary canal through mechano- and chemoreceptors that signal via the primary visceral afferents in the trigeminal (V), facial (VII), glossopharyngeal (IX), and vagus nerves (X), which mostly synapse directly in the nucleus of the solitary tract (Berthoud, 2002; Garcia-Diaz, Jimenez-Montufar, Guevara-Aguilar, Wayner, & Armstrong, 1988; Grill & Hayes, 2012). Importantly, the hindbrain contains the motor output nuclei, through which all neural signals have to be processed in order to produce or adjust behavior (Grill & Hayes, 2012). The hindbrain circuitries are elaborate, for purpose of this thesis only a general overview will be provided here.

The dorsal vagal complex (DVC) is comprised of **the nucleus of the solitary tract (NTS)**, **the area postrema (AP)** and the **dorsal motor nucleus of the vagus (DMX)**. The NTS is one of the most important hindbrain regions involved in the regulation feeding, which receives multiple inputs that are important for the regulation of food intake. Apart from cranial nerve input, it is located close to the AP, which lies next to the fourth ventricle and lacks a tight blood-brain-barrier. In addition, the NTS receives descending information from the hypothalamus such as the PVN (Guevara-Aguilar et al., 1988). This information is processed in the NTS and distributed via ascending projections to other hindbrain, forebrain, hypothalamic and cortical brain regions to regulate food intake (Halsell, Travers, & Travers,

1996; Riche, De Pommery, & Menetrey, 1990; Rinaman, 2010). In addition, the information can be directly relayed via descending projections to modulate the autonomic nervous system, e.g. via the sympathetic preganglionic neurons in the intermediolateral nucleus in the spinal cord, or to motor nuclei, such as the DMX that innervates several visceral structures to mediate vagal reflexes (Grill & Hayes, 2012).

Apart from nuclei that are distinguishable by gross anatomy, the hindbrain also contains several of the **catecholaminergic systems**, which are categorized into subnuclei that are distributed over different anatomical nuclei. The ascending noradrenergic and adrenergic projections arise from the NTS, parts of the reticular formation, and the locus coeruleus, and project heavily throughout the brain, ascending to regions of the hypothalamus, and descending to the spinal cord to provide behavioral output via the reticulospinal tract (Paxinos & Watson, 2007; Sawchenko & Swanson, 1982). The catecholaminergic systems are involved in the regulation of multiple processes including those related to food intake. Particularly, the C1/A1 catecholaminergic group in the ventrolateral medulla is important for coordinating physiological and feeding responses in response to physiological challenges (Ritter, Li, Wang, & Dinh, 2011), and the involvement of the C1/A1 → PVN projection in mediating the feeding response to glucoprivation has been researched intensively (A. J. Li & Ritter, 2004; A. J. Li, Wang, Dinh, & Ritter, 2009; Rinaman, 2003; Ritter, Bugarith, & Dinh, 2001).

Reward circuitries involved in food intake regulation

The reward circuitries of the brain mediate the hedonic and motivational aspects of feeding behavior. Hedonic aspects of food intake can arise from pleasant sensory factors and contribute to the palatability of a food, whereas the motivational aspects of food intake relate to the willingness of an organism to work for food. The direct hedonic value of food is also called 'liking', whereas 'wanting' relates to the motivational aspects of food intake (Berridge & Robinson, 2016).

The mesolimbic dopamine circuitry is the most studied circuitry related to food-motivated behavior. This circuitry consists of the dopamine neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc), as well as to other corticolimbic structures (see figure 2; [Morton et al., 2006]). The VTA receives input from many brain regions including hindbrain regions involved in visceral sensory processing, and hypothalamic regions, particularly the LHA (Geisler & Zahm, 2005; Meye & Adan, 2014; Watabe-Uchida, Zhu, Ogawa, Vamanrao, & Uchida, 2012; Yetnikoff, Lavezzi, Reichard, & Zahm, 2014). The mesolimbic VTA→NAc projection is implicated in mediating the motivational aspects of feeding behavior (Baik, 2013b; Hernandez & Hoebel, 1988; Liang, Hajnal, & Norgren, 2006; Martel & Fantino, 1996). Indeed, altering dopamine transmission specifically alters the motivation to work for food (i.e. 'wanting'; [Aberman, Ward, & Salamone, 1998; Boekhoudt

et al., 2017; Boekhoudt et al., 2018; Randall et al., 2012; Salamone & Correa, 2012; Treit & Berridge, 1990]). The effects of dopamine are mediated through five dopamine receptor subtypes (Baik, 2013b). The NAc has a high expression of the dopamine receptor 1 (DRD₁) and dopamine receptor 2 (DRD₂), which are mainly expressed on separate GABAergic medium spiny neurons (Meredith, Baldo, Andrezjewski, & Kelley, 2008). Diet composition, dysregulated feeding and obesity are associated with alterations in the dopamine circuitry, and particularly with changes in NAc DRD₂ signaling (Baik, 2013a; P. M. Johnson & Kenny, 2010; Kenny, 2011b; Soares-Cunha, Coimbra, David-Pereira, et al., 2016; van Galen, Ter Horst, Booij, la Fleur, & Serlie, 2018; Volkow, Wang, & Baler, 2011; G. J. Wang et al., 2001).

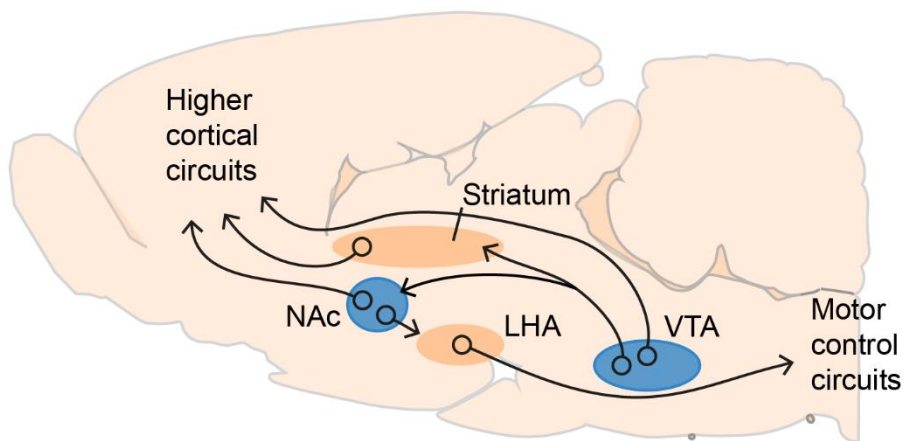


Figure 2. Schematic overview of the principal reward system. Neurons in the VTA of the midbrain project to forebrain areas, such as the NAc, striatum and cortex to process hedonic and motivational aspects of feeding behavior. Especially the VTA->NAc projection is important for dopaminergic signaling of reward (blue regions = mesolimbic dopamine system). Not all regions implicated in reward processing are drawn for clarity. In addition, input to the VTA from homeostatic regions, such as hindbrain or hypothalamic regions are not drawn for clarity. See text for details. LHA = lateral hypothalamic area, NAc = nucleus accumbens, VTA = ventral tegmental area. Adapted with permission from (Morton et al., 2006).

The opioid reward system consists of the opioid peptides enkephalin, dynorphin, and β -endorphin, signaling through the mu-, delta- and kappa-opioid receptor subtypes, which are expressed throughout the brain (Le Merrer, Becker, Befort, & Kieffer, 2009). Within the NAc, opioid-expressing medium spiny neurons consist of separate populations that either express enkephalin (mainly co-expressing the DRD₂), or dynorphin (mainly co-expressing the DRD₁) and project to the basal ganglia to coordinate voluntary motor movements (Gerfen et al., 1990; Kreitzer, 2009). Opioid neurotransmission, and in particular enkephalin/mu-opioid receptor signaling in the NAc, is important for processing hedonic properties, i.e. 'liking', of food. For example, NAc mu-opioid receptor stimulation specifically leads to increased intake

of fat, a palatable food item, via direct and indirect projections to the LHA (Higgs & Cooper, 1998; Kelley, Will, Steininger, Zhang, & Haber, 2003; Pecina & Berridge, 2000; M. Zhang, Gosnell, & Kelley, 1998). In addition, palatable diet consumption can specifically influence enkephalin expression in the NAc (Kelley et al., 2003).

Interactions between the homeostatic and reward circuitries

Though often conceptualized as separate systems, it is increasingly recognized that the homeostatic and reward circuitries interact in the coordination of feeding behavior (Berthoud, Munzberg, & Morrison, 2017; Ferrario et al., 2016). Anatomical substrates for the interactions between these systems include the presence of receptors for nutrients and hormones related to energy status in reward-related regions (Abizaid et al., 2006; Batch et al., 1992; Figlewicz, Evans, Murphy, Hoen, & Baskin, 2003; Krugel, 2003; Werther et al., 1987; Zigman et al., 2006), as well as the presence of receptors for neurotransmitters or neuro-modulators that signal from homeostatic circuitries such as orexin receptors or Neuropeptide Y receptors (Liu & Borgland, 2015; Quarta & Smolders, 2014). In addition, though generally sparse, there are (reciprocal) neuronal projections between areas of the homeostatic systems and the VTA or NAc with the largest input from the hypothalamus to the VTA coming from the LHA (Meye & Adan, 2014; M. A. Rossi & Stuber, 2018; Watabe-Uchida et al., 2012).

Neuropeptide Y

Homeostatic and reward-related roles in food intake regulation

Neuropeptide Y (NPY) is a small 36 amino-acid peptide that is expressed throughout the body and nervous system (Allen et al., 1983; Chronwall et al., 1985; de Quidt & Emson, 1986a; Tatemoto, 1982). NPY plays a role in a wide range of physiological functions, including food intake and energy homeostasis, by signaling through several NPY receptor subtypes (NPYRs). The NPYRs constitute a multi-ligand/multi-receptor system with overlapping ligand binding properties (Table 1; (Blomqvist & Herzog, 1997). Rats and humans express the $G_{i/o}$ -coupled NPY1R, NPY2R, NPY4R and NPY5R, and mice additionally express a functional NPY6R (Blomqvist & Herzog, 1997; Iyengar, Li, & Simmons, 1999; Michel, 1991; Michel et al., 1998). The NPY1R and NPY5R mediate the orexigenic effects of NPY, whereas NPY2R activation is anorexigenic, and NPY4R activation is orexigenic or anorexigenic depending on the brain region examined (Loh, Herzog, & Shi, 2015; MacNeil, 2007; Marsh, Hollopeter, Kafer, & Palmiter, 1998; Pedrazzini et al., 1998). The NPY1R and NPY5R are functionally linked as they are both required to mediate the orexigenic effect of NPY and their expression is regulated by the same gene promoter region (Mashiko et al., 2009; Nguyen et al., 2012).

Table 1. Neuropeptide Y receptor binding affinities.

Receptor subtype	High affinity ligands [$\leq 10^{-9}$ M]	Low affinity ligand [$\geq 10^{-6}$ M]
NPY1R	NPY, PYY	PP
NPY2R	NPY, PYY	PP
NPY4R	PYY*, NPY*, PP*	-
NPY5R	NPY, PYY	PP* [10^{-7} – 10^{-8} M]

NPY = Neuropeptide Y, NPYR = Neuropeptide Y receptor subtype, PP = pancreatic polypeptide (secreted by the islets of Langerhans in the pancreas after fasting), PYY = peptide YY (primarily released from cells of the ileum and colon after feeding). The affinity of ligands for a specific receptor can be affected by several assay-related factors, which is seen with ligands denoted with an asterisk (Blomqvist & Herzog, 1997). **Note:** In this thesis, 1 μ g NPY / 3 μ L saline [235 pmol; 78 μ M] is used for intraventricular cerebral infusion studies, and 0.3 μ g NPY / 0.3 μ L saline [71 pmol; 235 μ M] or 0.6 μ g NPY / 0.3 μ L saline [142 pmol; 470 μ M] for region-specific infusion studies.

NPY infusion into the ventricles increases food intake, which was subsequently localized to the hypothalamus (Stanley, Chin, & Leibowitz, 1985). The most prominent NPY neuronal population involved in the regulation of food intake resides in the arcuate nucleus of the hypothalamus (Arc; (L. Zhang et al., 2019). Arc NPY levels are modulated by hormonal and metabolic signals, and fluctuate with the energy status of an animal (Clark, Kalra, & Kalra, 1985; Kask, Rago, & Harro, 1998; Kohno & Yada, 2012; Sahu, Kalra, & Kalra, 1988; Stanley, Chin, et al., 1985; White & Kershaw, 1990). As mentioned above (section *Descending information stream*), the Arc NPY neurons project to several hypothalamic and extrahypothalamic regions to affect food intake and food intake-related behaviors. Especially the Arc->PVN projection is important in the regulation of feeding behavior, as illustrated by the fluctuations of PVN NPY output with energetic state, and the orexigenic effect of local infusion or overexpression of *Npy* in the PVN (Dube, Sahu, Kalra, & Kalra, 1992; S. P. Kalra, Dube, Sahu, Phelps, & Kalra, 1991; Sahu et al., 1988; Tiesjema, Adan, Luijendijk, Kalsbeek, & la Fleur, 2007; Tiesjema, la Fleur, Luijendijk, & Adan, 2009). Activation of the NPY system in the LHA by local NPY infusion or *Npy* overexpression also leads to increased food intake (Stanley, Magdalin, Seirafi, Thomas, & Leibowitz, 1993; Tiesjema et al., 2007).

Apart from effects in the hypothalamus, NPY signaling in regions of the reward system are also capable of modulating feeding behavior (Liu & Borgland, 2015; Quarta & Smolders, 2014). NPY stimulates the motivation to obtain food, in particular after infusion into the VTA or NAc (Flood & Morley, 1991; Jewett, Cleary, Levine, Schaal, & Thompson, 1995; Pandit, Luijendijk, Vanderschuren, la Fleur, & Adan, 2014a). For example, intra-VTA NPY increases the motivation to obtain sucrose pellets (Pandit et al., 2014a), and intra-NAc NPY infusion increases the motivation to obtain sucrose pellets, elicits a conditioned place preference, and also increases free-feeding of sucrose pellets (C. M. Brown, Coscina, & Fletcher, 2000;

Josselyn & Beninger, 1993; Pandit et al., 2014a). Furthermore, NAc infusion of NPY does not increase free-feeding of chow, suggesting a role for palatability in this effect (C. M. Brown et al., 2000). Accordingly, NPY interacts directly with components of the mesolimbic dopamine system by modulating neurotransmission of VTA dopamine neurons and by increasing NAc dopamine concentrations (Korotkova, Brown, Sergeeva, Ponomarenko, & Haas, 2006; Quarta, Leslie, Carletti, Valerio, & Caberlotto, 2011; Sorensen et al., 2009; K. S. West & Roseberry, 2017).

Neuropeptide Y, dietary composition and choice

The orexigenic effects of NPY are modulated by prior dietary preference, dietary intake, and/or the availability of dietary choice options. In the PVN, NPY is associated with carbohydrate intake, but also with fat intake. Intra-PVN NPY infusion specifically increases carbohydrate intake when rats are allowed to choose between different pure macronutrient options, as well as when they have a choice between chow (containing protein and high levels of complex carbohydrates) and a 10% sucrose solution (Giraud, Grace, Billington, & Levine, 1999; Smith, Berthoud, York, & Bray, 1997; Stanley, Daniel, Chin, & Leibowitz, 1985; J. Wang, Dourmashkin, Yun, & Leibowitz, 1999). In addition, NPY peptide levels in the PVN are increased in animals that are fed a high-carbohydrate diet compared to a low-carbohydrate high-fat diet, or in animals with a high baseline preference for carbohydrates (Beck, Stricker-Krongrad, Burlet, Cumin, & Burlet, 2001; J. Wang et al., 1999). In animals that had a high baseline intake of fat, intra-PVN infusion increased both carbohydrate and fat intake (Smith et al., 1997). Though less well-studied for the LHA and areas of the reward system, it has been shown that intra-LHA or intra-NAc NPY infusion increases the intake of sucrose pellets when it is the only option available (Pandit et al., 2014a). Intra-LHA NPY can also increase chow intake when it is the only option, as opposed to the NAc, where NPY infusion does not increase chow intake when it is the only option available (C. M. Brown et al., 2000; Stanley et al., 1993). Together, this suggests that the effect of NPY on food intake not only depends on prior dietary intake or choice options, but also on brain region-specific NPY signaling.

Neuropeptide Y dysregulation in obesity and particularly in free-choice high-fat high-sucrose-fed rats

Based on NPY's role in homeostatic and reward-related feeding behavior, and the reciprocal interactions of NPY and dietary composition, NPY may play an important role in the development of obesity. Indeed, it has been shown that chronic intracerebral NPY infusion or (hypothalamic) *Npy* overexpression leads to increased intake, body weight and fat percentage, whereas knockout of *Npy* decreases the susceptibility to develop diet-induced obesity (Beck, Stricker-Krongrad, Nicolas, & Burlet, 1992; E. J. Lin et al., 2006; H. R. Patel et al.,

2006; Stanley, Kyrkouli, Lampert, & Leibowitz, 1986; Tiesjema et al., 2007; Tiesjema et al., 2009).

The rapid increase in obesity prevalence suggests that environmental factors such as diet play an important role (see section *Obesity: a role for environmental factors*). In addition, freely available palatable foods are generally overconsumed and can lead to altered feeding behaviors such as snacking and larger portion sizes, which may further increase caloric intake. Animal models of diet-induced obesity should therefore allow an assortment of freely available foods and should elicit hyperphagic feeding behavior shown as an increase in snacking behavior and/or larger portion sizes. In contrast, a large body of studies modelling diet-induced obesity employ single-option pelleted diets in which chow pellets are fabricated that contain high percentages of fat and/or sugar. Though these diets often result in behavioral and physiological changes that mimic human obesity (Buettner et al., 2006; Hariri & Thibault, 2010; Panchal & Brown, 2011), they lack several important factors of the modern dietary environment such as choice options and variation in the sensory properties of the foods. Importantly, the addition of palatable choice options has been shown to persistently elicit hyperphagia as opposed to an initial transient period of hyperphagia as is seen with pelleted obesogenic diets (la Fleur, Luijendijk, van der Zwaal, Brans, & Adan, 2014). In this thesis, diet-induced obesity is therefore modeled by presenting the free-choice high-fat high-sucrose (fCHFHS) diet to male Wistar rats.

The fCHFHS diet consists of *ad libitum* access to chow (standard rat food containing mostly complex carbohydrates, essential levels of protein, a low percentage of fat, fiber, and essential nutritional additives such as minerals and vitamins), fat (a dish of mainly saturated fats), a 30 % sucrose water solution, and water (la Fleur et al., 2007). Consumption of the fCHFHS diet leads to behavioral changes that mimic those found in human obesity such as sustained hyperphagia and increased motivation even when sated (Giesen, Havermans, Douven, Tekelenburg, & Jansen, 2010; la Fleur et al., 2014; la Fleur et al., 2007). It also leads to physiological changes that are associated with human obesity, such as increased white adipose tissue, increased plasma insulin and leptin levels as well as glucose intolerance, decreased insulin sensitivity, and decreased peripheral leptin sensitivity compared to rats that have access to a control diet, consisting of *ad libitum* access to chow and tap water (la Fleur et al., 2014; la Fleur, Luijendijk, van Rozen, Kalsbeek, & Adan, 2011; la Fleur et al., 2007; Slomp et al., 2019; van den Heuvel, Eggels, van Rozen, et al., 2014). Importantly, allowing access to a free-choice high-sucrose (fCHS) or a free-choice high-fat (fCHF) diet with access to chow, tap water and either a 30 % sucrose solution or saturated fat respectively, also led to initial increases in intake, but these diets did not lead to persistent hyperphagia (la Fleur et al., 2014; la Fleur et al., 2011; la Fleur, van Rozen, Luijendijk, Groeneweg, & Adan, 2010; van den Heuvel, Eggels, van Rozen, et al., 2014). This indicates that the interaction between the effects of sucrose and fat intake are important for eliciting persistent hyperphagia.

Interestingly, changes were found in the central nervous system that may explain the persistent hyperphagia seen in animals on a fCHFS diet. Rats on a fCHFS diet showed increased *Npy* mRNA in the Arc after one week of diet consumption, whereas animals on a fCHS, a fCHF or a CHOW control diet did not (la Fleur et al., 2010). In addition, after four weeks of diet consumption, Arc *Npy* mRNA was normalized in fCHFS-fed rats, but the orexigenic effects of intraventricular NPY infusion were greater in fCHFS-fed rats compared to fCHS-, fCHF- or CHOW-fed rats (van den Heuvel, Eggels, van Rozen, et al., 2014). This suggests that the NPY brain circuitry is more sensitive to NPY release to elicit feeding. However, the region-specific functions of the NPY circuitry in regulating feeding and food-motivated behaviors are not fully elucidated. Also, the changes that underlie increased cerebral NPY sensitivity in rats that consume a fCHFS diet are not known.

(Dys)regulations of reward circuitries by homeostatic processes

Dopamine and opioid regulation during fasting

As mentioned above (see section *Interactions between the homeostatic and reward circuitries*), it is increasingly recognized that the homeostatic- and reward circuitries interact in the coordination of feeding behavior via receptors for nutrients and hormones as well as via neuronal projections. Fasting and chronic food deprivation are well-known methods to increase hunger as well as food reward-related behaviors (K. D. Carr, 2007; Jewett et al., 1995). Fasting increases the motivation for food in a manner that scales with the duration of the food deprivation (Hanlon, Baldo, Sadeghian, & Kelley, 2004; Jewett et al., 1995; Scheggi, Secci, Marchese, De Montis, & Gambarana, 2013), and leads to temporary hyperphagia or bingeing of calorie-rich foods (D. R. Brown & Holtzman, 1979; Smith et al., 1997; Will, Franzblau, & Kelley, 2003). Dopamine and opioid signaling may play a role in mediating these fasting-induced behaviors. However, only a few studies have studied the reward system after acute food deprivation, instead focusing on the effects of chronic food deprivation. Nevertheless, insight into how acute fasting changes dopamine and opioid signaling can indicate starting points where physiological perturbations of energy homeostasis can lead to long-term changes or (mal)adaptations in dopamine or opioid signaling. Many of the factors that can affect both homeostatic and reward-related behaviors signal via G-protein-coupled receptors, including ghrelin and Neuropeptide Y. Therefore, one of the mechanisms through which fasting can affect dopamine and opioid signaling is through changes in gene expression.

Diet-induced dysregulation of dopamine receptor subtype 2/3

In addition, diet composition, dysregulated feeding and obesity are associated with changes specifically in NAc DRD_{2/3} signaling (Baik, 2013a; P. M. Johnson & Kenny, 2010; Kenny, 2011b; Soares-Cunha, Coimbra, David-Pereira, et al., 2016; van Galen et al., 2018; Volkow et al.,

2011; G. J. Wang et al., 2001). However, the underlying causes leading to alterations in dopamine receptor levels have not been elucidated as of yet. In our fCHFS-model, rats show decreased DRD_{2/3} availability in the NAc (van de Giessen et al., 2013). Interestingly, this depends on the composition of the diet, as only rats that eat relatively high levels of fat show decreased NAc DRD_{2/3} binding levels (van de Giessen, la Fleur, de Bruin, van den Brink, & Booij, 2012; van de Giessen et al., 2013). This suggests that dietary fat intake plays an important role in the development of alterations in DRD_{2/3} signaling. However, difficulties in measuring the DRD_{2/3} have precluded studying the effects of diet on DRD_{2/3} levels over time.

Outline and aim of this thesis

In this thesis, disruption of the NPY system in diet-induced obesity, the role of central NPY in dietary selection, and the anatomical organization of the NPY neural circuitry was investigated. In **chapter II**, I reviewed the literature on the effects of obesogenic diet consumption on NPY levels in different brain regions. Changes in the NPY system of the arcuate nucleus (Arc) of the hypothalamus appeared to be related to diet-induced obesity in animals consuming a fCHFS diet. However, only NPY levels were assessed, leaving out potential changes in the NPY receptors. In addition, the results indicated sufficient rationale to investigate the NPY system in areas outside of the hypothalamus, in particular the reward-related system. In **chapter III**, the effects of prolonged consumption of a fCHFS diet on the NPY system in the hypothalamus and regions of the reward-related system were investigated. In addition, **chapter IV** addresses the question if the effect of an acute fast, which increases Arc NPY levels, also affects NPY-related gene expression in brain regions of the reward-related system.

In view of the stimulating effects of NPY on food intake and the changes in the NPY system in animals consuming the fCHFS diet, the following chapters investigated how NPY infusion in specific brain regions affects food intake. The effects of an NPY infusion in the nucleus accumbens of the reward circuit was investigated (**chapter V**), as well as of an NPY infusion in the lateral hypothalamic area (LHA; **chapter VI**), a brain region where NPY is known to stimulate intake in animals consuming a standard chow diet. In both chapters, it was also determined which NPY receptors mediate the effects on food intake. The effects of NPY infusions in the LHA suggested that the effect of NPY on food intake depend on the basal intake pattern of the rats. Therefore, **chapter VII** investigated if the effects of NPY infusion in the LHA were affected by dietary intake and preference by infusing NPY into the LHA in rats exposed to different choice diets.

Despite the clear evidence that NPY signaling in the reward-related brain regions is of importance in the regulation of feeding and motivation, the origin of NPY in these brain regions was unknown. Chapter V therefore also investigated forebrain NPY projections to the

nucleus accumbens. In **chapter VIII**, I systematically determined from which brain structures NPY projects to the ventral tegmental area.

Lastly, the previous chapters showed that diet can change the functionality of the central NPY system and that the reward-related brain regions may play an important role in mediating these changes. **Chapters IX and X** investigated whether physiological perturbations can change the reward system. Chapter IX describes the effect of fasting on gene expression in the dopamine and opioid systems, and chapter X further investigated if consumption of a fCHFHS diet can disrupt the dopamine system in the nucleus accumbens. In addition, chapter X explores the use of a relatively non-invasive method to determine DRD_{2/3} levels over time.