Brain circuitries in control of feeding behaviors

Focus on Neuropeptide Y

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Chapter II.
The effect of obesogenic diets on brain Neuropeptide Y

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Abstract

Obesity is a major health problem characterized by accumulated fat mass. The availability of an energy dense, highly palatable diet plays an important role in obesity development. Neuropeptide Y (NPY) is an orexigenic peptide. Brain NPY is affected by dietary composition, but NPY can also affect dietary preference. The hypothalamic NPY system is well characterized and has been studied in several models of obesity. However, findings from models of diet-induced obesity are not straightforward. In addition, NPY plays a role in (food)motivated behaviors, possibly by interacting with the mesolimbic dopamine system, both of which are altered in obesity. We here review the effect of obesogenic diet consumption on NPY levels in the hypothalamus and reward-related regions.

Introduction

Obesity, which is characterized by excessive fat mass accumulation, has been a rising problem for the past several decades (Stevens et al., 2012). Although genetic factors contribute to obesity development (Rankinen et al., 2006), the rapid increase in obesity prevalence suggests that environment also plays a significant role (Wright & Aronne, 2012). Important factors in obesity development include: a sedentary lifestyle, increased availability of highly palatable, energy dense foods, containing high percentages of fat and sugar (Carden & Carr, 2013; Malik et al., 2010; Popkin & Nielsen, 2003), larger portion sizes (Piernas & Popkin, 2011), and snacking behavior (Higgins, 2014; Kant & Graubard, 2015).

The brain plays a prominent role in food intake regulation where homeostatic and reward circuitries are involved in different aspects of food intake behavior (Shin, Zheng, & Berthoud, 2009; H. Zheng & Berthoud, 2007). The homeostatic system is comprised of the hypothalamus and brainstem, which function as sensors of the energy status of the internal environment and generate effectors of energy balance through autonomic and endocrine outflow (McMinn, Baskin, & Schwartz, 2000; G. J. Schwartz, 2000). The reward circuitry includes the mesocorticolimbic areas and, among others, the mesolimbic dopaminergic system (Berridge, 2007; Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003), which responds to and regulates (palatable) food intake (Lenard & Berthoud, 2008; Morton et al., 2006). Hyperphagia is thought to arise from dysregulation of hedonic processes (i.e. the motivation to work for food and the rewarding effects of highly palatable food) that drive food intake beyond homeostatic need (Kenny, 2011a, 2011b; Volkow et al., 2011; H. Zheng, Lenard, Shin, & Berthoud, 2009).

Neuropeptide Y (NPY) is one of the neuropeptides that potently increases food intake, is expressed in the homeostatic and hedonic systems, and is affected in obesity (Allen et al., 1983; Chronwall et al., 1985; de Quidt & Emson, 1986a; Pelletier, 1990). NPY has reciprocal effects on diet composition: NPY infusion in the brain affects macronutrient intake (Stanley, Anderson, Grayson, & Leibowitz, 1989; Stanley, Daniel, et al., 1985; van den Heuvel et al., 2015), and macronutrient preference is associated with brain NPY levels (Beck et al., 1994; Jhanwar-Uniyal, Beck, Jhanwar, Burlet, & Leibowitz, 1993). Furthermore, NPY has an effect on the motivation to work for a food reward (Jewett et al., 1995), specifically when infused into reward regions (C. M. Brown et al., 2000; Pandit et al., 2014a). In addition, the NPY system has been shown to be altered in many genetic and diet-induced rodent models of obesity. This review focuses on changes found in NPY peptide and mRNA levels in hypothalamic and reward regions when animals are exposed to highly palatable diets.

Neuropeptide Y circuitry of food intake regulation

Neuropeptide Y (NPY), a 36 amino acid peptide (Tatemoto, 1982), is expressed throughout the brain (Allen et al., 1983; Chronwall et al., 1985; de Quidt & Emson, 1986a; Pelletier, 1990).

In humans and rats, NPY signals via five G-protein coupled receptors (NPY1R, NPY2R, NPY4R, NPY5R; in mice NPY6R is also functional; [Blomqvist & Herzog, 1997; Iyengar et al., 1999; Michel et al., 1998]). Intracerebral NPY infusion increases food intake (Clark, Kalra, & Kalra, 1997; Flood & Morley, 1991; Kask et al., 1998; Levine & Morley, 1984) through its effects on appetitive (i.e. searching and acquiring food; e.g. meal frequency; [Benoit, Clegg, Woods, & Seeley, 2005; Seeley, Payne, & Woods, 1995]) and, under certain conditions, consummatory (i.e. ingestion; e.g. meal size) feeding behaviors (Baird, Gray, & Fischer, 2006; Benoit et al., 2005). $NPY^{-/-}$ animals have normal growth curves (i.e. food intake, body weight and adiposity compared to $NPY^{+/+}$ and $NPY^{+/-}$ animals; [Erickson, Clegg, & Palmiter, 1996]). However, they have reduced hyperphagic responses and delayed or failed body weight recovery after fasting (Bannon et al., 2000), although this has not been consistently demonstrated (Erickson, Clegg, et al., 1996). In addition, $NPY^{-/-}$ animals have reduced intake at the onset of the dark period and delayed hyperphagia in response to predictable palatable food compared to $NPY^{+/+}$ animals, despite unaltered approaching of the food (Sindelar, Palmiter, Woods, & Schwartz, 2005). Knock-down of NPY in adulthood reduces food intake and weight gain (Gardiner et al., 2005), demonstrating the importance of NPY as an orexigen when developmental compensation cannot take place. Thus, NPY may facilitate behaviors to eat more food in a short time window (Sindelar et al., 2005), and may be necessary during acute changes in energy availability. Interestingly, however, modest overexpression of NPY led to obesity only when the animals were put on a palatable diet (Kaga et al., 2001), indicating important interactions between NPY and diet composition in hyperphagia.

The role of hypothalamic NPY in food intake regulation has been studied extensively since injection of NPY into several hypothalamic nuclei produced orexigenic effects (R. E. Mercer, Chee, & Colmers, 2011; Stanley, Chin, et al., 1985). The arcuate nucleus of the hypothalamus (Arc) contains the majority of hypothalamic NPY neurons, which co-express for approximately 94% with agouti-related protein (AgRP; [Broberger, Johansen, Johansson, Schalling, & Hokfelt, 1998; Hahn, Breininger, Baskin, & Schwartz, 1998]). AgRP is an orexigenic peptide that antagonizes the melanocortin system (R. D. Cone, 2005). The Arc is situated close to the median eminence and third ventricle, allowing access to circulating signals (Broadwell & Brightman, 1976; Ciofi, 2011; Rodriguez et al., 2010). Accordingly, Arc NPY neurons are sensitive to changes in energy state. For example, NPY peptide levels (Sahu et al., 1988), the level of *Npy* mRNA, and the number of cells expressing NPY in the Arc are all increased after fasting (Hahn et al., 1998; Marks, Li, Schwartz, Porte, & Baskin, 1992; M. W. Schwartz, Erickson, Baskin, & Palmiter, 1998; White & Kershaw, 1990). Furthermore, they respond to endocrine signals of energy state, such as leptin, insulin, and ghrelin, and can also sense nutrients such as glucose (Kohno & Yada, 2012).

Within the Arc, NPY inhibits anorexigenic pro-opiomelanocortin-/cocaine-and-amphetamine-related-transcript (POMC/CART) neurons. POMC/CART neurons release alpha-

melanocyte stimulating hormone (α-MSH), a cleavage product of the POMC gene, which acts on melanocortin 3 and 4 receptors to inhibit feeding (R. D. Cone, 2005). POMC neurons express NPY1R, NPY2R, and NPY5R, of which NPY1R and NPY2R activation leads to membrane hyperpolarization and an inhibition of firing activity (Acuna-Goycolea, Tamamaki, Yanagawa, Obata, & van den Pol, 2005; Cowley et al., 2001; Ghamari-Langroudi et al., 2005; Roseberry et al., 2004). However, knockout of NPR1R, NPY2R, or NPY5R paradoxically leads to obesity during development, but attenuates (Naveilhan et al., 1999; Pedrazzini et al., 1998), or does not affect fasting-induced refeeding (Marsh et al., 1998). In addition to the effects within the Arc, Arc NPY neurons project to many hypothalamic and extra-hypothalamic regions (Bai et al., 1985; Silverman, Hoffman, & Zimmerman, 1981; van den Heuvel et al., 2015; D. Wang et al., 2015) that contain NPYR-expressing cells (Gray & Morley, 1986; Kishi et al., 2005; Pickel, Beck-Sickinger, Chan, & Weiland, 1998; Wolak et al., 2003).

Within the hypothalamus, the Arc-paraventricular nucleus (PVN) projection in particular, is implicated in food intake regulation (Jhanwar-Uniyal et al., 1993; J. Wang et al., 2013). The Arc and PVN are among the few hypothalamic regions in which levels of *Npy* mRNA (Arc; [Beck, Jhanwar-Uniyal, et al., 1990]) or NPY peptide release (PVN; [Dube et al., 1992]) increase after deprivation. Additionally, the PVN is the only hypothalamic region where NPY peptide levels (Sahu et al., 1988) and release (S. P. Kalra et al., 1991) decrease dynamically with refeeding, and where the release of NPY is augmented before the initiation of feeding (S. P. Kalra et al., 1991). In the PVN, NPY indirectly affects neuronal excitability via gamma-amino-butyric-acid (GABA) interneurons that synapse onto a subset of melanocortin-sensitive parvocellular PVN neurons (pPVN; [Cowley et al., 1999; Pronchuk, Beck-Sickinger, & Colmers, 2002]), which in turn project to the lateral parabrachial nucleus and suppress feeding when stimulated (Garfield et al., 2015; Shah et al., 2014). NPYergic inhibition of these neurons via GABAergic signaling would thus lead to inhibited suppression of intake. Second to the PVN, NPY elicits the strongest feeding response when infused into the perifornical area of the lateral hypothalamic area (pfLHA; [Stanley et al., 1993]). When overexpressed in the LHA, NPY increases daily food intake by increasing meal size (Tiesjema et al., 2007). Paradoxically, *ex vivo* experiments showed that NPY inhibits two types of LHA neurons that have stimulatory effects on feeding, hypocretin/orexin (Ox) neurons (Rodgers et al., 2001) and melanin concentrating neurons (MCH; [Tritos et al., 1998]). In these *ex vivo* preparations, NPY inhibits Ox neurons postsynaptically via the NPY1R and presynaptically via NPY2R/NPY5R (Fu, Acuna-Goycolea, & van den Pol, 2004), and inhibits melanin concentrating hormone (MCH) neurons pre- and postsynaptically (van den Pol, Acuna-Goycolea, Clark, & Ghosh, 2004). However, it therefore seems unlikely that NPY-induced feeding effects are mediated via MCH or Ox neurons. How NPY manipulations of the LHA can lead to hyperphagia needs further investigation and should address the role of other LHA neuronal populations in mediating this effect. Next to the LHA, NPY also acts in the ventromedial hypothalamus (VMH) to stimulate

intake by pre- and postsynaptically inhibiting the anorexigenic output of the VMH (Chee, Myers, Price, & Colmers, 2010), which consists of stimulating POMC neurons in the Arc (Sternson, Shepherd, & Friedman, 2005). In the dorsomedial hypothalamus (DMH), overexpression of *Npy* results in hyperphagia (F. Zheng, Kim, Chao, & Bi, 2013), but only when increased energy demands require increased feeding. For example, in lactating females or during chronic food restriction, which both increase DMH *Npy* mRNA levels (Bi, Robinson, & Moran, 2003; P. Chen & Smith, 2003; C. Li, Chen, & Smith, 1998b). Unlike the Arc NPY neurons, DMH NPY neurons do not express leptin receptors (Bi et al., 2003). Overall, it can be concluded that Arc NPY neurons can influence food intake via several hypothalamic areas, with a major role for the Arc->PVN and Arc->LHA projections. In addition, under specific circumstances NPY will be expressed in the DMH to influence food intake.

Arc NPY neurons also project to regions outside the hypothalamus, such as the ventral tegmental area (VTA), and the dorsal and ventral striatum, which are part of the reward circuitry and involved in motivation-related behaviors. Intracerebral (ICV) NPY increases the motivation for food (Jewett et al., 1995), which may involve these regions. Accordingly, studies from our lab and others have shown that intra-VTA NPY increases food-motivated behavior for sucrose, whereas intra-NAc NPY increases both food-motivated behavior and free-feeding of sucrose pellets (Pandit et al., 2014a), and can elicit a conditioned place preference (C. M. Brown et al., 2000). Furthermore, intra-NAc NPY infusions did not affect free-feeding of chow (C. M. Brown et al., 2000), suggesting that palatability may play a role in this effect. Accordingly, intra-NAc NPY infusion specifically increases the intake of fat in animals on an obesogenic choice diet via NPY1R receptors (van den Heuvel et al., 2015). The circuitries underlying these effects are, however, currently unknown.

Given its prominent orexigenic position, alterations in the NPY circuitry could be important in obesity. Studies have shown that increasing NPY levels by chronic intracerebral administration (Beck, Stricker-Krongrad, Nicolas, et al., 1992; Paez & Myers, 1991; Raposinho et al., 2001; Stanley et al., 1986; Zarjevski, Cusin, Vettor, Rohner-Jeanrenaud, & Jeanrenaud, 1993), hypothalamic overexpression (E. J. Lin et al., 2006), or PVN- or LHA specific overexpression (Tiesjema et al., 2007) leads to hyperphagia and subsequent obesity. Furthermore, knockout of NPY produces mice that are less susceptible to diet-induced obesity (H. R. Patel et al., 2006). In addition, obesity is accompanied by changes in motivation and reward-related behaviors, and the reward circuitry (Kenny, 2011a, 2011b; Volkow et al., 2011). Therefore, it can be expected that hyperphagia in obesity is accompanied by alterations in the hypothalamic and, possibly, the extrahypothalamic NPY system in the VTA and NAc. In the next section, we will briefly review changes in NPY levels in genetic models of obesity.

Genetic models of obesity are accompanied by changes in NPY levels

Genetic models of obesity show alterations in hypothalamic NPY levels. When leptin signaling is disrupted (e.g. in obese Zucker *fa/fa* rats or *ob/ob* mice) hyperphagia occurs in association with increased synthesis, transport, and release of NPY in the Arc and PVN (Beck, 2006), showing that leptin's inhibitory effects on Arc NPY levels are important for mediating its anorexigenic effects. On the other hand, adult obese tubby mice, with a disruption in the *tub* gene (an orphan gene accompanied by hyperleptinemia), have decreased levels of Arc *Npy* mRNA and increased *Npy* mRNA expression in the DMH and VMH (Guan, Yu, & Van der Ploeg, 1998), which could be explained by the finding that tubby Arc AgRP/NPY neurons are still responsive to injections of leptin (Wilson et al., 1999), which decrease Arc *Npy* mRNA levels. Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which lack functional cholecystokinin receptors (Miyasaka et al., 1994; Moran & Bi, 2006; Takiguchi et al., 1997; Takiguchi et al., 1998) have decreased *Npy* mRNA levels in the Arc (Bi, Ladenheim, Schwartz, & Moran, 2001) and DMH (Moran & Bi, 2006), but Arc *Npy* mRNA levels are not responsive to fasting (Bi & Moran, 2003) and OLETF rats are more sensitive to ICV NPY infusion as measured by food intake increases (Moran, Lee, Ladenheim, & Schwartz, 2002). This indicates that NPY-receptor-sensitivity or -quantity may be increased. Thus, genetic obesity is accompanied by a variety of alterations in the hypothalamic NPY system depending on the specific mutation. In the next section, we review the alterations in NPY levels after exposure to different obesogenic diets.

The effects of obesogenic diets on NPY levels in the brain

Animals can be exposed to a variety of high-energy and, often, highly palatable diets that contain high levels of fat and/or simple carbohydrates (Lutz & Woods, 2012). These components are mixed with standard chow or provided separately, such as in cafeteria-style diets, where animals are presented with a variety of high-calorie palatable foods. In addition, some paradigms also select animals for their susceptibility to diet-induced obesity (DIO). Sprague Dawley rats, when switched to a high-fat diet, have a bimodal distribution in body weight gain (Levin & Dunn-Meynell, 1997), and selection of DIO-prone and DIO-resistant animals can be done prior to obesity (Levin, 1995). DIO-prone *vs.* -resistant rats have increased Arc NPY levels prior to obesity, which are insensitive to fasting or restriction (Levin & Dunn-Meynell, 1997), and NPY levels stay increased after short-term DIO (Levin & Dunn-Meynell, 1997), but decrease after long-term DIO (Levin, 1999). Interestingly, others did not observe this bimodal distribution in their population of Sprague Dawley rats and describe similar changes in the NPY system as in other non-selected DIO models (as described below; [Archer, Rayner, Rozman, Klingenspor, & Mercer, 2003]).

We next describe changes in the hypothalamic NPY system in animals rendered obese using diet-induced obesity, where obesity is defined as high body weight or high fat mass

weight compared to the control group. Studies are arranged by diet type since exposure to different types of obesogenic diets leads to different obese phenotypes and NPY levels are affected by dietary composition. The few studies that have addressed changes in NPY levels after diet-induced obesity in regions outside of the hypothalamus will be mentioned separately.

High-fat diets and NPY levels

High-fat diets (HFDs) lead to body weight gain (Hariri & Thibault, 2010), and contribute to the development of metabolic syndrome (Ghibaudi, Cook, Farley, van Heek, & Hwa, 2002; Woods, Seeley, Rushing, D'Alessio, & Tso, 2003). In the studies reviewed, the control diet usually consisted of a high-carbohydrate diet (HCD) containing a high percentage of complex carbohydrates and a low percentage of fat compared to the control group.

After fasting, whole hypothalamic *Npy* mRNA expression was not different prior to obesity (i.e. no significant increases in body or fat pad weight) between HFD-fed and chow-fed animals (Heijboer et al., 2005). However, measuring NPY levels in the whole hypothalamus may occlude more subtle and region-specific effects (see e.g. [Wilding, Gilbey, Mannan, et al., 1992]). Assessing NPY levels in specific hypothalamic regions after dietary intervention revealed that Arc *Npy* mRNA was decreased (H. Wang, Storlien, & Huang, 2002), or unaltered (S. Lin, Storlien, & Huang, 2000) after one week of HFD. Comparing exposure to a HFD, a HCD, or an intermediate HFD or HCD, revealed that a HFD decreased levels of *Npy* mRNA in the Arc, whereas intermediate diets resulted in intermediate (slightly lowered) expression levels, and the HCD produced no change compared to chow controls (Giraudo, Kotz, Grace, Levine, & Billington, 1994). Thus, most studies report a decrease in Arc *Npy* mRNA after short-term HFD exposure, which is in line with HFD increasing leptin levels, which in turn decrease Arc *Npy* mRNA (Ahima & Hileman, 2000). However, within the PVN, NPY peptide levels did not change (Giraudo et al., 1994), indicating that the output of NPY is unaltered in the Arc->PVN projection. Interestingly, when measured at the end of the light period, increased NPY levels were found in the parvocellular part of the PVN (pPVN) of animals that were allowed access to both a HFD and a HCD (Beck, Stricker-Krongrad, Burlet, Nicolas, & Burlet, 1992), suggesting increased NPYergic output and possibly an increased drive to eat at a time when natural feeding commences in nocturnal animals. However, Arc NPY levels were unchanged in this study (Beck, Stricker-Krongrad, Burlet, et al., 1992). In addition, LHA NPY levels, but not VMH or DMH levels, were decreased, indicating a role for changes in LHA NPY in the development of diet-induced obesity (Beck, Stricker-Krongrad, Burlet, et al., 1992).

Prolonged access (5-24 weeks) to a HFD increases body weight and fat mass (Guan, Yu, Trumbauer, et al., 1998; S. Lin et al., 2000; Stricker-Krongrad, Cumin, Burlet, & Beck, 1998; H. Wang et al., 2002; J. Wang et al., 1998), and decreases Arc *Npy* mRNA (Guan, Yu, Trumbauer,

et al., 1998; S. Lin et al., 2000; H. Wang et al., 2002) and NPY peptide levels (Stricker-Krongrad et al., 1998), as well as PVN NPY peptide levels (Stricker-Krongrad et al., 1998). After an extended exposure to a HFD (i.e. 24 weeks), DMH/VMH *Npy* mRNA was increased (Guan, Yu, Trumbauer, et al., 1998), possibly translating into decreased anorexigenic output. Kinzig *et al.* (2005) found that long-term HFD exposure did not alter Arc *Npy* mRNA expression, but that a very high-fat (ketogenic) diet increases Arc *Npy* mRNA at the end of the light period after several weeks of exposure.

In the studies mentioned above, the control diet consisted of a low-fat chow control (maximally 17% fat). However, in several studies, the control diet contained a relatively high percentage of fat (e.g. 30% fat). This is sometimes considered a HFD in itself, as rats generally require 5% fat in their daily diet (National Research Council (US) Subcommittee on Laboratory Animal Nutrition, 1995), and diets containing >~13% fat can lead to obesity in rodents (Harrold, Williams, & Widdowson, 2000). Exposure to a HFD (60% fat) versus a relatively low-fat diet (30% fat) resulted in significant measures of obesity, while no differences in Arc *Npy* mRNA, or Arc and PVN NPY peptide levels were found (J. Wang et al., 1998). However, when compared with exposure to a typical control diet with a low fat content (10% fat/65% carbohydrate), decreased Arc *Npy* mRNA, and Arc and PVN NPY peptide levels were found (J. Wang et al., 1998). This shows that the composition of the control diet is very important for determining the effects of a certain macronutrient percentage on NPY levels in the brain. This is partly corroborated by another study using comparable HF, HC, and control diets based on macronutrient percentages. Measured at the end of the light period, NPY levels in the pPVN were increased in the HFD group versus the HCD, but not compared to the intermediate chow control group (Beck, Stricker-Krongrad, Burlet, Nicolas, & Burlet, 1990). In this study, the HFD led to increased body weight compared to the HCD and control diet, which had similar body weights. No additional differences in Arc, mPVN, VMH, or DMH NPY peptide levels were found between the three diets, although LHA NPY peptide levels were decreased compared to the chow diet. Of note, animals were fasted prior to decapitation, which might limit comparison with other studies.

In summary, short-term exposure to a HFD decreases NPY levels in the Arc, and does not affect NPY levels in the PVN, which may thus be interpreted as a physiological response to reduce food intake. However, NPY output in the pPVN may be increased at a time when normal feeding commences, which may translate in increased feeding behavior. Prolonged exposure to a HFD and an obese state decreases Arc and PVN NPY levels and eventually increases DMH/VMH *Npy* mRNA levels (see Table 1 for an overview). In addition, the studies reviewed above demonstrate the importance of measuring region-specific NPY levels and the composition of the control diet.

Table 1. The effect of high fat diets on hypothalamic NPY levels.

Diet: %fat/%carb (%sugar)	Control diet %fat/%carb (%sugar)	Duration (wks)	Species	Measures of obesity	NPY effect	Region/ measure	State	Intake (kcal)*	Ref
43/Na	Na	2	Mice	BW =	=	WH mRNA	Fasted 4h at end of DP	Na	1
58/21 (6)	10/68 (16)	1, 7, 11	Mice	BW, FM 1wk = 7, 11wk ↑	All ↓	Arc mRNA	Non-deprived	1, 7, 11wk ↑	2
59/14 (8)	10/63 (11)	1, 8, 19	Mice	BW, FM 1wk = 8, 19 wk ↑	1wk = 8, 19wk ↓	Arc mRNA	Na	1wk = 8wk ↓ 19wk ↑	3
HF 77/1 (0) IHF 59/19 (0) HC 0/78 (0) IHC 35/43 (0)	Na (chow)	1	Rats	BW gain C=IHF=HF>IHC>HC	Arc HF ↓ IHF, IHC (↓) PVN =	Arc mRNA PVN peptide	Na	All = But HC<C	4
HF 68/19 (0) and HC 14/70 (0)	37/55 (0)	2	Rats	BW =	Arc, mPVN, VMH, DMH = pPVN ↑ LHA ↓	Arc, pPVN, mPVN, VMH, DMH, LHA peptide	End of LP	↑	5
73/17 (na)	14/69 (Na)	~17	Rats	BW, FM ↑	Arc, PVN ↓	Arc, PVN peptide	Fed	Na Fat intake ↑	6
60/21 (3)	17/58 (Na)	24	Mice	BW ↑	Arc ↓ DMH/VMH ↑	Arc, DMH/VMH mRNA	Middle of LP	Na	7
HC 10/65 (37) HF 60/15 (Na)	30/45 (Na)	4-5	Rats	BW, FM HF>C>HC	HC>HF=C	Arc mRNA Arc, PVN peptide	Na	=	8
HF 60/25 (7) LC-HF 80/5 (2) (ketogenic)	16/66 (0)	7-9	Rats	BW HF>LC-HF=C FM LC-HF>HF>C	LC-HF >HF = C	Arc mRNA	End of light period	LC-HF ↓	9
HF 68/19 (0) HC 14/70 (0)	31/55 (0)	2	Rats	BW gain HF>HC=C	Arc, VMH, DMH, mPVN HF=C=HC pPVN HF=C>HC LHA HF<HC=C	Arc, pPVN, mPVN, VMH, DMH, LHA peptide	Fasted in LP	HF>HC=C	10

Table 1 (*Overleaf*). Listed in order of appearance in text. Unless noted otherwise, all comparisons are made versus values measured in the control diet group. Fat%/carb% = % kilocalories (kcal) from fat and % kcal from carbohydrate; %sugar = % kcal from sugar. C = control diet; LC-HF = low-carb, high-fat diet. BW = body weight; FM = fat mass. WH = whole hypothalamus, Arc= arcuate nucleus, PVN = paraventricular nucleus (m = magnocellular part, p = parvocellular part), DMH = dorsomedial hypothalamus, VMH = ventromedial hypothalamus, LHA = lateral hypothalamus. Non-deprived = sacrificed at the beginning of the light period; DP = dark period; LP = light period. Na = not available.
* Caloric intake versus intake in the control group. 1) Heijboer et al., 2005; 2) H. Wang et al., 2002; 3) S. Lin et al., 2000; 4) Giraudo et al., 1994; 5) Beck, Stricker-Krongrad, Burlet et al., 1992; 6) Stricker-Krongrad et al., 1998; 7) Guan, Yu, Trumbaier et al., 1998; 8) J. Wang et al., 1998 Exp. 4; 9) Kinzig et al., 2005; 10) Beck, Stricker-Krongrad et al., 1990.

High-carbohydrate diets and NPY levels

The control diets in the HFD studies reviewed above were usually chow diets with high levels of complex carbohydrates and resulted in less body weight gain and no change or higher NPY levels compared to HFD-fed animals. Therefore, only HCDs including high levels of simple carbohydrates are considered obesogenic in this review, as high levels of simple carbohydrates such as fructose, sucrose or glucose, can lead to body weight gain, increased fat mass (Alzamendi et al., 2009; Pandit, Mercer, Overduin, la Fleur, & Adan, 2012), and metabolic syndrome (R. J. Johnson et al., 2013; R. J. Johnson et al., 2009; Nakagawa et al., 2006; Roncal-Jimenez et al., 2011). No studies were found where NPY levels were assessed after exposure to a chow diet containing high levels of simple carbohydrates with a fat content comparable to the control diet. Instead, high levels of simple carbohydrates seem to be always combined with high fat content, which will be looked into in the next paragraph.

High-fat high-sugar diets, choice paradigms and NPY levels

Mixture diets, combining a high fat content with simple carbohydrates are often called high-energy diets or highly palatable diets (HPD). HPDs can, but do not always, result in increased fat mass after short-term exposure. Also, HPDs most reliably mimic human obesity-related metabolic syndrome (Panchal & Brown, 2011). Prior to weight change, a HPD did not change hypothalamic *Npy* mRNA (Staszkiewicz, Horswell, & Argyropoulos, 2007), or NPY peptide levels (Wilding, Gilbey, Jones, et al., 1992). However, decreased *Npy* mRNA levels were found in different study (Widdowson et al., 1999). Ziotopoulou et al. (2000) found that one day of HPD increased plasma leptin levels without changes in hypothalamic *Npy* mRNA, but on the 2nd day of diet exposure, *Npy* mRNA was increased. Thus, no consistent effects are found when measuring the whole hypothalamus. However, no studies were found that assessed region-specific NPY levels after exposure to a HPD, but prior to increased weight gain or the development of obesity.

Table 2. The effect of high-fat, high-sugar and choice diets on hypothalamic NPY levels.

Diet: %fat/%carb (%sugar)	Control: %fat/%carb (%sugar)	Duration (wks)	Species	Measures of obesity	NPY effect	Region/ measure	State	Intake (kcal)*	Ref
60/20 (7)	10/70 (35)	3, 5, 9 or 12.5	Mice	BW ↑ from 6wk	All =	WH mRNA	Na	Na	1
13/68 (>7 by weight) (HP chow)	9/66 (Na)	1, 4	Rats	BW 1wk = 4wks ↑	1wk peptide = mRNA X 4wk peptide ↑ mRNA =	mHyp/LHA peptide WH mRNA	Na	Na	2
HC 10/65 (37) HF 60/15 (Na)	-	4-5	Rats	BW, FM HF>HC	PVN HF<HC VMH, DMH =	PVN/VMH/DMH peptide	Na	=	3
13/68 (>7 by weight) (HP chow)	9/66 (Na)	7	Rats	BW ↑ from 1 wk	Arc, PVN ↑ VMH, DMH, LHA = WH mRNA =	Arc/PVN/VMH/ DMH/LHA peptide WH mRNA	Na	Na	4
13/68 (>7 by weight) (HP chow) (low energy density)	9/65 (Na)	2, 6-8	Rats	BW, FM 2wks = 6-8wks ↑	All ↓	WH mRNA	Na	↑	5
1) 43/43 (30) or 2) 45/35 (17)	10/70 (35)	1, 2, 7, 14 d	Mice	All BW ↑ from 2 wk	Day 1,7,14 = Day 2 HFD1 ↓ HFD2 ↓	WH mRNA	Non-deprived	Day 1 HFD1 = LFD Day 2 HFD1=HFD2 >LFD Day 7 HFD1= HFD2= LFD Day 14 HFD1 > LFD	6
24/54 (17)	9/69 (Na)	12	Mice	BW, FM ↑	↓	WH mRNA	Na	Na	7
35/23 (23)	5/64 (Na)	1	Rats	BW ↑	=	ARC mRNA	Na	↑	8
Combinations of: HE 33/53 (25) EN 22/64 (27) C 12/65 (4)	12/65 (4)	5	Rats	BW, FM ↑	All ↓	Arc NPY mRNA	LP	Na	9

Table continued on the following page.

Continuation Table 2. The effect of high-fat, high-sugar and choice diets on hypothalamic NPY levels.

Diet: %fat/%carb (%sugar)	Control: %fat/%carb (%sugar)	Duration (wks)	Species	Measures of obesity	NPY effect	Region/ measure	State	Intake (kcal)*	Ref
33/53 (25)	12/65 (4)	5	Rats	BW =, FM ↑	Arc, DMH ↓	Arc, DMH mRNA	LP	=	10
Control diet with access to 23 sucrose solution or 23 glucose solution or 23 fructose solution	20/Na (Na)	2	Rats	All BW ↑	All ↓	WH mRNA	Fasted o/n	↑	11
Free choice: fcHFHS 18/71* fcHF 54/35* fcHS 49/38*	9/69 (4)	1	Rats	BW: HF=HFHS=HS FM: fcHFHS ↑ fcHF ↑ fcHS =	fcHFHS ↑ fcHF ↓ fcHS =	Arc mRNA	Non-deprived	fcHFHS >C fcHF>C fcHS>C	12
Free choice diet: fcHFHS 31/59*	9/69 (4)	4	Rats	BW ↑ FM ↑	fcHFHS=fcHF=fcHS=C	Arc mRNA	Non-deprived	fcHFHS>fcHF=fcHS=C	13
49/36 (Na)	6/69 (Na)	10	Rats	BW, FM ↑	=	WH mRNA	Fasted?	Na	14
32/Na (Na) + Cafeteria(HP chow)	12/Na (Na)	2, 10	Mice	BW, FM 2, 10 wk ↑	Arc 2wk =, 10wk ↓ PVN 2wk =, 10wk =	Arc, PVN peptide	Non-deprived	↑	15
30/56 (Na) + cafeteria	5/75 (Na)	2, 12 9, 17	Rats	BW ↑ from 3 wk FM ↑ from 2 wk	Arc 2, 9, 12wk =; 17wk ↓ PVN 2, 9, 12wk =; 17wk ↓ AH 2wk ↑; 9, 12wk ↓; 17wk =	Arc, PVN, AH mRNA	Na	↑	16

Table 2 (Overleaf). Listed in order of appearance in the text. Unless noted otherwise, all comparisons are made versus values measured in the control diet group. Fat%/carb% = % kilocalories (kcal) from fat and % kcal from carbohydrate; %sugar = % kcal from sugar. C = control diet; EN = Ensure; fc = free choice; HE = high-energy diet; HF = high-fat; HFHS = high-fat high-sugar; HP chow = highly palatable chow made by mixing standard chow with palatable food such as condensed milk; HS = high-sugar. BW = body weight; FM = fat mass. AH = anterior hypothalamus, Arc= arcuate nucleus, DMH = dorsomedial hypothalamus, LHA = lateral hypothalamus, mHyp = medial hypothalamus (including the arcuate, paraventricular and ventromedial hypothalamic nuclei), PVN = paraventricular nucleus (p = parvocellular part), VMH = ventromedial hypothalamus, WH = whole hypothalamus. DP = dark period; LP = light period; Non-deprived = sacrificed at the beginning of the light period. Na = not available. *Caloric intake versus intake in the control group. For choice diets, mean intake in percentages is used. 1) Staszkiewicz et al., 2007; 2) Wilding, Gilbey, Jones et al., 1992; 3) J. Wang et al., 1998 Exp. 3; 4) Wilding, Gilbey, Mannan, et al., 1992; 5) Widdowson et al., 1999; 6) Ziotopoulou et al., 2000; 7) Briggs et al., 2010; 8) Kim et al., 1998; 9) Archer et al., 2007; 10) Archer et al., 2004 in weanlings; 11) Lindqvist et al., 2008; 12) la Fleur et al., 2010; 13) van den Heuvel, Eggels, Fliers, et al., 2014; 14) Plut et al., 2003; 15) M.J. Morris et al., 2008; 16) Hansen et al., 2004.

After HPD-induced obesity, hypothalamic *Npy* mRNA levels were not different (Staszkiewicz et al., 2007; Ziotopoulou et al., 2000), or decreased (Briggs, Enriori, Lemus, Cowley, & Andrews, 2010; Widdowson et al., 1999). NPY peptide levels were found to increase, but without alterations in *Npy* mRNA levels (Wilding, Gilbey, Jones, et al., 1992). Looking at specific hypothalamic regions, short-term HPD exposure leads to obesity, but does not change Arc *Npy* mRNA levels compared to chow controls (Kim, Welch, Grace, Billington, & Levine, 1998). However, more prolonged HPD exposure increases Arc NPY peptide levels (Wilding, Gilbey, Mannan, et al., 1992), and Arc decreases *Npy* mRNA levels (Archer et al., 2007). In the PVN, NPY peptide levels were either increased (Wilding, Gilbey, Mannan, et al., 1992), or decreased (J. Wang et al., 1998), and no change in VMH, DMH (J. Wang et al., 1998;
Wilding, Gilbey, Mannan et al., 1992), or LHA NPY peptide levels were found (Wilding, Gilbey, Mannan, et al., 1992). Archer, Rayner, and Mercer (2004) started HPD exposure early in development, which initially increased food intake, but was accompanied by decreased levels of Arc and DMH *Npy* mRNA when intake normalized (Archer et al., 2004). Together, and taking peptide levels as a more direct measure of neuronal output than mRNA levels, HPDs do not alter Arc *Npy* mRNA levels just after the onset of obesity, but do increase Arc and PVN NPY peptide levels after more prolonged exposure, which could contribute to increased intake.

HPD choice paradigms offering a variety of foods including palatable items that are high in fat and/or sugar, or presenting pure macronutrients as choice options in addition to chow (la Fleur et al., 2007), all lead to body weight gain and metabolic changes (Apolzan & Harris, 2012; la Fleur et al., 2007; Martire, Holmes, Westbrook, & Morris, 2013; Rothwell, Saville, & Stock, 1982; Rothwell & Stock, 1982). Although offering a variety of foods can make it more difficult to determine the contribution of different macronutrients exactly, HPD

choice diets mimic the human diet more accurately by offering palatability, variety, and choice (J. G. Mercer & Archer, 2008), which have additive effects on food consumption (la Fleur et al., 2014; Rogers & Blundell, 1984).

No studies were found that looked at NPY levels after exposure to a choice diet, but prior to weight gain or the development of obesity. After the onset of obesity, a choice diet comprising a normal chow diet including either a sucrose, glucose, or fructose solution, decreased hypothalamic *Npy* mRNA levels compared to a water control after an overnight fast (Lindqvist, Baelemans, & Erlanson-Albertsson, 2008). Focusing on the Arc, prolonged exposure to a choice HPD, comprising high-energy chow (created by mixing e.g. lard and sweetened condensed milk in normal chow) and access to liquid Ensure® (containing fat and sugar) also decreased Arc *Npy* mRNA levels (Archer et al., 2007). In our lab, in a direct comparison of different free-choice (fc) diets, providing a 30% sucrose solution (fcHS), a dish of lard (fcHF), or both (fcHFHS), in addition to chow and water, revealed that exposure to the fcHF or fcHFHS diet for one week resulted in obesity as measured by increased fat mass (la Fleur et al., 2010). However, a divergence was found in the *Npy* mRNA levels; a fcHF diet decreased, and a fcHS diet (not inducing obesity) did not change Arc *Npy* mRNA levels, whereas a fcHFHS diet increased Arc *Npy* mRNA levels compared to chow controls (la Fleur et al., 2010). This was reflected by food intake behavior: only fcHFHS-fed animals were hyperphagic, whereas fcHF and fcHS animals initially overate, but had normalized intake when *Npy* mRNA was measured (la Fleur et al., 2011; la Fleur et al., 2010). Interestingly, at four weeks exposure, when all groups were obese and showed stable food intake, Arc and DMH *Npy* mRNA levels were not different between the diet groups and their chow controls, but the sensitivity to ICV infusions of NPY was increased in fcHFHS animals (van den Heuvel, Eggels, van Rozen, et al., 2014; see Figure 1).

Cafeteria-style diet paradigms may mimic the human diet most closely, but may hamper assessment of macronutrient intake. No studies were found that measured NPY levels prior to the manifestation of obesity due to a cafeteria diet. In Morris et al. (M. J. Morris, Chen, Watts, Shulkes, & Cameron-Smith, 2008), animals were exposed to a cafeteria diet for several weeks, and obesity developed after two weeks of dietary intervention. Arc and PVN NPY peptide levels were measured at 2 and 10 weeks of exposure; Arc NPY peptide levels were decreased at 10 weeks of exposure, whereas PVN peptide levels were unchanged at both time points (M. J. Morris et al., 2008). In another study, rats kept on a cafeteria diet for 2-17 weeks were obese after 3 weeks. Up to 12 weeks of diet exposure did not result in changes in Arc or PVN *Npy* mRNA levels, although animals were more sensitive to NPY infusion (Hansen, Jovanovska, & Morris, 2004). Levels in both regions were decreased after 17 weeks on the diet ([Hansen et al., 2004]; see Table 2). Prolonged exposure to a cafeteria diet did not result in changes in hypothalamic *Npy* mRNA levels compared to chow controls when animals were fasted overnight (Plut, Ribiere, Giudicelli, & Dausse, 2003).

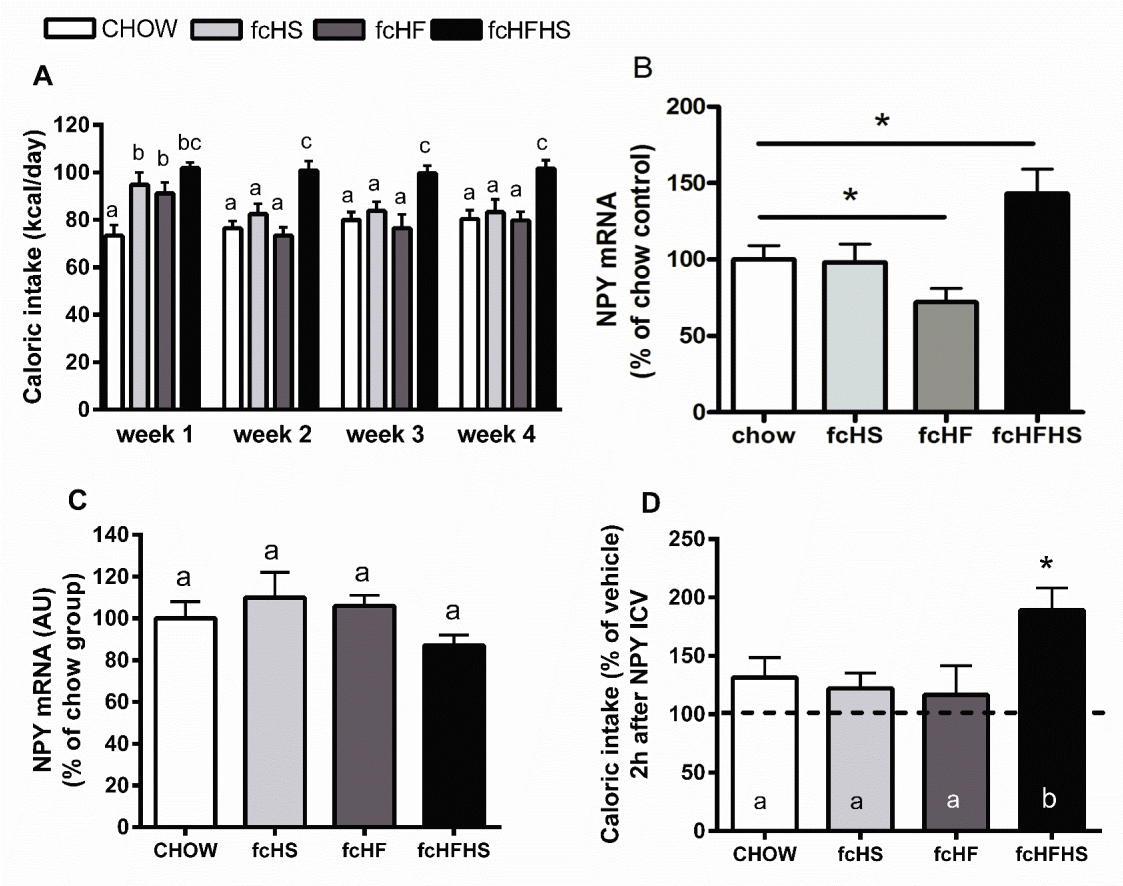

Figure 1. The effects of obesogenic free-choice diets on Arc *Npy* mRNA and NPY sensitivity. A) Average daily total caloric intake in rats on a CHOW diet, free-choice high-sugar (fcHS), free-choice high-fat (fcHF) or free-choice high-fat high-sugar (fcHFHS) diet for 4 weeks. N = 7-9 rats per group. Different letters represent significant differences between bars ($p < 0.05$) as indicated by repeated measures analysis of variance (ANOVA). **B)** Arc *Npy* mRNA levels after 1 week diet exposure showing increased *Npy* mRNA in fcHFHS-, and decreased *Npy* mRNA in fcHF-fed rats compared to fcHS- and CHOW-fed control rats. Letters represent significant differences between bars ($p < 0.05$) after two-way ANOVA indicated a difference ($p < 0.05$). Numbers on bars represent the number of rats per group. **C)** Arc *Npy* mRNA levels after 4 weeks diet exposure do not differ between the diet groups. **D)** NPY sensitivity is increased in fcHFHS-fed rats compared to fcHS-, fcHF-, and CHOW-fed control rats after 4 weeks diet exposure. *Significantly different from vehicle injection in the same diet ($p < 0.05$). All data are mean ± *SEM*. Figures A, C and D are from (van den Heuvel, Eggels, van Rozen, et al., 2014). Figure B is adapted from (la Fleur et al., 2010).

In conclusion, short-term exposure to non-choice HPDs results in unchanged Arc NPY levels compared to chow controls, whereas prolonged exposure leads to increased levels of NPY peptide in the Arc and PVN, although there is a discrepancy with Arc *Npy* mRNA levels. After exposure to several choice diets, NPY levels are decreased in the Arc with no change in the PVN. The effect on NPY levels in other hypothalamic regions is unknown. In addition, we have found that different choice diets can differentially affect NPY levels. Specifically, exposure to

fat and sugar led to increased Arc *Npy* mRNA levels. In addition, the sensitivity of the brain to NPY infusions is increased after more prolonged exposure, suggesting that even if changes in NPY levels are not observed, the system might be altered and responding to perturbation more readily.

Obesogenic diets and NPY levels in the striatum/nucleus accumbens and ventral tegmental area

Very few studies have looked at diet-induced changes in NPY peptide or mRNA levels outside of the hypothalamus (see Table 3 for an overview). Only one study was found that addressed NPY levels in the NAc. In this study, long-term exposure to a HFD, a HCD supplemented with a sucrose solution, or a chow diet containing 31% fat, did not change NPY peptide levels in the NAc of weanlings (Beck et al., 1994), whereas Arc and PVN(p) NPY peptide levels were decreased in the HFD and HCD versus the chow group. In this study, animals were separated based on their macronutrient intakes (i.e. those that ate fat were HFD animals, etc.), which addresses the additional effects of preference. In the VTA, prior to obesity, the choice between a HFD and a HCD did not change NPY peptide levels compared to relatively high-fat (37% fat) chow-fed animals (Beck, Stricker-Krongrad, Burlet, et al., 1992). After HFD-induced obesity, no change in VTA peptide levels was found, compared to a high-fat chow (37% fat), or a HCD diet (Beck, Stricker-Krongrad, et al., 1990). Although the striatum and VTA are regions where NPY may mediate motivation and food-reward, it remains to be determined how these areas are affected by obesogenic diets and whether there is a role for these alterations in motivational changes observed when animals are obese. Especially as studies measuring NPY levels in the hypothalamus show a lot of variation.

Conclusions on the role of NPY changes in diet induced obesity

Since hypothalamic NPY levels are affected by dietary composition, we expected that different obesogenic diets would affect NPY levels differently. In addition, as NPY infusion can affect feeding and motivation-related behaviors when infused into the NAc and VTA, we expected that NPY levels in these regions are affected in obesity, as obesity is known to be accompanied by alterations in motivational behaviors (Kenny, 2011a, 2011b; Volkow et al., 2011). The variety of experimental paradigms and the lack of studies on the effects of exposure to certain obesogenic diets on NPY in specific hypothalamic and reward regions, make a straightforward conclusion difficult. In addition, we found discrepancies between peptide and mRNA levels, and observe that the resolution of measurement (whole hypothalamus versus specific regions), as well as the choice of the control diet are important factors when determining changes in NPY levels due to a specific dietary interventions. Still, some main trends can be noted.

Obesogenic diets increase the sensitivity to NPY

Arc NPY levels are decreased after exposure to a HFD, pointing to negative feedback at the level of the Arc, with no change in output at the level of the PVN. This is in accordance with increased leptin levels shortly after exposure to a HFD (e.g. [Widdowson et al., 1999; Ziotopoulou et al., 2000]), and the inhibitory effect of leptin on Arc NPY neurons (Baver et al., 2014; Korner, Savontaus, Chua, Leibel, & Wardlaw, 2001; van den Heuvel, Eggels, Fliers, et al., 2014; van den Top, Lee, Whyment, Blanks, & Spanswick, 2004; Yang et al., 2010). pPVN NPY levels are increased in HFD fed animals at a time when normal feeding commences, which may indicate the start of altered regulation. Future studies could address whether this translates to behavior, i.e. that HFD-fed animals eat more during this period than control animals. Decreased Arc and PVN NPY levels after HFD exposure are, however, not always sufficient to inhibit feeding.

The effects of sugar content alone, i.e. apart from the additive effects of increased fat content or of a choice element such as when supplementing it as a solution, on NPY levels are unknown. It should be recognized that a glucose injection increases Arc NPY levels after a transient decrease (Chang, Karatayev, Davydova, Wortley, & Leibowitz, 2005), brief access to a sucrose solution or a glucose injection increases Arc and PVN peptide NPY levels (J.Wang et al., 1999), and a sugar preload increases subsequent intake (Gaysinskaya, Karatayev, Shuluk, & Leibowitz, 2011). Taken together, this indicates that sugar consumption affects both NPY levels and food intake. During obesity due to prolonged access to a sugar solution, NPY levels are, however, decreased (Lindqvist et al., 2008). This has not yet been localized to a specific hypothalamic region, but it is clear that decreased NPY levels are again not sufficient to reduce intake.

Non-choice HPDs increase Arc and PVN NPY peptide levels, which is in contrast to the effects found after exposure to a diet containing only high-sugar or high-fat levels. Over-activity of the Arc->PVN NPY projection may, in part, explain why this type of diet can lead to increased body weight and fat gain in less time. Increased NPY levels leading to increased food intake is reminiscent of genetic models in which leptin signaling is disrupted and where reduction of NPY levels reduces intake (Erickson, Hollopeter, & Palmiter, 1996). On the other hand, HPD choice diets either led to decreased or increased Arc and PVN NPY levels, but the variety of choice diets interferes with a making a firm conclusion. It is, however, clear that choice-HPD-DIO animals are more sensitive to NPY as measured by their feeding response after NPY infusion (Hansen et al., 2004; van den Heuvel, Eggels, van Rozen, et al., 2014). Although NPY sensitivity has not been assessed in animals on the other obesogenic diets, changes in sensitivity might explain how decreased NPY levels still lead to hyperphagic behavior and may indicate that after prolonged exposure, the NPY system adapts (appearing normal or downregulated), whereas it is actually more sensitive to perturbation. For instance, when DIO animals are put back on a chow diet, they have increased NPY levels compared to

Table 3. The effect of obesogenic diets on NPY levels in reward regions.

Diet: %fat/%carb (%sugar)	Control: %fat/%carb (%sugar)	Duration (wks)	Species	Measures of obesity	NPY effect	Region/ Measure*	State	Intake (kcal)**	Ref
HF 68/19 (0) and HC 14/70 (0) + 25% sucrose solution	31/55 (0)	14	Rats	BW HF=C, HC=C, HF>HC FM HF>C>HC	HF=HC=C	NAc peptide	Na	HC>HF=C Last week	1
HF 68/19 (0) and HC 14/70 (0)	37/55 (0)	2	Rats	BW =	=	VTA peptide	End of LP	↑	2
HF 68/19 (0) HC 14/70 (0)	31/55 (0)	2	Rats	BW gain HF>HC=C	HF=HC=C	VTA peptide	Fasted in LP	HF>HC=C	3

Listed in order of appearance in the text. Unless noted otherwise, all comparisons are made versus values measured in the control diet group. Fat%/carb% = % kilocalories (kcal) from fat and % kcal from carbohydrate; %sugar = % kcal from sugar. C = control diet; HC =high-carbohydrate; HF = high-fat. BW = body weight; FM = fat mass. NAc = nucleus accumbens, VTA = ventral tegmental are. LP = light period. Na = not available.*The studies in this table are also mentioned in Tables 2 and 3 as NPY levels were measured in several brain regions. For clarity, only effects in reward regions are listed here. ** Caloric intake versus intake in the control group. For choice diets, the mean intake in percentages is used. 1) Beck et al., 1994 in weanlings; 2) Beck, Stricker-Krongrad, Burlet, et al., 1992; 3) Beck, Stricker-Krongrad, et al., 1990.

chow controls (Archer et al., 2005). Increased sensitivity could be explained by increased hypothalamic NPY2R and/or NPY5R (Huang, Han, & Storlien, 2003; Widdowson et al., 1997) and/or NPY1R receptors (P. S. Kalra, Dube, Xu, Farmerie, & Kalra, 1998; P. S. Kalra, Dube, Xu, & Kalra, 1997). Gene transcription may also play a role; for instance, *in vitro* studies have shown that stimulation of NPY1R increases phosphorylation of cAMP-responsive element binding protein (CREB) and induces the expression of cAMP-responsive element (CRE) containing genes, including the NPY1R gene (Sheriff, A, W, Kasckow, & Balasubramaniam, 2002; Sheriff, Chance, Fischer, & Balasubramaniam, 1997). The underlying mechanisms of receptor upregulation or sensitization are, however, unknown, and more research is required to elucidate the mechanisms underlying the increased sensitivity to NPY. Especially, as exposure to an obesogenic diet actually leads to increased excitability of NPY neurons in *ex vivo* experiments (Baver et al., 2014; W. Wei et al., 2015). Since only a few studies have measured NPY levels in other hypothalamic regions, we can also not exclude the possibility that changes in the DMH, VMH, or LHA play a role in the maintenance of hyperphagia in DIO animals.

NPY beyond the hypothalamus may play a role in palatability-induced hyperphagia

As mentioned above, NPY's effects on feeding behavior extend beyond the hypothalamus; NPY interacts with elements of the reward system to mediate feeding behavior as well as feeding-related motivation. In addition, the effects of NPY are modulated by palatability, which may be mediated by the reward circuitry. For instance, NPY-/- mice show delayed hyperphagia in response to the presentation of palatable food (Sindelar et al., 2005), and modest overexpression of brain-wide overexpression of *Npy* only leads to obesity when mice are put on a highly palatable diet (Kaga et al., 2001). Furthermore, intra-NAc infusion of NPY increases the motivation to work for sucrose pellets (Pandit et al., 2014a), or the choice for fat on a fcHFHS diet (van den Heuvel et al., 2015), but does not increase intake when animals are only offered chow (C. M. Brown et al., 2000). Since the reward circuitry and specifically the mesolimbic dopamine system are altered in obesity (Kenny, 2011a, 2011b; Volkow et al., 2011), a change was expected in NPY. Only a few studies investigated whether DIO influences NPY in the reward areas and did not find changes, but more research is needed to make definite conclusions. NPY neurons and its receptors are found in the NAc and VTA (Gray & Morley, 1986; Kishi et al., 2005; Korotkova et al., 2006; Pickel et al., 1998; Wolak et al., 2003), thus NPY may affect these regions locally or through projections. For instance, Arc NPY neurons project to the NAc (van den Heuvel et al., 2015), and AgRP neurons project to the VTA (Dietrich et al., 2012), making it likely that NPY does as well. The hypothalamic changes in NPY described above may affect the reward areas. NPY's stimulatory effects on intake and motivational aspects of intake (Pandit, la Fleur & Adan, 2013; Quarta & Smolders, 2015; van den Heuvel et al., 2015) are in accordance with its stimulatory effects on accumbal dopamine

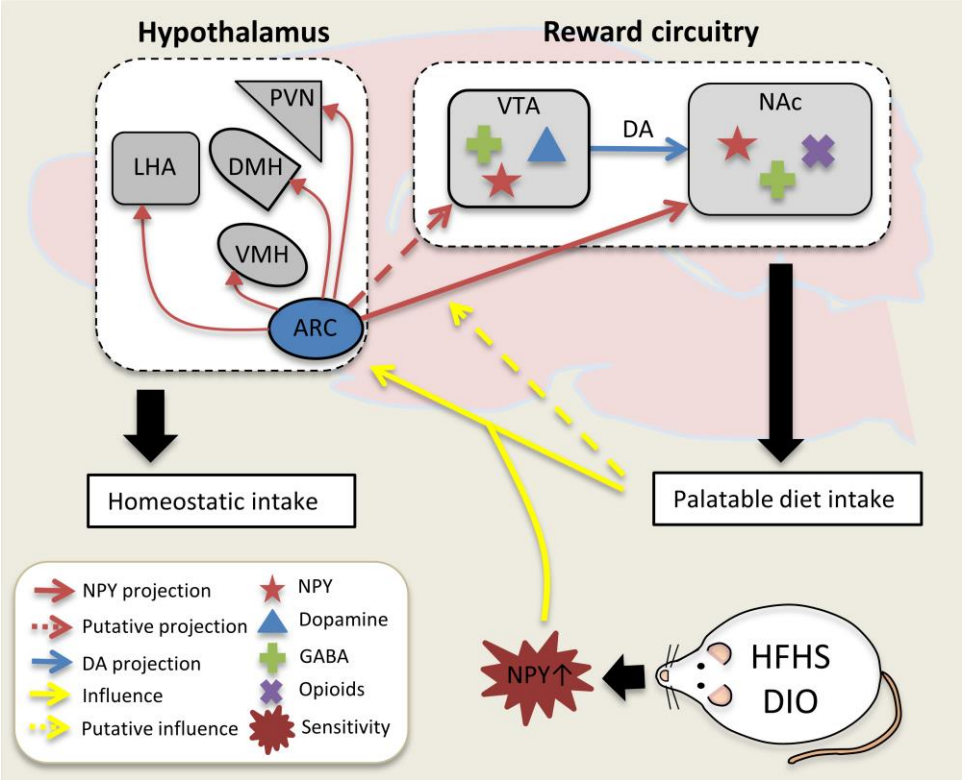

Figure 2. Interactions of diet and brain Neuropeptide Y in regulating different aspects of food intake. Arc NPY neurons project to hypothalamic regions to mediate homeostatic feeding. The Arc->PVN projection is most implicated in short-term intake regulation. The Arc NPY neurons also project to the NAc and possibly to the VTA, where they interact with the mesolimbic DA system. Especially in the NAc, NPY may interact with GABA and opioid signaling to influence palatable food intake. Next to this, palatable diet consumption influences the NPY system in the hypothalamic and possibly also in the reward circuitry, either via NPYergic projections or direct effects on local NPY neurons. Furthermore, in (HFHS) DIO, NPY sensitivity is increased, which may lead to increased homeostatic and hedonic intake. The interrelations between these regions have been omitted for clarity. See text for details. ARC = arcuate nucleus, DA = dopamine, DIO = diet-induced obesity, DMH = dorsomedial hypothalamus, GABA = gamma-amino-butyric acid, HFHS = high-fat high-sugar, LHA = lateral hypothalamic area, NAc = nucleus accumbens, NPY= Neuropeptide Y, PVN = paraventricular hypothalamic nucleus, VMH = ventromedial hypothalamic nucleus, VTA = ventral tegmental area.

levels (Adewale, Macarthur, & Westfall, 2007; Beal, Frank, Ellison, & Martin, 1986; Kerkerian-Le Goff et al., 1992). Paradoxically, NPY inhibits both GABA and dopamine neurons in the VTA in an *ex vivo* preparation (Korotkova et al., 2006). These findings may be reconciled when considering that only a subset of VTA dopamine and GABA neurons expresses NPY or NPYR, that only a subset of neurons is inhibited by NPY (Korotkova et al., 2006), and that more GABAergic cells were inhibited more strongly (Korotkova et al., 2006), which may thus result in overall increased dopaminergic output. As VTA neurons are highly heterogeneous based on their expression profiles and projection areas, which can be measured at the behavioral level

(Lammel, Lim, & Malenka, 2014; Sanchez-Catalan, Kaufling, Georges, Veinante, & Barrot, 2014), it is necessary to replicate these findings and address this heterogeneity (e.g. if the effects of NPY on dopaminergic cells depends on the project target of the dopamine neurons). Possibly local circuit dynamics or projection-specific effects eventually result in increased dopamine release in the NAc. Future studies can also address whether the effects of NPY in the NAc and VTA on dopamine translate to different roles in behavior.

Of particular interest to NPY's putative role in palatability-induced hyperphagia are a few studies that suggest an interaction between NPY, GABA and opioid signaling in the NAc in regulating palatable food intake. Increasing GABA, opioid (Kelley, Baldo, Pratt, & Will, 2005) or NPY signaling in the NAc (van den Heuvel et al., 2015) all lead to food intake, preferably of palatable food (i.e. fat). Also, decreased firing in the NAc is necessary for food intake (Krause, German, Taha, & Fields, 2010), and intra-NAc NPY infusion decreases neuronal activity (van den Heuvel et al., 2015). NPY infusion also affects enkephalin neurons in the NAc (van den Heuvel et al., 2015), and blocking the opioid system prevents NPY-induced feeding (Israel et al., 2005). Interestingly, exposure to a palatable diet can sensitize the effects of accumbal GABAergic stimulation (Newman, Pascal, Sadeghian, & Baldo, 2013). This suggests an important role for diet composition and palatability in increasing the sensitivity of this system (see Figure 2 for an overview). In line with the effects of diet composition on hypothalamic NPY sensitivity, it would be interesting to pursue this further and determine whether NPY plays a role in this effect.

Conclusions

We have reviewed obesogenic diet-induced changes in the NPY system in the hypothalamus and the reward system. Diet composition affects NPY levels; high-fat diets decrease hypothalamic NPY levels before and after the onset of obesity, whereas high-fat high-sugar (choice) diets increase hypothalamic NPY levels. In addition, the obese state is characterized by increased sensitivity of the NPY system, into which future research is required. Additionally, limited evidence indicated no change in NPY levels in the reward system due to exposure to obesogenic diets. Yet, NPY interacts with the mesolimbic reward system to modulate feeding and motivation-related behaviors, which are altered in obesity. Therefore, this may prove to be an important circuitry in explaining how homeostatic and hedonic alterations manifest as hyperphagia in obesity.