THE “Y” AND HOW OF STRESS EATING

Neuropeptide Y on the crossover between feeding and stress

Abstract

Chronic stress is detrimental to the health and increases the risk of obesity. Stress can disrupt feeding behaviour, which is regulated by a complex neuronal and hormonal network. The neuronal and endocrinal network of stress overlaps with the feeding network at some points, leading to stress eating. One of these overlapping points is neuropeptide Y (NPY), a potent orexigenic and anxiolytic agent.

In this review, the overlap between the role of NPY in homeostatic feeding and in the stress response will be compared. Stress can elicit varied responses on both feeding behaviour and NPY expression and signalling. Acute stress inhibits feeding, whereas chronic stress tends to promote feeding. The stress eating network is responsible for stress eating, usually through the direct or indirect modification of NPY/AgRP neurons in the arcuate nucleus of the hypothalamus, and could be a potential target for the treatment of stress-induced obesity.

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Layman's summary

Obesity, defined as a body mass index of 30 or higher, can lead to several major health issues, like cardiovascular diseases and type 2 diabetes. In the recent years, the prevalence of obesity has increased severely. Obesity can be caused by an imbalance in the energy intake and expenditure, for instance by eating too much calories. The eating behaviour is regulated by a complex network in the brain that is not yet fully understood. A key player in this network is the hypothalamus, a brain region that plays an important role in keeping the body balanced. Within this structure, there is a group of neurons that inhibits eating when activated, which can be recognised by the expression of the proteins POMC and CART. Another group of neurons in the hypothalamus has the opposite effect on eating: this group will increase feeding behaviour when the cells are activated. This group can be recognised by the proteins Neuropeptide Y (NPY) and Agouti-related protein (AgRP).

Stress is a known risk factor for obesity and can influence feeding behaviours. Stress is regulated by a large network that involves certain regions in the brain and several stress hormones, like adrenalin and cortisol. The release of cortisol is regulated by the HPA-axis, which consist of the Hypothalamus, Pituitary gland and Adrenal gland. When stressed, the hypothalamus releases the hormone CRH (corticotropin-releasing hormone). This signal is relayed through the pituitary gland to the adrenal gland, which then releases cortisol in the bloodstream. Cortisol has effects throughout the body, like increasing the heart rate, but also in the brain. It inhibits the release of CRH from the hypothalamus to form a negative feedback loop.

The networks for eating and for feeding overlap at some points. This can lead to stress eating. Most notable is the hypothalamus, where acute stress can inhibit the NPY/AgRP neurons, while chronic stress activates this neuron group. However, this effect is variable: Different types of stress, or even the same type of stress in a slightly different situation, can alter the amount of NPY signalling in this brain region. However, there might be a notable correlation between how stress affects feeding and how it affects NPY. If the stress caused more food intake, the levels of NPY would likely also be increased. On the other hand, if the stress reduced the food intake, the levels of NPY signalling in the hypothalamus likely were also decreased. This means that, if stress alters feeding behaviour (either more or less eating), the effect likely depends on signalling of NPY in the hypothalamus.

There are also other brain regions that can modulate the activity of NPY/AgRP cells. A lot of these routes also use NPY in some way. This shows that the network that regulates stress eating in the brain is complex and dependent on NPY. Understanding this network could help prevent stress-induced obesity or other stress and eating disorders.

Introduction: Stress, obesity, and Neuropeptide Y

Stress has been called the "Health epidemic of the 21st century", which is supported by the fact that stress levels have increased by 10 to 30% in the USA from 1983 to 2009 (Fink, 2016). Stress can be defined as "a real or perceived threat to the homeostasis of the body by adverse forces, called the stressors" (Charmandari *et al.*, 2005). The stress response is essential for survival, as it motivates the avoidance of dangerous situations e.g. by running away from a predator. The stress response is variable and dependent on a multitude of factors, such as the type of stressor and the internal state of the organism. However, one can generally distinguish between acute stress, which lasts relatively short, and chronic stress, that last over a longer period. These two systems are regulated by their own networks. Acute stress could have a beneficial effect on the organism (Dhabhar, 2018). On the other hand, excessive or chronic stress is maladaptive (Glaser & Kiecolt-Glaser, 2005) and is a risk factor for multiple pathologies, like anxiety disorders, addiction and obesity (Sinha & Jastreboff, 2013).

Obesity, defined as a body-mass index of 30 kg/m^2 or higher, is an increasing problem worldwide. Since 1975, the prevalence of obesity has almost tripled (World Health Organisation, 2021). In 2016, the World Health Organisation (WHO) estimated that globally more than 650 million adults, or approximately 13% of the world population, were obese. It is a health risk that can lead to cardiac diseases, type 2 diabetes and an overall increase in mortality (Must et al., 1999). Obesity can be caused by an imbalance in the energy balance of the body, for instance by the overconsumption of high-caloric foods. The network that regulates eating is a complex process involving multiple neural and hormonal pathways (Ahima & Antwi, 2008).

Neuropeptide Y (NPY) was found to be a key player in the network of feeding behaviour (Stanley et al., 1985) and, also, to act as an anxiolytic agent (Heilig et al., 1989), among other function. As NPY is involved in both feeding and stress regulation, this neurohormone has been the subject of numerous studies that looked at stress eating (Chigr et al., 2014; S.-X. Wang et al., 2012). In this thesis, I will review the role of NPY in feeding and the stress response, and the role this neuropeptide can play in stress-induced hyper- and hypophagia. First, NPY will be introduced, then the neural mechanisms of feeding behaviour will be discussed, with a special focus on NPY. Next, I will provide the different mechanisms involved in the stress response, and the role of NPY in these processes. Finally, the overlap between these systems will be discussed and a framework for the effects stress can exert on feeding, and vice versa, will be proposed.

Neuropeptide Y

NPY was discovered in 1982 in porcine brains (Carlquist et al., 1982). This protein is the most prevalent peptide in the brain and has been implicated in numerous functions (Stanley et al., 1985). It consists of 38 amino acids and its sequence is highly conserved (Larhammar, 1996; Larhammar et al., 1993). It belongs to the same protein family as the Pancreatic Polypeptide (PP) and the Peptide YY (PYY), that are both involved in eating and feeding behaviour. All the members of the NPY protein family are characterised by a similar and distinct tertiary structure motif, known as the PP-fold (Nygaard et al., 2006).

Over time, eight different receptors for NPY have been discovered, going by the names of Y_1 to Y_8 (Bromée et al., 2006). These can be further divided into the subfamilies Y_1, consisting of Y_1, Y_4 and Y_6, and Y_2, consisting of Y_2 and Y_7. The Y_5 receptor is considered a separate subfamily. The proposed Y_3 was later redefined as CXCR4, and therefore is no longer considered an NPY receptor (Herzog et al., 1993). In mammals, only the receptor subtypes Y_1, Y_2, Y_4, Y_5 and Y_6 are found and in humans, only

receptors Y_1, Y_2, Y_4 and Y_5 are functional (Bromée et al., 2006). NPY receptors are observed throughout the body (e.g. in kidney, heart and colon tissue) and brain (Wahlestedt & Reis, 1993).

All the NPY receptors are G-protein coupled receptors (Yi et al., 2018). A schematic overview of the intracellular signalling cascade after NPY binding is shown in Figure 1. Upon binding of NPY, these receptors release the G-protein complex, which will break down into two different subunits. The two parts are involved in a different intracellular cascade, but both can affect the gene expression. Not shown in the figure is that binding of NPY can modulate Ca^{2+} and K^+ ion channels (Cabrele & Beck-Sickinger, 2000). Via this mechanism, NPY signalling can elicit an immediate change in the electrophysiological properties of cells, which could for instance make neurons more prone to hyperpolarisation.

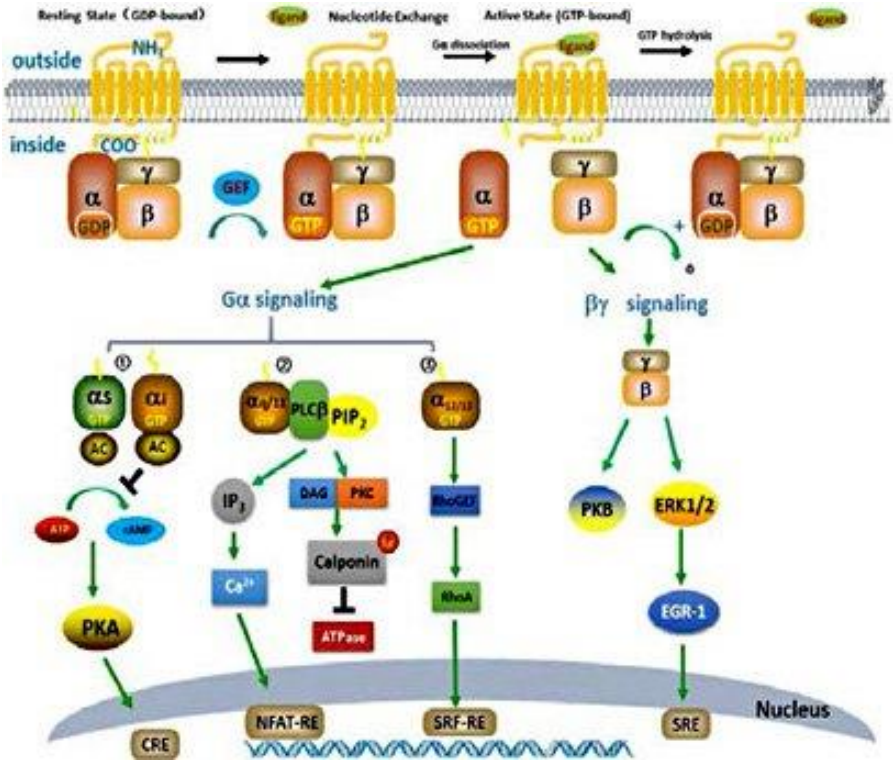

Figure 1: Intracellular pathways of NPY signalling.
NPY binds to the NPY receptor (top). This releases the Gα- and βγ-subunit of the coupled G-protein from the receptor. Both these subunits activate intracellular signalling cascades. Image taken from Yi et al., 2018.

Regulation of feeding behaviour

The mechanisms that urge an organism to feed or to stop feeding can be split into two main networks: the homeostatic network and the hedonic network (Liu & Kanoski, 2018). The homeostatic network revolves around the regulation of the energy balance. This network alters between hunger and satiety to drive the body to eat or stop eating. The hedonic feeding network is intertwined with the dopaminergic reward system and is involved in the rewarding value of food. These networks might appear distinct, but they are heavily interconnected and both involved in any and all decisions about feeding (Liu & Kanoski, 2018). Although the hedonic feeding network is often considered to drive stress eating (Yau & Potenza, 2013), stress also affects the homeostatic feeding network (Maniam & Morris,

2012). NPY plays a role in both the homeostatic and hedonic feeding pathway. Below, I will discus first the homeostatic feeding pathway, as this pathway and the role of NPY therein have been well-studied.

Input for the feeding network: Hunger and satiety signals from the periphery

The brain relies on signals from the periphery to regulate the homeostatic drive to eat. Through these signals, the brain receives an indication of the homeostatic state of the body and can react accordingly to adjust the energy intake and expenditure. The communication between the gut and the brain, and therefore between the periphery and the central nervous system (CNS), can work through two mechanisms: 1) through the *nervus vagus* or 2) through circulating nutrients and hormones in the bloodstream, released prior, during, and after food intake.

The nervus vagus originates in the Nucleus of the Solitary Tract (NTS) in the medulla oblongata and innervates a multitude of peripheral targets, such as the digestive tract (Baker & Lui, 2021). This nerve is in control of the parasympathetic nervous system. It contains both afferent and efferent connections, although the majority of these are afferent (Prechtl & Powley, 1990). These efferent connections mediate gastrointestinal parasympathetic responses such as the release of insulin. The vagal afferents contain a multitude of different mechano- and chemoreceptors (G. J. Schwartz, 2000). These monitor the state of the walls of the gastrointestinal tract and of the levels of nutrients and hunger and satiety signalling factors in the periphery. The importance of the vagus with regards to hunger and satiety can be shown by blocking the vagal signals to the brain. Rats exhibited longer meal durations after food deprivation when these signals were blocked by *N*-methyl-D-aspartate antagonist MK-801 (Treece et al., 1998). After a vagotomy, a procedure where the vagus has been surgically severed, rats increased their meal size but lowered the frequency of their meals (la Fleur et al., 2010). These opposing effects kept the daily caloric intake of these rats constant and the animals showed no overall weight gain. These results indicate a role of the vagus for the signalling of satiety during a feeding bout. Also notable is that the effect of abdominal satiety factors are abolished after a vagotomy (G. P. Smith et al., 1985). Intraperitoneal injection of the satiety factor cholecystokinin in the rats from these experiments did not alter their meal size, or even increased their meal size after their vagal nerve had been severed.

More than twenty different hormones are involved in the endocrine signalling of eating, including the aforementioned cholecystokinin. Of these factors, only ghrelin functions as a hunger signal, with all other factors serving as satiety factors. However, most of these exert a paracrine effect that is then transmitted to the CNS by the vagus, as discussed previously. Therefore, this section will focus on ghrelin, insulin and leptin, as these are known to directly affect the brain.

Ghrelin is the only gut hormone that has an orexigenic function (Bewick et al., 2009). This hormone is secreted by the stomach and works to communicate a negative energetic state to the brain (Mani & Zigman, 2017). Fasting elevates the circulating levels of ghrelin and the levels of ghrelin are inversely correlated with a person's body mass index (Ariyasu et al., 2001). Insulin is secreted by beta cells in the pancreas and maintains the glucose balance in the blood by promoting the hydrolysis of glucagon (Loh et al., 2017). Furthermore, it regulates the uptake of glucose on a cellular level (Ebeling et al., 1998). Its levels in the blood correlate well with the amount of adipose tissue (G. J. Schwartz, 2000). The hormone Leptin is often implicated in the context of obesity. Mice homozygous for a leptin mutation, the so-called *ob/ob* genotype, were found to develop an obese phenotype. This hormone is secreted by white adipose tissue and the levels of leptin in the blood plasma correlate well with the amount of adipose tissue (Park & Ahima, 2015).

These circulating factors can moderate multiple brain areas, both directly and indirectly. These brain regions and their function in feeding behaviour will be discussed next.

The homeostatic feeding network

There are two brain regions that play a major role in the homeostatic regulation of feeding behaviour: the brainstem and the hypothalamus. These regions are heavily connected, with connections going both from the hypothalamus to the hindbrain (Geerling et al., 2010) and from the hindbrain to the hypothalamus (Ritter et al., 2001). The hypothalamus and brainstem also share several common regions they project to, like the parabrachial nucleus (PBN)(Broberger et al., 1998).

The brainstem

The brainstem, or more specifically the NTS in the medulla oblongata, receives signals about hunger and satiety from the *nervus vagus* (Grill & Hayes, 2012). The NTS can relay its information to other brain areas or directly intercede by efferent signalling via the *nervus vagus*. One of the main target regions of the NTS is the *Paraventricular Nucleus* (PVN) of the hypothalamus (Bailey et al., 2006), which is innervated by a distinct population of NTS neurons (Hermes et al., 2006). Receptors for leptin, insulin and ghrelin are expressed in the NTS (Cabral et al., 2013; Pardini et al., 2006; Park & Ahima, 2015). These hormones can diffuse to this region through the fourth ventricle and there affect feeding behaviour by affecting the signalling to and from the ARC.

Hypothalamus

The other brain region, the hypothalamus, is often indicated as the central hub in energy balance (G. J. Schwartz, 2000). This brain structure allows for the exchange of bloodborne factors between the hypothalamus and the circulatory circuit (Campbell et al., 2017): The median eminence contains a mesh of capillaries and has blood-brain barrier free zones where this exchange can take place. This makes the median eminence a suitable place for signalling molecules, like hormones, to influence the hypothalamus, and for the hypothalamus to release signalling factors in the bloodstream. The hypothalamus contains several distinct nuclei, of which the most notable are the *Arcuate nucleus* (ARC), the dorso- & ventromedial hypothalamus (DMH & VMH, respectively), and the PVN, with the ARC located directly adjacent to the median eminence. In humans, NPY-containing neurons are scattered throughout the human hypothalamus, with high expression in the DMH and ARC (Dudas et al., 2010)

Arcuate nucleus

Within the ARC, two distinct populations of neurons can be found that are the main actors in eating and that have an opposing effect on feeding behaviour. The medial ARC hosts a population of neurons that are positive for NPY and Agouti-related protein (AgRP), while the lateral part of the ARC contains neurons positive for proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). The role of the AgRP/NPY neurons in homeostatic feeding has been elegantly shown by (Luquet et al., 2005). They ablated this population of neurons by applying diphtheria toxin in mice with a transgenic, AgRP-dependent diphtheria toxin receptor. Adult mice would starve themselves to death

after this treatment, indicating that AgRP/NPY signalling is essential for the stimulation of feeding. Furthermore, the intensity of AgRP/NPY cell signalling is directly linked to the food intake in mice: Optogenetic stimulation at varying intensity, frequency or number of neurons correlated with the food intake of mice (Aponte et al., 2011).

The POMC/CART neurons release α-melanocyte-stimulating hormone, which is a product of POMC and has a strong anorexigenic effect (M. S. Kim et al., 2000). When the POMC/CART neurons were selectively ablated, mice developed obesity (Xu et al., 2005). In this paper, a slightly different approach is used compared to Luquet *et al.* (2005). The model used by Xu *et al.* depends on the deletion of mitochondrial transcription factor A (*Tfam*) by a tissue specific Cre-recombinase. When this gene is removed, the cell will survive for four to five months. However, when the mitochondrial gene products are depleted, the cell dies. This process results in a more gradual cell death, which also results in a dampened phenotype, as could be seen when they applied this same method to AgRP neurons. The mice in this group showed a non-significant lower body weight than the controls, which stands out to the extreme hypophagic phenotype that Luquet *et al.* found. A possible explanation could be that the method of neuronal ablation used by Xu *et al.* allows the brain to compensate for this loss by potentiation of the remaining neurons, while this is not possible when the ablation occurs suddenly, as in the research by Luquet *et al.*

Both cell populations in the ARC mainly project to the PVH. Here, the POMC/CART neurons activate melanocortin receptors, thereby inhibiting feeding, while the NPY/AgRP neurons inhibit several subpopulations of cells through the release of GABA and NPY, driving food intake (Engström Ruud et al., 2020; Shi et al., 2013). These AgRP/NPY cells also inhibit the POMC/CART neurons in the ARC through similar NPY and GABA release. The activity of both these populations can be influenced by peripheric factors, both directly and indirectly. Ghrelin receptors are found in the ARC that can, upon activation, increase the NPY mRNA levels in this brain area (Kamegai et al., 2001). Leptin, on the other hand, can depolarise the POMC/CART neurons whilst also inhibiting AgRP/NPY neurons by hyperpolarising the somata and preventing the release from NPY/GABA terminals (Cowley et al., 2001; Elias et al., 1999). Insulin has an inhibitory effect on NPY expression (Loh et al., 2017) and hyperpolarises these neurons (Williams et al., 2010). However, insulin also hyperpolarises a subset of POMC-expressing cells (Williams *et al.*, 2010).

Within the ARC, Y_1 is found on POMC/CART neurons, but not on AgRP/NPY neurons, while Y_2 is primarily found on AgRP/NPY neurons, but not on POMC/CART neurons (Broberger et al., 1997). Y_1 and Y_5 have an orexigenic effect when activated, while Y_2 has the opposite effect. A possible explanation for this is that Y_1 and Y_5 are only found post-synaptically, while Y_2 can be located pre-synaptically. This can lead to an autoinhibitory feedback loop: NPY released by a neuron will bind on Y_2 receptors located on the synapse of this same neuron to inhibit the release of more NPY (King et al., 2000). The Y receptors can also be activated by PYY, which can therefore inhibit the NPY/AgRP neurons (le Roux & Bloom, 2005).

Dorsomedial Hypothalamus:

This hypothalamic nucleus has been known as regulator of energy expenditure and intake since the sixties. Ablation of this region resulted in hypophagia (Bellinger & Bernardis, 2002; Bernardis et al., 1963), whereas stimulation of the DMH was followed by thermogenesis and an increase in the body temperature (DiMicco & Zaretsky, 2007; Morrison & Nakamura, 2011).

The overexpression of NPY in this region resulted in a hyperphagic and obese phenotype in rats(Bi et al., 2012), while knockdown of this neuropeptide prevented this phenotype (Chao et al., 2011; Yang et al., 2009). Furthermore, knockdown of NPY in the DMH also returned food intake, insulin sensitivity and glucose tolerance in obese rat models to normal (Y. J. Kim & Bi, 2016), indicating a role of NPY in DMH-mediated feeding. NPY expressing neurons do not express leptin receptors and are therefore not responsive to this satiety factor. However, these cells do express CCK-receptors and can be inhibited by CCKs (Bi et al., 2001, 2004). It is likely that the cells are responsive to other satiety factors as well, although it has not been experimentally established what factors that could be.

The cells in the DMH are inhibited by ARC-POMC-GABA neurons (Trotta et al., 2020). A majority of the efferent DMH projections lead to the PVN. Other cell types in the DMH project to the NTS and the dorsal motor nucleus of the vagus (DMV), through which this area can suppress the sympathetic nervous system and modulate peripheral satiety signals (Yang et al., 2009), However, it is not yet clear whether this modulation of the vagus is directly NPY dependent, as one study found no efferent NPY projections from the DMH to brainstem (Lee et al., 2013).

Periventricular nucleus

The PVN acts as a relay between the brainstem and the hypothalamus and is a target region for both the ARC and DMH (Trotta et al., 2020). Lesions in this area result in hyperphagia and increased body weight (Leibowitz et al., 1981). Increases in the levels of NPY, noradrenalin and AGRP in this region also results in more food consumption (de Backer et al., 2011; Taylor et al., 2007), indicating a modulatory role of these factors in the PVN for feeding behaviour. This effect might be influenced by the day/night cycle, as the orexigenic effect of NPY in this region only present during the early light phase and not during the late light phase (van Dijk & Strubbe, 2003). After sustained overexpression of NPY in the PVN, the food intake first increases but returns to a level similar to control animals after approximately 40 days (Tiesjema et al., 2007). The increase in food intake is driven by a higher meal frequency in the test animals, while the meal size remained constant under these conditions. A possible explanation for the return of food intake to baseline over time is Y5 receptor mediated insulin resistance in adipose tissue (Long et al., 2015, p. 20).

<u>The hedonic feeding network</u>

Food intake and reward are intricately linked. This overlap is integrated in the hedonic feeding network. The reward circuitry revolves around the neurotransmitter dopamine (DA), usually stemming from the ventral tegmental area (VTA). The main dopaminergic pathways that starts in the VTA are the mesolimbic and mesocortical dopamine pathways. The first connects the VTA to multiple regions in the limbic system, most notably the Nucleus accumbens (NAc) and the amygdala. The mesocortical pathway leads from the VTA to the frontal cortex. In the next section, I will focus on the role of NPY in different parts of the mesolimbic dopamine pathway within the in the context of hedonic feeding.

Ventral tegmental area

The VTA consists of heterogenous cells that receive input from and project to a multitude of brain regions (Morales & Margolis, 2017). The most notable efferent pathways of the VTA are the mesolimbic and mesocortical dopamine pathways, but also the ARC and PVH, among others, are

innervated by VTA neurons. The VTA has been implicated in the hedonic aspects of feeding. It contains receptors for ghrelin and leptin, which can moderate the firing rate of the dopaminergic cells in the VTA (West & Roseberry, 2017) Activation of the ghrelin receptor increases the firing rate, resulting in a higher motivation for palatable food and an increased food intake, whereas the activation of the leptin receptor inhibits firing of VTA cells.

The cells in the VTA are also sensitive to NPY, as injection of this neuropeptide into this region increased the motivation for food, although the food intake itself remained unchanged (Pandit et al., 2014). This motivational effect is dopamine dependent, as it does not occur in the presence of a dopamine antagonist. NPY elicits modifications both pre- and postsynaptically when injected into the VTA (West & Roseberry, 2017), by lowering the membrane potential or decreasing glutamatergic and GABAergic transmission. These effects hint at the involvement of both the Y1 and the Y2 receptor. NPY is not synthesised in the VTA, but stems from afferent connections from the ARC and the ventrolateral medulla of the brainstem (Gumbs et al., 2019).

Nucleus accumbens

The Nucleus accumbens (NAc) is the target for the dopaminergic connections from the VTA in the reward pathway and an important region for appetitive behaviour (Tanaka et al., 2021). Eating palatable food increases the levels of extracellular DA and this effect is increased by food deprivation (Sørensen et al., 2012), which is in line with the activation of the ghrelin receptor in the VTA. Infusions of glutamate receptor antagonists in the shell of the Nac increased food intake in a dose-dependent manner (Maldonado-Irizarry et al., 1995), whereas activation of these receptors suppressed feeding (Stratford et al., 1998). Likewise, infusion of NPY into the NAc increases the motivation for food, similar to VTA infusions, while also increasing the food intake (Pandit et al., 2014). The increase in food intake is likely Y1 and Y5 receptor mediated, as antagonists of these receptors were able to inhibit the increase in food intake (Stratford & Wirtshafter, 2004). Furthermore, infusions of NPY into the shell of the NAc increases extracellular DA (Sørensen et al., 2009) and specifically increases fat intake through Y1-mediated inhibition of cell activity in the Nac (van den Heuvel et al., 2015). This indicates a role for the NAc in food preference. The NAc is innervated by local interneurons and by projections from the ARC (Gumbs et al., 2019). These ARC-neurons project NPY into the NAc, which drives the intake of palatable food (Tanaka et al., 2021).

Extended amygdala

The amygdala plays an integral role in fear processing and emotional memory. This structure consists of 13 nuclei, which can be roughly divided into the basolateral amygdala (BLA), the centromedial amygdala (CeA) and the cortical subgroup (J. E. Kim et al., 2012). The BLA integrates the information of fear stimuli, like auditory and visual inputs, and projects the integrated information to the CeA, which acts as the main output node of the amygdala. The central nucleus of the amygdala and the bed nucleus of the stria terminalis (BNST) are often referred to as the central extended amygdala. This region contains a relatively high density of Y2 receptors (Wood et al., 2016). However, these receptors were not present on cells expressing NPY themselves. Activation of these receptors inhibited both glutamatergic and GABAergic transmission, while removal of NPY or Y2 resulted in increased GABA signalling. In line with these results, the addition of NPY in the amygdala increased cell activity in the CeA (Pomonis et al., 1997).

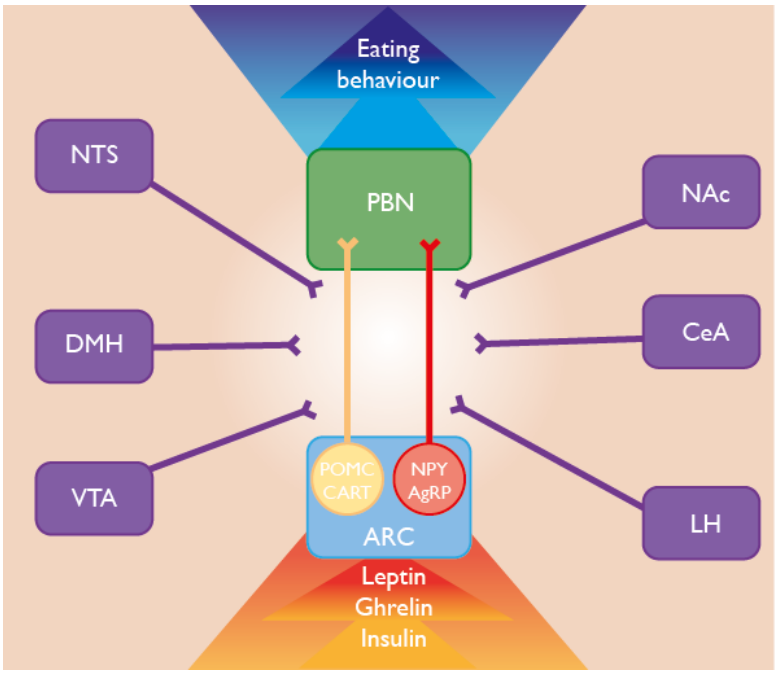

Figure 2: Schematic overview of the feeding network. *The two distinct populations in the ARC project to the PBN and drive feeding behaviour. They are modulated by blood borne factors like leptin, ghrelin and insulin, and by multiple other brain regions. Arc: Arcuate nucleus; CeA: Centromedial amygdala; DMH: Dorsomedial hypothalamus; NAc: Nucleus accumbens; LH: Lateral hypothalamus; NTS: Nucleus of the solitary tract; PBN: Parabranchial nucleus; VTA: Ventral tegmental area.*

The central nucleus of the amygdala is likely involved in the inhibitory control of food intake, as lesions of this area result in increased feeding and body weight gain (Hajnal et al., 1992). The precise mechanisms through which the amygdala alters feeding under homeostatic conditions have not yet been elucidated, although a recent paper shows a role for NPY in the CeA in the development of obesity (Ip et al., 2019). Furthermore, a recent MRI-study links central amygdala thickness with obesity and dietary self-control (M. S. Kim et al., 2020), implying a role of the amygdala in the preference for palatable food over healthy food. Outputs from the amygdala are also known to override satiety, possibly by the involvement or modulation of NPY (Petrovich et al., 2002).

Lateral hypothalamus

The LH receives projections from the PBN, NAc, BNST and frontal cortex (Jennings et al., 2013). This region itself projects to multiple other brain regions, including the PBN and NST (Wu et al., 2015). Ablation of the LH resulted in decreased food intake (Bernardis & Bellinger, 1996), whereas electrical stimulation of the LH results in ravenous eating behaviour and increased motivation for food (Stuber & Wise, 2016). These cells respond to NPY, as infusion of this neuropeptide results in increased food intake (Gumbs et al., 2020; Pandit et al., 2014), although the motivation for food remains unaffected by NPY (Pandit et al., 2014). Sustained NPY overexpression in the LH raises the daily food intake (Tiesjema et al., 2007). This effect attenuates over time, but remains above baseline and control measures. Although the meal frequency remains constant, the average meal size of these animals is increased and therefore the driving force behind the increased food intake. This is likely mediated by the Y1 and Y5 receptors, as the increase was attenuated by selective Y1 and Y5 antagonists (Gumbs et al., 2020). Diet induced obesity disrupts this network, as Y1 inhibition is no longer sufficient to prevent increased food intake in rats on a free-choice high-fat high-sucrose diet. Notable is that the effects on food intake in this research only apply to chow. The researchers also provided the animals with fat and sucrose solution, but there was no significant change in the intake of these food types. The LH is responsive to palatable and aversive stimuli (J. X. Li et al., 2013), with the neurons involved with reward located caudally and neurons in the rostral portion biased towards aversive stimuli. It is likely that the LH is, at least partly, responsible for the encoding of palatability (J. X. Li et al., 2013).

This section investigated the mechanisms of feeding behaviour, looking at the hedonic and homeostatic network. Although the homeostatic and hedonic feeding network have been described separately, it is important to note that these networks are heavily intertwined and reliant on each other (Rossi & Stuber, 2018). Some brain areas, like the LH, serve hedonic as well as homeostatic functions, so no clear distinction can be made between the two networks (Liu & Kanoski, 2018). Furthermore, it is possible to expand upon the described model with other brain areas. However, this would be beyond the scope of this thesis, as I focused on NPY signalling. The NPY peptide was discovered to have a strong orexigenic effect when injected into the brains of rats, most notably in areas of the hypothalamus (Stanley et al., 1985). Within the ARC in the hypothalamus, two distinct and antagonistic cell populations play an integral role in feeding: the POMC/CART neurons and the NPY/AgRP neurons. Activation of the latter leads to increased food intake, while removal of this neuron population resulted in the ablation of feeding behaviour, causing rats to starve themselves. The ARC projects to and is moderated by multiple brain regions that are involved in homeostatic and hedonic regulation of food intake, forming a complex and intertwined network.

NPY and stress

Like feeding behaviour, the stress response is regulated by a complex and adaptive network that involves multiple brain regions and hormones. NPY is also involved in this network, as it was discovered that NPY knockout mice increases stress-related behaviours (Bannon et al., 2000), whereas overexpression results in a decrease (Erickson et al., 1996). Multiple studies have investigated the effects of stress on central NPY levels (Lin et al., 2015; Melhorn et al., 2010). Central NPY levels are highly dependent on the type of stress that is used in the experiments and the brain region that is being measured; see (Reichmann & Holzer, 2016) for a table of different types of stress and their effect on NPY expression.

Furthermore, the NPY protein exerts an anxiolytic effect on rats when it was injected into the brain (Heilig et al., 1989). This anxiolytic effect is mostly visible when NPY has been increased in the amygdala or hippocampus (Bannon et al., 2000; Lin et al., 2010). Most, although not all, of the anxiolytic effects of NPY can be ascribed to the activation of the Y_1 receptor (Lach & de Lima, 2013). On the other hand, activation of the Y_2 receptor can have an anxiogenic effect (Sajdyk et al., 2002). However, there are some brain regions where Y_2 activation leads to a decrease in anxious behaviours (Kask et al., 1998). In the following section, I will go over the different brain areas involved in the stress response and how NPY can influence these.

Hypothalamus

The hypothalamus is the main player in the chronic stress response as instigator of Hypothalamus-Pituitary-Adrenal (HPA)-axis (S. M. Smith & Vale, 2006). Upon activation as by a stressor, the PVN in the hypothalamus releases corticotropin-releasing hormone (CRH, also known as corticotropin-releasing factor, CRF) and vasopressin into hypophysial portal vessels. These vessels connect to the anterior pituitary gland, where adrenocorticotropic hormone (ACTH) is released into the bloodstream when stimulated by CRH. Although vasopressin cannot activate these cells directly like CRH, it can potentiate the cells to the activating effect of CRH (Rivier & Vale, 1983). ACTH in turn can evoke the

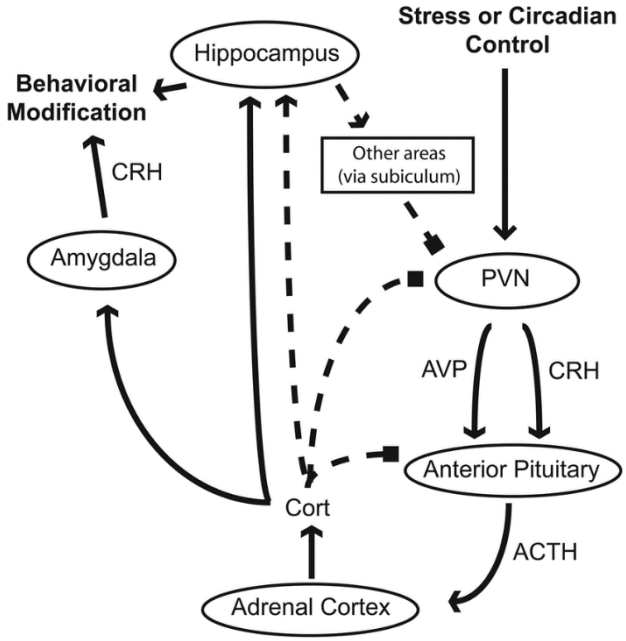

Figure 3: Schematic overview of the HPA axis and related effects of stress.
Stress activates the release of CRH and AVP from the PVN, hereby activating the HPA-axis. These activate the Anterior Pituitary, which in turn releases ACTH. ACTH in turn activates the adrenal cortex, which releases Cort. Cort has variable effects throughout the body and brain. It also inhibits the PVN and anterior pituitary, thereby creating a negative feedback loop.
ACTH: Adrenocorticotropic hormone; AVP: Vasopressin; CRH: Corticotropin releasing hormone; Cort: Cortisol or corticosterone; PVN: Paraventricular nucleus.
Image taken from Kolber et al., 2008.

release of glucocorticoids from the cortex of the adrenal glands, located above the kidneys. A schematic overview of the effects and components of this response can be found in Figure 2.

Glucocorticoids can activate mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) that are expressed ubiquitously throughout the body and brain (De Kloet et al., 1998). These receptors are primarily localised in the cytosol as part of a large protein complex (Bamberger et al., 1996). When a ligand binds to a GR or MR, the receptor is released from the complex and transported to the nucleus of the cell. Here, it can bind directly to the DNA in so called mineralocorticoid or glucocorticoid response elements and thereby alter the gene expression of these cells. The MR, also referred to as the type I glucocorticoid receptor, has an affinity for glucocorticoids that is approximately ten times higher than the affinity of the GR, or type II glucocorticoid receptor (De Kloet et al., 1998). This means that MRs are already activated at low levels of glucocorticoids, while GRs are only activated at higher levels of glucocorticoids, which allows for two different signalling pathways, depending on the concentration of glucocorticoids. The type II receptors are the main force of feedback in the HPA-axis. Circulating glucocorticoids will activate these receptors in CRH releasing neurons in the PVN and ACTH releasing neurons in the pituitary gland, inhibiting their release and dampening the stress response (Dallman et al., 1992). This negative feedback allows for the regulation of the HPA-axis and eventual termination of the stress response.

Some trends can be observed concerning the effects of stress on the levels of NPY in the hypothalamus. In this region, acute stress leads to an increase in the levels of NPY mRNA (Reichmann et al., 2015), notably also within the ARC (Sweerts et al., 2001). However, the effect of chronic stress on the levels of NPY within the hypothalamus has not been conclusively proven, as these levels are found to be either increased (McDougall et al., 2005) or unchanged by the stress response (Reichman et al., 2015).

Although stress can have an effect on NPY, this protein can in itself also get engaged in or even activate the stress response. Injections of NPY in the PVN of rats resulted in an activation of the HPA-axis, as could be seen by an increase in the levels of corticosterone and ACTH (Wahlestedt et al., 1987). It is likely that NPY affects CRH releasing neurons, as the levels of CRH increase in a dose-dependent manner after the injection of increasing doses of NPY (Suda et al., 1993). This occurs through CRH releasing neurons in the PVN, that are directly mediated by NPY through the Y_1 receptor present on

these cells (Dimitrov et al., 2007), although other studies suggest the involvement of a different Y-receptor (C. Li et al., 2000). It is also possible that this effect occurs independently, by an inhibitory intermediate cell, as the NPY/AgRP projections are predominantly inhibitory in nature (Comeras et al., 2019).

The brainstem

The brainstem is mainly involved in and a key player of the acute stress response. The main effectors for this response are the hormones adrenalin and noradrenalin (also known as epinephrine and norepinephrine, respectively), which are released through the sympathetic-adrenal medullary (SAM)-axis (Turner et al., 2012). This system has two separate pathways that both start in the brainstem. When the brainstem stimulates preganglionic neurons, a subset will activate postganglionic neurons, that in turn will release noradrenaline in their target tissue. A different subset of preganglionic neurons will project directly to the adrenal medulla. This structure will release adrenalin and noradrenalin in the blood stream when excited through acetylcholine secreted by these preganglionic projections (Turner et al., 2012). These hormones have effects throughout the body, preparing for a possible exertion, for instance by increasing the heart rate and respiratory rate. Overall, activation of the SAM-axis leads to an immediate and transient response to stress.

On the other hand, certain nuclei in the brainstem also play a part in the modulation of the chronic stress response. The NTS innervates the CRF neurons in the hypothalamus (Herman, 2011). These neurons release noradrenalin into the PVN, which stimulates the release of CRF at the start of the HPA-axis. The locus coeruleus (LC) is located in the hindbrain and contains noradrenalinergic projecting cells. This noradrenalin signalling could be inhibited by NPY in zebrafish, hypothesised to be part of the wake/sleep cycle (Singh et al., 2017). It is important to note that the NPY mRNA expression in the LC occurs in a circadian dependent pattern (Cedernaes et al., 2019), hinting at an interaction with the cortisol network as this factor also follows a diurnal pattern as part of the sleep/wake cycle. The corticosterone of the chronic stress response increases the plasma levels of NPY and noradrenalin when infused into the dorsal hindbrain (Daubert et al., 2014). Higher levels of noradrenalin can, in turn, affect food intake by increasing activity of NPY/AgRP neurons and decreasing the activity of POMC neurons in the ARC (Paeger et al., 2017).

Amygdala

The amygdala is involved in fear processing and is therefore intricately connected with the stress network. GABAergic from this structure can activate the HPA-axis and modulate the central response to stress (Martijena et al., 2002). The anxiolytic properties of NPY were discovered when it was injected into the amygdala (Heilig et al., 1989). Furthermore, NPY levels in the amygdala were downregulated in animals that were severely affected by trauma, whereas post-trauma treatment with a central injection of NPY significantly reduced stress behaviour in these animals (H. Cohen et al., 2012). Further investigation revealed that the Y1 and Y5 receptors were the driving force behind the anxiolytic effect of NPY, whereas the Y2 receptor was responsible for an anxiogenic phenotype (Sajdyk et al., 2002). The afferent and efferent projections of the CEA can be modulated by distinct neuronal populations through Y2 mediated signalling (Wood et al., 2016).

The anxiolytic effect of amygdalar NPY-injections appears to be subregion-specific, as this effect was present when NPY was injected into the CeA and BLA, but absent when NPY was injected into the MeA

(Kornhuber & Zoicas, 2021). Furthermore, the CeA injection also resulted in reduced expression of social fear in the animals, whereas this effect was not observed when NPY was injected into the BLA or MeA. The different regions of the amygdala are modulated by connections from the prefrontal cortex (McGuire et al., 2011). Many of these connections exhibit an inhibiting function of amygdalar cells and express Y2 receptors at their synapses. Activation of the Y2 receptor by NPY can therefore inhibit this inhibition and result in increased excitation of neurons in the amygdala.

Nucleus accumbens

The NAc has one of the highest NPY mRNA concentrations in the human brain (Adrian et al., 1983; Morris, 1989). NPY-immunoreactive fibres form a complex, heterogeneous distribution in the NAc (de Quidt & Emson, 1986), whereas cell bodies containing the NPY protein can be found in both the shell and core of the NAc (Aoki et al., 2016). NPY expression colocalises with somatostatin in interneurons (Beal et al., 1987; Kawaguchi et al., 1995). These NPY expressing cells are under the control of dopaminergic connections, as fewer NPY-expressing neurons are found when these connections were lesioned (Salin et al., 1990). The NPY expressing cells in the NAc almost exclusively project to the lateral hypothalamus (LH) and receive projections from the midline thalamic nuclei and the basomedial amygdala (Yamada et al., 2021). The expression of NPY protein is decreased in a mouse model for anxiety (Aoki et al., 2016), whereas overexpression of NPY in the NAc has an anxiolytic effect (Yamada et al., 2020). Complete removal of NPY neurons in this region by selective ablation increases anxiety-like behaviour, indicating an anxiolytic role of NPY neurons in the NAc.

In this section, the stress response was explored, with a focus on the role of NPY. Stress has a variable effect on the levels of NPY expression in the hypothalamus, while NPY itself can also exert an anxiolytic response. Overall, NPY appear to have a modulatory role of the stress response in several brain areas. Many of these brain areas overlap with the network of feeding behaviour. Therefore, the next section will analyse this overlap and place it within the context of stress eating and obesity.

Stress eating: the overlap in stress and feeding

Stress is a known risk factor for obesity (Sinha & Jastreboff, 2013), with stress eating as one of the factors that can play a role in this pathology. Acute stress represses food intake, whereas chronic stress tends to promote food intake and a preference for palatable food (Ans et al., 2018; Sominsky & Spencer, 2014). The stress induced accelerated obesity, as seen in the research by Ip *et al.* can also be observed in humans; Chronic stress increases vulnerability to obesity/fat increase when on a high-palatable diet (Aschbacher et al., 2014). These subjects also showed increased levels of peripheral NPY compared to a non-stressed control group. A peripheric injection of CRH in humans increases food intake in direct correlation with their cortisol response (George et al., 2010) and chronic glucocorticoid administration increases *ad libitum* food intake (Tataranni et al., 1996). Furthermore, chronic stress is an indicator for weight gain (Mason et al., 2018).

The effect of stress on NPY is highly variable and dependent on the type of stress, although even similar stress paradigms can result in different effects on NPY (Reichmann & Holzer, 2016). This variable effect of stress is also visible when looking at the effect of stress on body weight and feeding behaviour (see (Hardaway et al., 2015) for a table summarising the effects of multiple stress paradigms on food intake and body weight). Stress eating can stem from the intertwining of the stress network with the feeding

network, which occurs at multiple locations. Below, I will delve deeper into the most important areas of overlap and focus on the role of NPY in these areas within the context of stress eating. This framework will then be expanded by other NPY-dependent mechanisms that can drive stress eating and obesity, but are not restricted to a single brain area.

Hypothalamus

In models for obesity, the HPA-axis was dysregulated (Werdermann et al., 2020). This was accompanied by a change in expression of NPY and AgRP in the hypothalamus. CRH released through activation of the HPA-axis inhibits the NPY/AgRP neurons in the ARC (Sominsky & Spencer, 2014), resulting in the acute stress-mediated decrease in food intake. The glucocorticoids released at the end of the HPA-axis interact with the NPY/AgRP neurons in the ARC as well, although these factors have a stimulatory effect (Savontaus et al., 2002). This increase in NPY/AgRP cell activity is a driving effect behind chronic stress-induced feeding. The increase in food intake is likely Y1 receptor mediated, as the effect was not present when this receptor was inhibited (Goebel-Stengel et al., 2014).

It must be noted that there is a large variety in the effects of stress on feeding in animal model research. The connection between NPY and stress eating is equally variable. Table 1 shows an overview of research that investigated stress, feeding and hypothalamic NPY. Several of these studies report increased food intake (Goebel-Stengel et al., 2014; Melhorn et al., 2010), while others reported decreased feeding after stress (Chigr et al., 2014; S.-X. Wang et al., 2012) or no effect of stress on food intake (Lin et al., 2015). However, when considering the research in this table, there appears to be some correlation between the direction of change in NPY and the effect of stress on food intake: Decreased food intake was linked to a decrease in NPY mRNA (S.-X. Wang et al., 2012) or number of NPY-expressing cells (S. Wang et al., 2013) after stress, whereas the complete removal of NPY exacerbates this effect (Forbes et al., 2012). On the other hand, social stress lead to an increase in NPY expression and an increase in feeding behaviour, although this effect was only significant in submissive males (Melhorn et al., 2010). These males also showed a 3- to 4-fold increase in corticosterone as response to the stress. A similar effect was found to be attenuated by the application of a Y_1 antagonist (Goebel-Stengel et al., 2014). A likely explanation for this variation in results is the type of stressor that is used, as stress responses can be distinctly different dependent on the type of stressor. Overall, these results indicate an adaptive stress response that can mediate feeding through hypothalamic NPY.

The effect of hypothalamic NPY in stress eating might persist long after the stressor has subsided. NPY mRNA was upregulated for up to 48 hours after stress in the dorso-vagal complex (DVC, a group of brain stem nuclei including the NTS and DMV) and up to 72 hours after stress in the hypothalamus, while acute stress lead to a decrease in food intake that had returned to baselevel at 72 hours after stress (Chigr et al., 2014). Although the stress does not elicit an initial NPY response, the NPY mRNA expression does steadily increase until at least 72 hours after stress. Therefore, this NPY response might be the driving force behind the food intake returning to baseline.

There was no clear difference between acute and chronic stress in the presented articles, although overexpression of CRH decreased food intake (L. Wang et al., 2017), reminiscent of the initiation of the stress response. The mechanism behind the differential effect of acute and chronic stress might rely on the activation of distinct glucocorticoid receptors. Type II glucocorticoid receptors have a relatively low affinity for glucocorticoids, so they are only activated by high concentrations of these hormones, which occur after activation of the HPA-axis. Activation of the type II glucocorticoid receptors on NPY-producing neurons has a stimulating effect on the release of NPY, whereas blocking of this receptor attenuates the increased food intake after NPY injection into the PVN (Tempel & Leibowitz, 1993). This

effect was not found when the type I glucocorticoid receptors were blocked. Glucocorticoids inhibit the previous steps of the HPA-axis, including the release of CRH (Kolber et al., 2008). The dynamics of CRH and glucocorticoids therefore follow the changes in appetite during stress, with an initial drop in food intake by the initiating peak in CRH release, which is gradually suppressed by the increasing concentration of glucocorticoids, resulting in an increase in food intake through both the stimulatory effect of the glucocorticoids and removal of the inhibitory CRH.

Amygdala

The amygdala is a player in the stress response as fear centre. Further results suggest a controlling role of the amygdala in hedonic food intake as well. This area has therefore been investigated in the context of stress eating (Hu et al., 2016; Martín-Pérez et al., 2020). A recent paper by (Ip et al., 2019) elucidated on a circuit that drives accelerated obesity as response to stress. Weight gain can be accelerated by stress, resulting mostly in an increase in visceral adipose tissue. This process is NPY-dependent, as NPY-knock out mice do not show this accelerated weight gain. The circuit relies on a population of NPY-expressing neurons in the central amygdala. A selective knockout of NPY in this region was enough to attenuate the accelerated weight gain under stress. On the other hand, overexpression of NPY in this region was enough to accelerate obesity. These NPY neurons project to the ARC and likely onto NPY-expressing neurons in this region.

There is also evidence that hunger and satiety can influence stress. Most notable is that hunger appears to suppress fear and anxiety (Comeras et al., 2019). Hunger increases NPY/AgRP cell activity in the ARC. These cells project to the amygdala, among other regions, where they have an inhibitory effect on signalling. This hunger driven reduced stress could be beneficial for an organism; high stress can prevent exploration, so lower stress encourages active foraging for food, thereby increasing its chances for survival.

The brainstem

Structures in the brainstem can modulate the stress response and indirectly influence stress eating via that route. Noradrenalinergic connections from the NTS innervate the CRF neurons in the PVN (Herman, 2011). These connections stimulate the release of CRF at the start of the HPA-axis and can therefore induce the CRF-mediated inhibition of feeding, as discussed in the previous section on the hypothalamus. These conditions are reminiscent of the acute stress response, as CRF levels are high and not yet inhibited by glucocorticoids. This indirect inhibition of the NPY/AgRP neurons in the ARC could partly explain the decreased appetite during acute stress.

On the other hand, the corticosterone of the chronic stress response increases the plasma levels of NPY and noradrenalin when infused into the dorsal hindbrain (Daubert et al., 2014). Higher levels of noradrenalin can, in turn, affect food intake by increasing activity of NPY/AgRP neurons and decreasing the activity of POMC neurons in the ARC (Paeger et al., 2017).

Insulin resistance

Insulin can modulate NPY signalling (Cowley et al., 2001; Williams et al., 2010). Within the brain, insulin receptors are highly expressed in the ARC. Selective deletion of these receptors increases the expression of NPY and AgRP, but not of POMC (Obici et al., 2002). In line with this, injection of insulin

in the third ventricle reduced NPY expression (M. W. Schwartz et al., 1991), although this effect could not be replicated in obese rats. This is due to insulin resistance (Ono, 2019). When cells are exposed to high levels of insulin for longer periods of time, the cells become less sensitive to this hormone. This often occurs in obese individuals, as insulin levels are correlated with body fat (G. J. Schwartz, 2000). Insulin resistance is mediated by NPY in NPY/AgRP neurons, as NPY deficient mice were protected against this phenomenon (Engström Ruud et al., 2020). When NPY was selectively reintroduced into AgRP cells, the protection against insulin resistance in these animals was reversed.

Insulin is released during the stress response. In acute stress, this insulin inhibits the NPY/AgRP neuron population in the ARC, inhibiting feeding. However, under chronic stress, insulin resistance will occur, driven by glucocorticoids (Qi & Rodrigues, 2007). The levels of NPY mRNA in the ARC inversely correlate with the levels of insulin after acute and repeated immobilisation stress (Makino et al., 2000). In this situation, NPY/AgRP neurons in the ARC can no longer be inhibited by insulin, resulting in an increased NPY release into the PVN, which in turn can lead to more feeding behaviour. Furthermore, the NPY neurons in the amygdala are inhibited by insulin. Insulin resistance following stress can disinhibit these neurons, leading to a further increase in feeding behaviour and accelerated weight gain (Ip et al., 2019).

NPY and food preference

Another way how NPY can drive the development of obesity under stress is by shifting food preference towards palatable and energy-rich food. Injections of NPY increases not just the total food intake, but also the composition of the menu, although this food preference is brain-region dependent (Baird et al., 2008); NPY regulates food preference for carbohydrates through the activation of the Y1 receptor in the NAc (van den Heuvel et al., 2015) and the PVN (Tempel & Leibowitz, 1993). Preference for chow is regulated in the LH, which is also dependent on NPY (Gumbs et al., 2020). Furthermore, the central nucleus of the amygdala can modify the preference for palatable food, although it is still unclear whether this effect is NPY-dependent (M. S. Kim et al., 2020).

Stress generates a preference for comfort foods over healthy foods (Dallman, 2010; Oliver et al., 2000), likely involving NPY signalling. This can happen directly (e.g. through a GC-induced release of NPY in the PVN), or indirectly (e.g. when Nac Y1 receptors are activated). The calorie-rich food can provide energy quickly during an acute fight-or-flight situation, or can be used to replenish energy reserves after such an exertion. However, under chronic stress conditions, this preference for comfort food can become maladaptive (Dallman et al., 2005), especially in our current society where food is no longer a scarce commodity. This preference for palatable food exacerbates the problem of stress eating and speed up stress-induced obesity.

<u>The stress eating network</u>

The stress eating network occurs at the overlap of the feeding network and the stress network, with a key role for NPY signalling. Several of the main centres of this network have been discussed above, although I do not mean to imply that the proposed framework is complete, as there are other brain areas that are involved in both the stress response and in feeding behaviour. For these brain regions, like the NAc, the role of NPY on stress eating has not been excessively studied in these areas, and therefore they have not yet been included in this model. It is important to note that the network is heavily interconnected and therefore the described effects will not occur independently, but can be

influenced by each other. This implies that the eventual outcome (i.e. whether or not to eat) usually cannot be attributed to a single event, but is determined by all the different modulatory influences on the NPY/AgRP neurons in the ARC.

Understanding this network can help find new treatments for stress-induced obesity. The integration of rewarding and homeostatic processes, as well as most of the stress response, occur primarily outside awareness (Berthoud et al., 2017), underlining the difficulty of a completely will-based treatment of obesity. New anti-obesity treatments could focus on regulating stress and feeding networks, with a special focus on the inhibition of NPY, thereby supressing its orexigenic effects and reducing the shifted food preference.

NPY in stress resilience, post-traumatic stress disorder, and eating disorders

The network of stress eating might be subjected to plastic modulation mechanisms that can lead to (mal-)adaptive responses to stress. The study by Sweerts et al. (2001) showed that the stress from a daily 60-minute restraint paradigm elicits a peak in NPY mRNA in the ARC on the first day of stress. This peak is decreased on the third consecutive day of stress. On the tenth day of stress, the levels of NPY mRNA in the ARC have returned to baseline. However, they could not recreate this effect in a rat model for hypertension, as those animals did not show an initial NPY-increase but a sustained increase over a longer time frame. The differences between these strains indicate a potential role of NPY in stress resilience. The BLA plays a key role in stress resilience (Silveira Villarroel et al., 2018); NPY signalling in the BLA hyperpolarises BLA neurons, inhibiting the fear response they encode, and therefore preventing a stress-reaction. This effect is mediated by the Y5 receptor, as injection of NPY or a Y5 agonist resulted in an increase in non-stressed behaviour, while a Y5 antagonist was able to prevented this increase (Michaelson et al., 2020). These effects of NPY are counteracted by CRH.

The role of NPY in stress resilience has often been investigated in the context of post-traumatic stress disorder (PTSD), an acquired mental condition induced by a traumatic experience (American Psychiatric Association, 2013). Not all traumatic experiences lead to PTSD, but the chance of acquiring this disorder seem to be dependent on the stress resilience of an individual (S. Cohen et al., 2015). It was found that the NPY levels in the cerebrospinal fluid of soldiers with PTSD were lower than those of controls (Sah et al., 2014). Therefore, NPY has gained interest as a potential treatment for PTSD, due to its anxiolytic function. Treatment of an animal PTSD model with NPY during or after the trauma seems to increase the chance of a full recovery and reduced anxiety (Sah et al., 2014).

PTSD and eating disorders share a large comorbidity (Vanzhula et al., 2019) and patients suffering from PTSD have a predisposition towards obesity (Masodkar et al., 2016). Furthermore, the vast majority of eating disorder patients report some form of trauma in their past (Mitchell et al., 2012). However, the underlying networks of this comorbidity have not been elucidated. I would like to propose that (at least some incidences of) these disorders stem from a disruption in the stress feeding network as discussed in this thesis, affecting NPY signalling. This could explain why the patients show disruptions in both their stress regulation and feeding behaviour and why NPY seems to alleviate the symptoms of PTSD. However, this is so far purely hypothetical and would require more research into this network.

Limitations and cautions

There are some cautions that should be exerted when looking at the discussed results. Overall, it is important to keep in mind that the complexity of both the stress and feeding networks makes it more

difficult to interpret; the observed results could be primary effectors in the pathology or simply the effect of changes occurring elsewhere. The causal relationships can usually only be determined through direct interference within the brain, often with (invasive) methods. However, the growing volume of research on this subject allows for the gradual elucidation of the precise mechanisms and how they interact with each other. Furthermore, it is important to note that the continual application of NPY reduces the responsiveness to this neuropeptide (Corder et al., 2020). This poses challenges for the implementation of NPY as a therapeutic for PTSD, as long term exposure would decrease the effectiveness of the NPY therapy. On the other hand, this means that one should be careful when interpreting results of experiments where NPY was overexpressed for an extended period of time, as the measured effects here could reflect a reduced responsiveness to the signal rather than an increase in NPY signalling. It is possible that this reduced sensitivity is brain region dependent, as food intake returned to baseline after overexpression in the PVN, but not in the LH (Tiesjema et al., 2007).

Secondly, it is always difficult to translate animal data directly to the human situation. Especially chronic stress is difficult to recreate, as human chronic stress is often of the result of complex social and psychological stressors, like too many responsibilities or financial problems, which is hard to validate and recreate in animals (Harris, 2015). Furthermore, the effect of NPY can differ between different strains of animal models for obesity (Beck, 2006).

Part of the variation in the NPY response of different knockout and knock-down studies can be explained by the method of genetic manipulation of NPY levels. The NPY system is still developing postnatally (Nilsson et al., 2005). If the genetic deletion of NPY were to happen before birth (for instance by an NPY-full knock out mouse), it would have no effect on feeding behaviour or body weight (Mercer et al., 2011). If NPY was deleted postnatally, the food intake would decrease.

Furthermore, it is important to note that differences in sex can have a big influence on feeding behaviour and stress (Forbes et al., 2012). However, this fact is often overlooked or ignored, as most animal research is performed on male animals. Reproductive hormones, especially female hormones, influence the pathology of obesity (Asarian & Geary, 2013; Qiu et al., 2018).

Concluding remarks

The goal of this thesis was to review the role of NPY in the stress response, feeding behaviour and stress eating. NPY/AgRP projecting cells in the ARC drive food intake and are modulated by a multitude of other brain regions, forming a complex network that integrates homeostatic and hedonic systems. A similar complex network regulates the stress response, which is heavily interconnected with the feeding network. A molecular candidate for this crosstalk is NPY. This evolutionary conserved neuropeptide features a prominent role in feeding as an orexigenic factor. NPY was found to have an anxiolytic effect and is also referred to as the functional opposite of CRH, the starting point of the HPA-axis. Acute stress results in hypophagia, likely due to the inhibition of NPY/AgRP neurons in the ARC by CRH. Chronic stress generally leads to hyperphagia, likely by stimulation of NPY/AgRP neurons in the

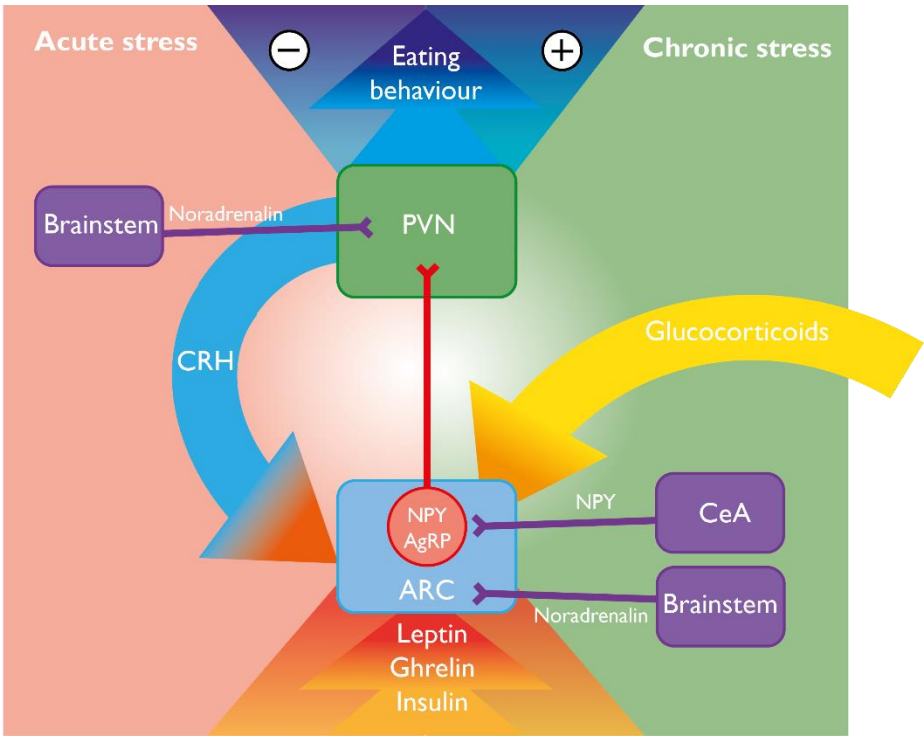

Figure 4: Proposed model of the interaction of the feeding and stress network under acute and chronic stress conditions. *The above figure represents a circuit for an NPY-mediated reaction on stress. Left: acute stress triggers the release of noradrenalin from the brainstem. This stimulates the release of CRH, which inhibits NPY/AgRP neurons in the ARC, inhibiting eating behaviour. Right: Chronic stress results in high levels of glucocorticoids in the body. These directly stimulate NPY/AgRP neurons in the ARC. This is strengthened by NPY from the CeA and Noradrenalin from the Brainstem.*
AgRP: Agouti-related protein; ARC: Arcuate nucleus of the hypothalamus; CeA: Centromedial Amygdala; CRH: Corticotropin releasing hormone; NPY: Neuropeptide Y; PVH: Paraventricular nucleus of the hypothalamus.

ARC by glucocorticoids, although this effect is highly variable and dependent on the type of stress. Although several studies have looked into the connection between NPY, stress and feeding, no unambiguous conclusion can be drawn from these studies, as there exists a large variety in the effects of stress on both other factors. Further research is still needed to determine how different types of stressors affect the stress response and, subsequently, affect stress eating. However, there does appear to be some relation between the direction of the stress-induced change in feeding and the direction of changes in NPY expression in the hypothalamus. This brain area is heavily modulated by the other regions in the feeding and stress networks. Besides the direct effects of the stress hormones on the NPY/AgRP-expressing neurons in the ARC, this population is indirectly modulated by other stress-related brain regions, like noradrenalinergic connections from the brainstem and NPY-expressing cells in the amygdala, leading to a large and complex modulatory network for stress eating. Furthermore, multiple other brain regions, like the amygdala and the brainstem, can play a NPY-mediated modulatory role on stress eating, for instance through the shifting of food preference towards palatable food. This implies that, although stress can have varied effects on eating behaviour, stress-induced hyper- and hypophagia is mediated by NPY. The network of stress eating is highly complex, containing feedback loops, inhibitions of inhibitions and multiple seemingly redundant relays. However, this complexity has lead to a highly adaptive stress response and feeding behaviour with a key role for NPY. Elucidating this network will potentially provide treatments for obesity, stress disorders and stress eating.

Table 1: Literature overview concerning stress, food intake and NPY. The table has been organised by model organism used. The third column states the type of stress used in the described research. The fourth column states the effect of the used stress paradigm on levels of corticosterone of the organism. The fifth column describes the effect this stress paradigm had on the feeding behaviour of the model organisms. The last column describes the effects the stress paradigm had on the levels of NPY.

Paper	Model organism	Type of stress	Effects of stress	Effect on eating behaviour	Effect on NPY
Rat					
(Chigr et al., 2014)	Adult rat	Immobilisation (acute, restraint)	N.D.	Decreased food intake, back to baseline after 72 hrs.	Gradual increase in mRNA over time in the hypothalamus
(Goebel-Stengel et al., 2014)	Adult rat	Tail pinch (acute) and repeated tail pinch (chronic)	No effect on ACTH levels in the blood (corticosterone levels were not monitored)	Increased food intake in both acute and chronic stress, stress eating response was attenuated when Y_1 antagonist was present	N.D.
(Krolow et al., 2013)	Prepubertal and adult rats	Isolation stress (chronic)	N.D.	Increased food intake when stressed and had access to palatable food.	Increase in NPY only in juvenile male that had no access to palatable food. Decrease in NPY only in adult males that had access to palatable food.
(S.-X. Wang et al., 2012)	Adult rat	Immobilisation (chronic, restraint)	N.D.	Decrease in body weight in stressed rats Decreased food intake in the stressed groups compared to control	No effect on NPY content in the hypothalamus However, a decrease in relative mRNA levels in the hypothalamus in the stress groups
(S. Wang et al., 2013)	Adult rat	Immobilisation (chronic, restraint)	N.D.	Smaller increase in body weight	Decrease in NPY-positive cells in the ARC

				compared to control Decreased food intake	
(Melhorn et al., 2010)	Adult rat	Visible Burrow System (chronic, social)	VBS increases the basal corticosterone levels in submissive males to about 3- to 4-fold increase, which was returned to baseline in recovery	Increase in meal size in submissive males	NPY mRNA levels in the ARC are increased in both the dominant and submissive males compared to control
Mouse					
(Forbes et al., 2012)	NPY KO mice and WT Mice	Restraint stress (acute)	Stress increased corticosterone levels. In males, this effect was significantly greater in NPY-KO mice.	Food intake decreased after stress and was even further decreased in NPY-KO mice.	N.D.
(L. Wang et al., 2017)	Transgenic mouse (BL6 background)	Chronic activation of the HPA axis by overexpression of CRH Also in combination with fasting (chronic, metabolic)	CRH-OE increased the corticosterone levels. Fasting increases the corticosterone levels to approximately the same levels. The combination of fasting and CRH-OE does not increase the corticosterone levels significantly more than both separate options.	Chronic HPA-activation abolished the sex-differences in body composition. CRH-OE had a lower food intake	Increase in NPY mRNA in fasted WT vs. NF WT, both sexes. Increase in NPY mRNA in NF CRH-OE in females (both in hypothalamus)
(Lin et al., 2015)	Mouse (BL6)	Social overcrowding (chronic, social)	Mice showed an increase in anxiety-like behaviours.	No effect on eating, but large changes	Increase in NPY and NPY1R, but not NPY2R

			Corticosterone levels were approximately doubled in SC mice	in body(fat) composition	
Chicken					
(Ito et al., 2015)	Chicken	Heat stress	Corticosterone levels were comparable at 2 hours of heat stress, but increased after 5 hours of heat stress.	Food intake in the stress group is lower than in the control group.	Diencephalic NPY was significantly increased by stress treatment.

References:

Adrian, T. E., Allen, J. M., Bloom, S. R., Ghatei, M. A., Rossor, M. N., Roberts, G. W., Crow, T. J., Tatemoto, K., & Polak, J. M. (1983). Neuropeptide Y distribution in human brain. *Nature*, *306*(5943), 584–586. https://doi.org/10.1038/306584a0

Ahima, R. S., & Antwi, D. A. (2008). Brain Regulation of Appetite and Satiety. *Endocrinology and Metabolism Clinics of North America*, *37*(4), 811–823. https://doi.org/10.1016/j.ecl.2008.08.005

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders* (Fifth Edition). American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

Ans, A. H., Anjum, I., Satija, V., Inayat, A., Asghar, Z., Akram, I., & Shrestha, B. (2018). Neurohormonal Regulation of Appetite and its Relationship with Stress: A Mini Literature Review. *Cureus*, *10*(7), e3032. https://doi.org/10.7759/cureus.3032

Aoki, M., Watanabe, Y., Yoshimoto, K., Tsujimura, A., Yamamoto, T., Kanamura, N., & Tanaka, M. (2016). Involvement of serotonin 2C receptor RNA editing in accumbal neuropeptide Y expression and behavioural despair. *European Journal of Neuroscience*, *43*(9), 1219–1228. https://doi.org/10.1111/ejn.13233

Aponte, Y., Atasoy, D., & Sternson, S. M. (2011). AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training. *Nature Neuroscience*, *14*(3), 351–355. https://doi.org/10.1038/nn.2739

Ariyasu, H., Takaya, K., Tagami, T., Ogawa, Y., Hosoda, K., Akamizu, T., Suda, M., Koh, T., Natsui, K., Toyooka, S., Shirakami, G., Usui, T., Shimatsu, A., Doi, K., Hosoda, H., Kojima, M., Kangawa, K., & Nakao, K. (2001). Stomach Is a Major Source of Circulating Ghrelin, and Feeding State Determines Plasma Ghrelin-Like Immunoreactivity Levels in Humans. *The Journal of Clinical Endocrinology & Metabolism*, *86*(10), 4753–4758. https://doi.org/10.1210/jcem.86.10.7885

Asarian, L., & Geary, N. (2013). Sex differences in the physiology of eating. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *305*(11), R1215–R1267. https://doi.org/10.1152/ajpregu.00446.2012

Aschbacher, K., Kornfeld, S., Picard, M., Puterman, E., Havel, P. J., Stanhope, K., Lustig, R. H., & Epel, E. (2014). Chronic stress increases vulnerability to diet-related abdominal fat, oxidative stress, and metabolic risk. *Psychoneuroendocrinology*, *46*, 14–22. https://doi.org/10.1016/j.psyneuen.2014.04.003

Bailey, T. W., Hermes, S. M., Andresen, M. C., & Aicher, S. A. (2006). Cranial Visceral Afferent Pathways through the Nucleus of the Solitary Tract to Caudal Ventrolateral Medulla or Paraventricular Hypothalamus: Target-Specific Synaptic Reliability and Convergence Patterns. *Journal of Neuroscience*, *26*(46), 11893–11902. https://doi.org/10.1523/JNEUROSCI.2044-06.2006

Baird, J.-P., Rios, C., Loveland, J. L., Beck, J., Tran, A., & Mahoney, C. E. (2008). Effects of hindbrain melanin-concentrating hormone and neuropeptide Y administration on licking for water, saccharin, and sucrose solutions. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *294*(2), R329–R343. https://doi.org/10.1152/ajpregu.00611.2006

Baker, E., & Lui, F. (2021). Neuroanatomy, Vagal Nerve Nuclei. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK545209/

Bamberger, C. M., Schulte, H. M., & Chrousos, G. P. (1996). Molecular Determinants of Glucocorticoid Receptor Function and Tissue Sensitivity to Glucocorticoids. *Endocrine Reviews*, *17*(3), 245–261. https://doi.org/10.1210/edrv-17-3-245

Bannon, A. W., Seda, J., Carmouche, M., Francis, J. M., Norman, M. H., Karbon, B., & McCaleb, M. L. (2000). Behavioral characterization of neuropeptide Y knockout mice. *Brain Research*, *868*(1), 79–87. https://doi.org/10.1016/S0006-8993(00)02285-X

Beal, M. F., Mazurek, M. F., & Martin, J. B. (1987). A comparison of somatostatin and neuropeptide Y distribution in monkey brain. *Brain Research*, *405*(2), 213–219. https://doi.org/10.1016/0006-8993(87)90290-3

Beck, B. (2006). Neuropeptide Y in normal eating and in genetic and dietary-induced obesity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *361*(1471), 1159–1185. https://doi.org/10.1098/rstb.2006.1855

Bellinger, L. L., & Bernardis, L. L. (2002). The dorsomedial hypothalamic nucleus and its role in ingestive behavior and body weight regulation. *Physiology & Behavior*, *76*(3), 431–442. https://doi.org/10.1016/S0031-9384(02)00756-4

Bernardis, L. L., & Bellinger, L. L. (1996). The lateral hypothalamic area revisited: Ingestive behavior. *Neuroscience and Biobehavioral Reviews*, *20*(2), 189–287. https://doi.org/10.1016/0149-7634(95)00015-1

Bernardis, L. L., Box, B. M., & Stevenson, J. A. F. (1963). Growth Following Hypothalamic Lesions in the Weanling Rat1. *Endocrinology*, *72*(5), 684–692. https://doi.org/10.1210/endo-72-5-684

Berthoud, H.-R., Münzberg, H., & Morrison, C. D. (2017). Blaming the brain for obesity: Integration of hedonic and homeostatic mechanisms. *Gastroenterology*, *152*(7), 1728–1738. https://doi.org/10.1053/j.gastro.2016.12.050

Bewick, G. A., Kent, A., Campbell, D., Patterson, M., Ghatei, M. A., Bloom, S. R., & Gardiner, J. V. (2009). Mice With Hyperghrelinemia Are Hyperphagic and Glucose Intolerant and Have Reduced Leptin Sensitivity. *Diabetes*, *58*(4), 840–846. https://doi.org/10.2337/db08-1428

Bi, S., Kim, Y. J., & Zheng, F. (2012). Dorsomedial hypothalamic NPY and energy balance control. *Neuropeptides*, *46*(6), 309–314. https://doi.org/10.1016/j.npep.2012.09.002

Bi, S., Ladenheim, E. E., Schwartz, G. J., & Moran, T. H. (2001). A role for NPY overexpression in the dorsomedial hypothalamus in hyperphagia and obesity of OLETF rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *281*(1), R254–R260. https://doi.org/10.1152/ajpregu.2001.281.1.R254

Bi, S., Scott, K. A., Kopin, A. S., & Moran, T. H. (2004). Differential Roles for Cholecystokinin A Receptors in Energy Balance in Rats and Mice. *Endocrinology*, *145*(8), 3873–3880. https://doi.org/10.1210/en.2004-0284

Broberger, C., Johansen, J., Johansson, C., Schalling, M., & Hökfelt, T. (1998). The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(25), 15043–15048. https://doi.org/10.1073/pnas.95.25.15043

Broberger, C., Landry, M., Wong, H., Walsh, J. N., & Hökfelt, T. (1997). Subtypes Y1 and Y2 of the Neuropeptide Y Receptor Are Respectively Expressed in Pro-Opiomelanocortin- and Neuropeptide-Y-Containing Neurons of the Rat Hypothalamic Arcuate Nucleus. *Neuroendocrinology*, *66*(6), 393–408. https://doi.org/10.1159/000127265

Bromée, T., Sjödin, P., Fredriksson, R., Boswell, T., Larsson, T. A., Salaneck, E., Zoorob, R., Mohell, N., & Larhammar, D. (2006). Neuropeptide Y-family receptors Y6 and Y7 in chicken. *The FEBS Journal*, *273*(9), 2048–2063. https://doi.org/10.1111/j.1742-4658.2006.05221.x

Cabral, A., Fernandez, G., & Perello, M. (2013). Analysis of brain nuclei accessible to ghrelin present in the cerebrospinal fluid. *Neuroscience*, *253*, 406–415. https://doi.org/10.1016/j.neuroscience.2013.09.008

Cabrele, C., & Beck-Sickinger, A. G. (2000). Molecular characterization of the ligand–receptor interaction of the neuropeptide Y family. *Journal of Peptide Science*, *6*(3), 97–122. https://doi.org/10.1002/(SICI)1099-1387(200003)6:3<97::AID-PSC236>3.0.CO;2-E

Campbell, J. N., Macosko, E. Z., Fenselau, H., Pers, T. H., Lyubetskaya, A., Tenen, D., Goldman, M., Verstegen, A. M. J., Resch, J. M., McCarroll, S. A., Rosen, E. D., Lowell, B. B., & Tsai, L. T. (2017). A molecular census of arcuate hypothalamus and median eminence cell types. *Nature Neuroscience*, *20*(3), 484–496. https://doi.org/10.1038/nn.4495

Carlquist, M., Jörnvall, H., Tatemoto, K., & Mutt, V. (1982). A Porcine Brain Polypeptide Is Identical to the Vasoactive Intestinal Polypeptide. *Gastroenterology*, *83*(1, Part 2), 245–249. https://doi.org/10.1016/0016-5085(82)90182-2

Cedernaes, J., Huang, W., Ramsey, K. M., Waldeck, N., Cheng, L., Marcheva, B., Omura, C., Kobayashi, Y., Peek, C. B., Levine, D. C., Dhir, R., Awatramani, R., Bradfield, C. A., Wang, X. A., Takahashi, J. S., Mokadem, M., Ahima, R. S., & Bass, J. (2019). Transcriptional Basis for Rhythmic Control of Hunger and Metabolism within the AgRP Neuron. *Cell Metabolism*, *29*(5), 1078-1091.e5. https://doi.org/10.1016/j.cmet.2019.01.023

Chao, P.-T., Yang, L., Aja, S., Moran, T. H., & Bi, S. (2011). Knockdown of NPY expression in the dorsomedial hypothalamus promotes development of brown adipocytes and prevents diet-induced obesity. *Cell Metabolism*, *13*(5), 573–583. https://doi.org/10.1016/j.cmet.2011.02.019

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annual Review of Physiology*, *67*, 259–284. https://doi.org/10.1146/annurev.physiol.67.040403.120816

Chigr, F., Rachidi, F., Tardivel, C., Najimi, M., & Moyse, E. (2014). Modulation of orexigenic and anorexigenic peptides gene expression in the rat DVC and hypothalamus by acute immobilization stress. *Frontiers in Cellular Neuroscience*, *8*, 198. https://doi.org/10.3389/fncel.2014.00198

Cohen, H., Liu, T., Kozlovsky, N., Kaplan, Z., Zohar, J., & Mathé, A. A. (2012). The Neuropeptide Y (NPY)-ergic System is Associated with Behavioral Resilience to Stress Exposure in an Animal Model of Post-Traumatic Stress Disorder. *Neuropsychopharmacology*, *37*(2), 350–363. https://doi.org/10.1038/npp.2011.230

Cohen, S., Vainer, E., Matar, M. A., Kozlovsky, N., Kaplan, Z., Zohar, J., Mathé, A. A., & Cohen, H. (2015). Diurnal Fluctuations in HPA and Neuropeptide Y-ergic Systems Underlie Differences in Vulnerability to Traumatic Stress Responses at Different Zeitgeber Times. *Neuropsychopharmacology*, *40*(3), 774–790. https://doi.org/10.1038/npp.2014.257

Comeras, L. B., Herzog, H., & Tasan, R. O. (2019). Neuropeptides at the crossroad of fear and hunger: A special focus on neuropeptide Y. *Annals of the New York Academy of Sciences*, *1455*(1), 59–80. https://doi.org/10.1111/nyas.14179

Corder, K. M., Li, Q., Cortes, M. A., Bartley, A. F., Davis, T. R., & Dobrunz, L. E. (2020). Overexpression of neuropeptide Y decreases responsiveness to neuropeptide Y. *Neuropeptides*, *79*, 101979. https://doi.org/10.1016/j.npep.2019.101979

Cowley, M. A., Smart, J. L., Rubinstein, M., Cerdán, M. G., Diano, S., Horvath, T. L., Cone, R. D., & Low, M. J. (2001). Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature*, *411*(6836), 480–484. https://doi.org/10.1038/35078085

Dallman, M. F. (2010). Stress-induced obesity and the emotional nervous system. *Trends in Endocrinology & Metabolism*, *21*(3), 159–165. https://doi.org/10.1016/j.tem.2009.10.004

Dallman, M. F., Akana, S. F., Scribner, K. A., Bradbury, M. J., Walker, C.-D., Strack, A. M., & Cascio, C. S. (1992). Stress, Feedback and Facilitation in the Hypothalamo-Pituitary-Adrenal Axis. *Journal of Neuroendocrinology*, *4*(5), 517–526. https://doi.org/10.1111/j.1365-2826.1992.tb00200.x

Dallman, M. F., Pecoraro, N., Akana, S. F., Fleur, S. E. la, Gomez, F., Houshyar, H., Bell, M. E., Bhatnagar, S., Laugero, K. D., & Manalo, S. (2003). Chronic stress and obesity: A new view of "comfort food". *Proceedings of the National Academy of Sciences*, *100*(20), 11696–11701. https://doi.org/10.1073/pnas.1934666100

Dallman, M. F., Pecoraro, N. C., & la Fleur, S. E. (2005). Chronic stress and comfort foods: Self-medication and abdominal obesity. *Brain, Behavior, and Immunity*, *19*(4), 275–280. https://doi.org/10.1016/j.bbi.2004.11.004

Daubert, D. L., Looney, B. M., Clifton, R. R., Cho, J. N., & Scheuer, D. A. (2014). Elevated corticosterone in the dorsal hindbrain increases plasma norepinephrine and neuropeptide Y, and recruits a vasopressin response to stress. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *307*(2), R212–R224. https://doi.org/10.1152/ajpregu.00326.2013

de Backer, M. W. A., la Fleur, S. E., & Adan, R. A. H. (2011). Both overexpression of agouti-related peptide or neuropeptide Y in the paraventricular nucleus or lateral hypothalamus induce obesity in a neuropeptide- and nucleus specific manner. *European Journal of Pharmacology*, *660*(1), 148–155. https://doi.org/10.1016/j.ejphar.2010.12.021

De Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joëls, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews*, *19*(3), 269–301. https://doi.org/10.1210/edrv.19.3.0331

de Quidt, M. E., & Emson, P. C. (1986). Distribution of neuropeptide Y-like immunoreactivity in the rat central nervous system—II. Immunohistochemical analysis. *Neuroscience*, *18*(3), 545–618. https://doi.org/10.1016/0306-4522(86)90057-6

Dhabhar, F. S. (2018). The short-term stress response – Mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Frontiers in Neuroendocrinology*, *49*, 175–192. https://doi.org/10.1016/j.yfrne.2018.03.004

DiMicco, J. A., & Zaretsky, D. V. (2007). The dorsomedial hypothalamus: A new player in thermoregulation. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *292*(1), R47–R63. https://doi.org/10.1152/ajpregu.00498.2006

Dimitrov, E. L., DeJoseph, M. R., Brownfield, M. S., & Urban, J. H. (2007). Involvement of Neuropeptide Y Y1 Receptors in the Regulation of Neuroendocrine Corticotropin-Releasing Hormone Neuronal Activity. *Endocrinology*, *148*(8), 3666–3673. https://doi.org/10.1210/en.2006-1730

Dudas, B., Baker, M., Rotoli, G., Grignol, G., Bohn, M. C., & Merchenthaler, I. (2010). Distribution and morphology of the catecholaminergic neural elements in the human hypothalamus. *Neuroscience*, *171*(1), 187–195. https://doi.org/10.1016/j.neuroscience.2010.08.050

Ebeling, P., Koistinen, H. A., & Koivisto, V. A. (1998). Insulin-independent glucose transport regulates insulin sensitivity. *FEBS Letters*, *436*(3), 301–303. https://doi.org/10.1016/S0014-5793(98)01149-1

Elias, C. F., Aschkenasi, C., Lee, C., Kelly, J., Ahima, R. S., Bjorbaek, C., Flier, J. S., Saper, C. B., & Elmquist, J. K. (1999). Leptin differentially regulates NPY and POMC neurons projecting to the lateral hypothalamic area. *Neuron*, *23*(4), 775–786. https://doi.org/10.1016/s0896-6273(01)80035-0

Engström Ruud, L., Pereira, M. M. A., de Solis, A. J., Fenselau, H., & Brüning, J. C. (2020). NPY mediates the rapid feeding and glucose metabolism regulatory functions of AgRP neurons. *Nature Communications*, *11*, 442. https://doi.org/10.1038/s41467-020-14291-3

Erickson, J. C., Clegg, K. E., & Palmiter, R. D. (1996). Sensitivity to leptin and susceptibility to seizures of mice lacking neuropeptide Y. *Nature*, *381*(6581), 415–418. https://doi.org/10.1038/381415a0

Fink, G. (2016). Stress: Concepts, Cognition, Emotion, and Behavior: Handbook of Stress. In *Stress: Concepts, Cognition, Emotion, and Behavior: Handbook of Stress* (p. 487).

Forbes, S., Herzog, H., & Cox, H. (2012). A role for neuropeptide Y in the gender-specific gastrointestinal, corticosterone and feeding responses to stress. *British Journal of Pharmacology*, *166*(8), 2307–2316. https://doi.org/10.1111/j.1476-5381.2012.01939.x

Geerling, J. C., Shin, J.-W., Chimenti, P. C., & Loewy, A. D. (2010). Paraventricular hypothalamic nucleus: Axonal projections to the brainstem. *Journal of Comparative Neurology*, *518*(9), 1460–1499. https://doi.org/10.1002/cne.22283

George, S. A., Khan, S., Briggs, H., & Abelson, J. L. (2010). CRH-stimulated cortisol release and food intake in healthy, non-obese adults. *Psychoneuroendocrinology*, *35*(4), 607–612.

https://doi.org/10.1016/j.psyneuen.2009.09.017

Glaser, R., & Kiecolt-Glaser, J. K. (2005). Stress-induced immune dysfunction: Implications for health. *Nature Reviews Immunology*, *5*(3), 243–251. https://doi.org/10.1038/nri1571

Goebel-Stengel, M., Stengel, A., Wang, L., & Taché, Y. (2014). Orexigenic response to tail pinch: Role of brain NPY1 and corticotropin releasing factor receptors. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *306*(3), R164–R174. https://doi.org/10.1152/ajpregu.00335.2013

Grill, H. J., & Hayes, M. R. (2012). Hindbrain Neurons as an Essential Hub in the Neuroanatomically Distributed Control of Energy Balance. *Cell Metabolism*, *16*(3), 296–309. https://doi.org/10.1016/j.cmet.2012.06.015

Gumbs, M. C. R., Eggels, L., Kool, T., Unmehopa, U. A., van den Heuvel, J. K., Lamuadni, K., Mul, J. D., & la Fleur, S. E. (2020). Neuropeptide Y Signaling in the Lateral Hypothalamus Modulates Diet Component Selection and is Dysregulated in a Model of Diet-Induced Obesity. *Neuroscience*, *447*, 28–40. https://doi.org/10.1016/j.neuroscience.2019.12.014

Gumbs, M. C. R., Vuuregge, A. H., Eggels, L., Unmehopa, U. A., Lamuadni, K., Mul, J. D., & la Fleur, S. E. (2019). Afferent neuropeptide Y projections to the ventral tegmental area in normal-weight male Wistar rats. *The Journal of Comparative Neurology*, *527*(16), 2659–2674. https://doi.org/10.1002/cne.24698

Hajnal, A., Sándor, P., Jandö, G., Vida, I., Czurkó, A., Karádi, Z., & Lénárd, L. (1992). Feeding disturbances and EEG activity changes after amygdaloid kainate lesions in the rat. *Brain Research Bulletin*, *29*(6), 909–916. https://doi.org/10.1016/0361-9230(92)90165-T

Hardaway, J. A., Crowley, N. A., Bulik, C. M., & Kash, T. L. (2015). Integrated circuits and molecular components for stress and feeding: Implications for eating disorders. *Genes, Brain, and Behavior*, *14*(1), 85–97. https://doi.org/10.1111/gbb.12185

Harris, R. B. S. (2015). Chronic and acute effects of stress on energy balance: Are there appropriate animal models? *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *308*(4), R250–R265. https://doi.org/10.1152/ajpregu.00361.2014

Heilig, M., Söderpalm, B., Engel, J. A., & Widerlöv, E. (1989). Centrally administered neuropeptide Y (NPY) produces anxiolytic-like effects in animal anxiety models. *Psychopharmacology*, *98*(4), 524–529. https://doi.org/10.1007/BF00441953

Herman, J. P. (2011). Central Nervous System Regulation of the Hypothalamic–Pituitary–Adrenal Axis Stress Response. In *The Handbook of Stress* (pp. 29–46). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118083222.ch2

Hermes, S. M., Mitchell, J. L., & Aicher, S. A. (2006). Most neurons in the nucleus tractus solitarii do not send collateral projections to multiple autonomic targets in the rat brain. *Experimental Neurology*, *198*(2), 539–551. https://doi.org/10.1016/j.expneurol.2005.12.028

Herzog, H., Hort, Y. J., Shine, J., & Selbie, L. A. (1993). Molecular Cloning, Characterization, and Localization of the Human Homolog to the Reported Bovine NPY Y3 Receptor: Lack of NPY Binding and Activation. *DNA and Cell Biology*, *12*(6), 465–471. https://doi.org/10.1089/dna.1993.12.465

Hu, M. H., Bashir, Z., Li, X. F., & O'Byrne, K. T. (2016). Posterodorsal Medial Amygdala Mediates Tail-Pinch Induced Food Intake in Female Rats. *Journal of Neuroendocrinology*, *28*(5), n/a. https://doi.org/10.1111/jne.12390

Ip, C. K., Zhang, L., Farzi, A., Qi, Y., Clarke, I., Reed, F., Shi, Y.-C., Enriquez, R., Dayas, C., Graham, B., Begg, D., Brüning, J. C., Lee, N. J., Hernandez-Sanchez, D., Gopalasingam, G., Koller, J., Tasan, R., Sperk, G., & Herzog, H. (2019). Amygdala NPY Circuits Promote the Development of Accelerated Obesity under Chronic Stress Conditions. *Cell Metabolism*, *30*(1), 111-128.e6. https://doi.org/10.1016/j.cmet.2019.04.001

Ito, K., Bahry, M. A., Hui, Y., Furuse, M., & Chowdhury, V. S. (2015). Acute heat stress up-regulates neuropeptide Y precursor mRNA expression and alters brain and plasma concentrations of free amino acids in chicks. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *187*, 13–19. https://doi.org/10.1016/j.cbpa.2015.04.010

Jennings, J. H., Rizzi, G., Stamatakis, A. M., Ung, R. L., & Stuber, G. D. (2013). The Inhibitory Circuit Architecture of the Lateral Hypothalamus Orchestrates Feeding. *Science*, *341*(6153), 1517–1521. https://doi.org/10.1126/science.1241812

Kamegai, J., Tamura, H., Shimizu, T., Ishii, S., Sugihara, H., & Wakabayashi, I. (2001). Chronic Central Infusion of Ghrelin Increases Hypothalamic Neuropeptide Y and Agouti-Related Protein mRNA Levels and Body Weight in Rats. *Diabetes*, *50*(11), 2438–2443. https://doi.org/10.2337/diabetes.50.11.2438

Kask, A., Rägo, L., & Harro, J. (1998). Anxiolytic-like effect of neuropeptide Y (NPY) and NPY13–36 microinjected into vicinity of locus coeruleus in rats. *Brain Research*, *788*(1), 345–348. https://doi.org/10.1016/S0006-8993(98)00076-6

Kawaguchi, Y., Wilson, C. J., Augood, S. J., & Emson, P. C. (1995). Striatal interneurones: Chemical, physiological and morphological characterization. *Trends in Neurosciences*, *18*(12), 527–535. https://doi.org/10.1016/0166-2236(95)98374-8

Kim, J. E., Dager, S. R., & Lyoo, I. K. (2012). The role of the amygdala in the pathophysiology of panic disorder: Evidence from neuroimaging studies. *Biology of Mood & Anxiety Disorders*, *2*(1), 20. https://doi.org/10.1186/2045-5380-2-20

Kim, M. S., Luo, S., Azad, A., Campbell, C. E., Felix, K., Cabeen, R. P., Belcher, B. R., Kim, R., Serrano-Gonzalez, M., & Herting, M. M. (2020). Prefrontal Cortex and Amygdala Subregion Morphology Are Associated With Obesity and Dietary Self-control in Children and Adolescents. *Frontiers in Human Neuroscience*, *14*, 563415. https://doi.org/10.3389/fnhum.2020.563415

Kim, M. S., Rossi, M., Abusnana, S., Sunter, D., Morgan, D. G., Small, C. J., Edwards, C. M., Heath, M. M., Stanley, S. A., Seal, L. J., Bhatti, J. R., Smith, D. M., Ghatei, M. A., & Bloom, S. R. (2000). Hypothalamic localization of the feeding effect of agouti-related peptide and alpha-melanocyte-stimulating hormone. *Diabetes*, *49*(2), 177–182. https://doi.org/10.2337/diabetes.49.2.177

Kim, Y. J., & Bi, S. (2016). Knockdown of neuropeptide Y in the dorsomedial hypothalamus reverses high-fat diet-induced obesity and impaired glucose tolerance in rats. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, *310*(2), R134–R142. https://doi.org/10.1152/ajpregu.00174.2015

King, P. J., Williams, G., Doods, H., & Widdowson, P. S. (2000). Effect of a selective neuropeptide Y Y2 receptor antagonist, BIIE0246 on neuropeptide Y release. *European Journal of Pharmacology*, *396*(1), R1–R3. https://doi.org/10.1016/S0014-2999(00)00230-2

Kolber, B. J., Wieczorek, L., & Muglia, L. J. (2008). Hypothalamic–pituitary–adrenal axis dysregulation and behavioral analysis of mouse mutants with altered glucocorticoid or mineralocorticoid receptor function. *Stress*, *11*(5), 321–338. https://doi.org/10.1080/10253890701821081

Kornhuber, J., & Zoicas, I. (2021). Brain Region-Dependent Effects of Neuropeptide Y on Conditioned Social Fear and Anxiety-Like Behavior in Male Mice. *International Journal of Molecular Sciences*, *22*(7), 3695. https://doi.org/10.3390/ijms22073695

Krolow, R., Noschang, C., Arcego, D. M., Huffell, A. P., Marcolin, M. L., Benitz, A. N., Lampert, C., Fitarelli, R. D., & Dalmaz, C. (2013). Sex-specific effects of isolation stress and consumption of palatable diet during the prepubertal period on metabolic parameters. *Metabolism*, *62*(9), 1268–1278. https://doi.org/10.1016/j.metabol.2013.04.009

Kuo, L. E., Kitlinska, J. B., Tilan, J. U., Li, L., Baker, S. B., Johnson, M. D., Lee, E. W., Burnett, M. S., Fricke, S. T., Kvetnansky, R., Herzog, H., & Zukowska, Z. (2007). Neuropeptide Y acts directly in the periphery on fat tissue and mediates stress-induced obesity and metabolic syndrome. *Nature Medicine*, *13*(7), 803–811. https://doi.org/10.1038/nm1611

la Fleur, S. E., van Rozen, A. J., Luijendijk, M. C. M., Groeneweg, F., & Adan, R. a. H. (2010). A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia. *International Journal of Obesity*, *34*(3), 537–546. https://doi.org/10.1038/ijo.2009.257

Lach, G., & de Lima, T. C. M. (2013). Role of NPY Y1 receptor on acquisition, consolidation and extinction on contextual fear conditioning: Dissociation between anxiety, locomotion and non-emotional memory behavior. *Neurobiology of Learning and Memory*, *103*, 26–33. https://doi.org/10.1016/j.nlm.2013.04.005

Larhammar, D. (1996). Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. *Regulatory Peptides*, *65*(3), 165–174. https://doi.org/10.1016/0167-0115(96)00110-3

Larhammar, D., Söderberg, C., & Blomquist, A. G. (1993). Evolution of the Neuropeptide Y Family of Peptides. In W. F. Colmers & C. Wahlestedt (Eds.), *The Biology of Neuropeptide Y and Related Peptides* (pp. 1–41). Humana Press. https://doi.org/10.1007/978-1-59259-465-8_1

le Roux, C. W., & Bloom, S. R. (2005). Why do patients lose weight after Roux-en-Y gastric bypass? *The Journal of Clinical Endocrinology and Metabolism*, *90*(1), 591–592. https://doi.org/10.1210/jc.2004-2211

Lee, S. J., Kirigiti, M., Lindsley, S. R., Loche, A., Madden, C. J., Morrison, S. F., Smith, M. S., & Grove, K. L. (2013). Efferent projections of NPY expressing neurons of the dorsomedial hypothalamus in chronic hyperphagic models. *The Journal of Comparative Neurology*, *521*(8), 1891–1914. https://doi.org/10.1002/cne.23265

Leibowitz, S. F., Hammer, N. J., & Chang, K. (1981). Hypothalamic paraventricular nucleus lesions produce overeating and obesity in the rat. *Physiology & Behavior*, *27*(6), 1031–1040. https://doi.org/10.1016/0031-9384(81)90366-8

Li, C., Chen, P., & Smith, M. S. (2000). Corticotropin releasing hormone neurons in the paraventricular nucleus are direct targets for neuropeptide Y neurons in the arcuate nucleus: An anterograde tracing study. *Brain Research*, *854*(1–2), 122–129. https://doi.org/10.1016/S0006-8993(99)02324-0

Li, J. X., Yoshida, T., Monk, K. J., & Katz, D. B. (2013). Lateral Hypothalamus Contains Two Types of Palatability-Related Taste Responses with Distinct Dynamics. *Journal of Neuroscience*, *33*(22), 9462–9473. https://doi.org/10.1523/JNEUROSCI.3935-12.2013

Lin, E.-J. D., Lin, S., Aljanova, A., During, M. J., & Herzog, H. (2010). Adult-onset hippocampal-specific neuropeptide Y overexpression confers mild

anxiolytic effect in mice. *European Neuropsychopharmacology*, *20*(3), 164–175. https://doi.org/10.1016/j.euroneuro.2009.08.004

Lin, E.-J. D., Sun, M., Choi, E. Y., Magee, D., Stets, C. W., & During, M. J. (2015). Social overcrowding as a chronic stress model that increases adiposity in mice. *Psychoneuroendocrinology*, *51*, 318–330. https://doi.org/10.1016/j.psyneuen.2014.10.007

Liu, C. M., & Kanoski, S. E. (2018). Homeostatic and non-homeostatic controls of feeding behavior: Distinct vs. common neural systems. *Physiology & Behavior*, *193*(Pt B), 223–231. https://doi.org/10.1016/j.physbeh.2018.02.011

Loh, K., Zhang, L., Brandon, A., Wang, Q., Begg, D., Qi, Y., Fu, M., Kulkarni, R., Teo, J., Baldock, P., Brüning, J. C., Cooney, G., Neely, G. G., & Herzog, H. (2017). Insulin controls food intake and energy balance via NPY neurons. *Molecular Metabolism*, *6*(6), 574–584. https://doi.org/10.1016/j.molmet.2017.03.013

Long, M., Zhou, J., Li, D., Zheng, L., Xu, Z., & Zhou, S. (2015). Long-Term Over-Expression of Neuropeptide Y in Hypothalamic Paraventricular Nucleus Contributes to Adipose Tissue Insulin Resistance Partly via the Y5 Receptor. *PLOS ONE*, *10*(5), e0126714. https://doi.org/10.1371/journal.pone.0126714

Luquet, S., Perez, F. A., Hnasko, T. S., & Palmiter, R. D. (2005). NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science (New York, N.Y.)*, *310*(5748), 683–685. https://doi.org/10.1126/science.1115524

Makino, S., Baker, R. A., Smith, M. A., & Gold, P. W. (2000). Differential regulation of neuropeptide Y mRNA expression in the arcuate nucleus and locus coeruleus by stress and antidepressants. *Journal of Neuroendocrinology*, *12*(5), 387–395. https://doi.org/10.1046/j.1365-2826.2000.00451.x

Maldonado-Irizarry, C. S., Swanson, C. J., & Kelley, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *Journal of Neuroscience*, *15*(10), 6779–6788. https://doi.org/10.1523/JNEUROSCI.15-10-06779.1995

Mani, B. K., & Zigman, J. M. (2017). Ghrelin as a Survival Hormone. *Trends in Endocrinology and Metabolism: TEM*, *28*(12), 843–854. https://doi.org/10.1016/j.tem.2017.10.001

Maniam, J., & Morris, M. J. (2012). The link between stress and feeding behaviour. *Neuropharmacology*, *63*(1), 97–110. https://doi.org/10.1016/j.neuropharm.2012.04.017

Martijena, I. d., Rodríguez Manzanares, P. a., Lacerra, C., & Molina, V. a. (2002). Gabaergic modulation of the stress response in frontal cortex and amygdala. *Synapse*, *45*(2), 86–94. https://doi.org/10.1002/syn.10085

Martín-Pérez, C., Contreras-Rodríguez, O., Verdejo-Román, J., Vilar-López, R., González-Pérez, R., & Verdejo-García, A. (2020). Stressing diets? Amygdala networks, cumulative cortisol, and weight loss in adolescents with excess weight. *International Journal of Obesity*, *44*(10), 2001–2010. https://doi.org/10.1038/s41366-020-0633-4

Masodkar, K., Johnson, J., & Peterson, M. J. (2016). A Review of Posttraumatic Stress Disorder and Obesity: Exploring the Link. *The Primary Care Companion for CNS Disorders*, *18*(1), 22710. https://doi.org/10.4088/PCC.15r01848

Mason, A. E., Schleicher, S., Coccia, M., Epel, E. S., & Aschbacher, K. (2018). Chronic stress and impulsive risk-taking predict increases in visceral fat over 18 months. *Obesity (Silver Spring, Md.)*, *26*(5), 869–876. https://doi.org/10.1002/oby.22150

McDougall, S. J., Widdop, R. E., & Lawrence, A. J. (2005). Differential gene expression in WKY and SHR brain following acute and chronic air-puff stress. *Brain Research. Molecular Brain Research*, *133*(2), 329–336. https://doi.org/10.1016/j.molbrainres.2004.10.010

McGuire, J. L., Larke, L. E., Sallee, F. R., Herman, J. P., & Sah, R. (2011). Differential Regulation of Neuropeptide Y in the Amygdala and Prefrontal Cortex during Recovery from Chronic Variable Stress. *Frontiers in Behavioral Neuroscience*, *5*, 54. https://doi.org/10.3389/fnbeh.2011.00054

Melhorn, S. J., Krause, E. G., Scott, K. A., Mooney, M. R., Johnson, J. D., Woods, S. C., & Sakai, R. R. (2010). Meal patterns and hypothalamic NPY expression during chronic social stress and recovery. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *299*(3), R813-822. https://doi.org/10.1152/ajpregu.00820.2009

Mercer, R. E., Chee, M. J. S., & Colmers, W. F. (2011). The role of NPY in hypothalamic mediated food intake. *Frontiers in Neuroendocrinology*, *32*(4), 398–415. https://doi.org/10.1016/j.yfrne.2011.06.001

Michaelson, S. D., Miranda Tapia, A. P., McKinty, A., Silveira Villarroel, H., Mackay, J. P., Urban, J. H., & Colmers, W. F. (2020). Contribution of NPY Y5 Receptors to the Reversible Structural Remodeling of Basolateral Amygdala Dendrites in Male Rats Associated with NPY-Mediated Stress Resilience. *The Journal of Neuroscience*, *40*(16), 3231–3249. https://doi.org/10.1523/JNEUROSCI.2621-19.2020

Mitchell, K. S., Mazzeo, S. E., Schlesinger, M. R., Brewerton, T. D., & Smith, B. N. (2012). Comorbidity of Partial and Subthreshold PTSD among Men and Women with Eating Disorders in the National Comorbidity Survey-Replication Study. *The International Journal of Eating Disorders*, *45*(3), 307–315. https://doi.org/10.1002/eat.20965

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nature Reviews Neuroscience*, *18*(2), 73–85. https://doi.org/10.1038/nrn.2016.165

Morris, B. J. (1989). Neuronal localisation of neuropeptide Y gene expression in rat brain. *The Journal of Comparative Neurology*, *290*(3), 358–368. https://doi.org/10.1002/cne.902900305

Morrison, S. F., & Nakamura, K. (2011). Central neural pathways for thermoregulation. *Frontiers in Bioscience (Landmark Edition)*, *16*, 74–104. https://doi.org/10.2741/3677

Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G., & Dietz, W. H. (1999). The Disease Burden Associated With Overweight and Obesity. *JAMA*, *282*(16), 1523–1529. https://doi.org/10.1001/jama.282.16.1523

Nilsson, I., Johansen, J. E., Schalling, M., Hökfelt, T., & Fetissov, S. O. (2005). Maturation of the hypothalamic arcuate agouti-related protein system during postnatal development in the mouse. *Brain Research. Developmental Brain Research*, *155*(2), 147–154. https://doi.org/10.1016/j.devbrainres.2005.01.009

Nygaard, R., Nielbo, S., Schwartz, T. W., & Poulsen, F. M. (2006). The PP-fold solution structure of human polypeptide YY and human PYY3-36 as determined by NMR. *Biochemistry*, *45*(27), 8350–8357. https://doi.org/10.1021/bi060359l

Obici, S., Feng, Z., Karkanias, G., Baskin, D. G., & Rossetti, L. (2002). Decreasing hypothalamic insulin receptors causes hyperphagia and insulin resistance in rats. *Nature Neuroscience*, *5*(6), 566–572. https://doi.org/10.1038/nn0602-861

Oliver, G., Wardle, J., & Gibson, E. L. (2000). Stress and food choice: A laboratory study. *Psychosomatic Medicine*, *62*(6), 853–865. https://doi.org/10.1097/00006842-200011000-00016

Ono, H. (2019). Molecular Mechanisms of Hypothalamic Insulin Resistance. *International Journal of Molecular Sciences*, *20*(6), 1317. https://doi.org/10.3390/ijms20061317

Paeger, L., Karakasilioti, I., Altmüller, J., Frommolt, P., Brüning, J., & Kloppenburg, P. (2017). Antagonistic modulation of NPY/AgRP and POMC neurons in the arcuate nucleus by noradrenalin. *ELife*, *6*, e25770. https://doi.org/10.7554/eLife.25770

Pandit, R., Luijendijk, M. C. M., Vanderschuren, L. J. M. J., la Fleur, S. E., & Adan, R. A. H. (2014). Limbic substrates of the effects of neuropeptide Y on intake of and motivation for palatable food. *Obesity*, *22*(5), 1216–1219. https://doi.org/10.1002/oby.20718

Pardini, A. W., Nguyen, H. T., Figlewicz, D. P., Baskin, D. G., Williams, D. L., Kim, F., & Schwartz, M. W. (2006). Distribution of insulin receptor substrate-2 in brain areas involved in energy homeostasis. *Brain Research*, *1112*(1), 169–178. https://doi.org/10.1016/j.brainres.2006.06.109

Park, H.-K., & Ahima, R. S. (2015). Physiology of leptin: Energy homeostasis, neuroendocrine function and metabolism. *Metabolism: Clinical and Experimental*, *64*(1), 24–34. https://doi.org/10.1016/j.metabol.2014.08.004

Pecoraro, N., Reyes, F., Gomez, F., Bhargava, A., & Dallman, M. F. (2004). Chronic Stress Promotes Palatable Feeding, which Reduces Signs of Stress: Feedforward and Feedback Effects of Chronic Stress. *Endocrinology*, *145*(8), 3754–3762. https://doi.org/10.1210/en.2004-0305

Petrovich, G. D., Setlow, B., Holland, P. C., & Gallagher, M. (2002). Amygdalo-Hypothalamic Circuit Allows Learned Cues to Override Satiety and Promote Eating. *The Journal of Neuroscience*, *22*(19), 8748–8753. https://doi.org/10.1523/JNEUROSCI.22-19-08748.2002

Pomonis, J. D., Levine, A. S., & Billington, C. J. (1997). Interaction of the Hypothalamic Paraventricular Nucleus and Central Nucleus of the Amygdala in Naloxone Blockade of Neuropeptide Y-Induced Feeding Revealed by c-fos Expression. *The Journal of Neuroscience*, *17*(13), 5175–5182. https://doi.org/10.1523/JNEUROSCI.17-13-05175.1997

Prechtl, J. C., & Powley, T. L. (1990). The fiber composition of the abdominal vagus of the rat. *Anatomy and Embryology*, *181*(2), 101–115. https://doi.org/10.1007/BF00198950

Qi, D., & Rodrigues, B. (2007). Glucocorticoids produce whole body insulin resistance with changes in cardiac metabolism. *American Journal of Physiology. Endocrinology and Metabolism*, *292*(3), E654-667. https://doi.org/10.1152/ajpendo.00453.2006

Qiu, J., Rivera, H. M., Bosch, M. A., Padilla, S. L., Stincic, T. L., Palmiter, R. D., Kelly, M. J., & Rønnekleiv, O. K. (2018). Estrogenic-dependent glutamatergic neurotransmission from kisspeptin neurons governs feeding circuits in females. *ELife*, *7*, e35656. https://doi.org/10.7554/eLife.35656

Reichmann, F., Hassan, A. M., Farzi, A., Jain, P., Schuligoi, R., & Holzer, P. (2015). Dextran sulfate sodium-induced colitis alters stress-associated behaviour and neuropeptide gene expression in the amygdala-hippocampus network of mice. *Scientific Reports*, *5*, 9970. https://doi.org/10.1038/srep09970

Reichmann, F., & Holzer, P. (2016). Neuropeptide Y: A stressful review. *Neuropeptides*, *55*, 99–109. https://doi.org/10.1016/j.npep.2015.09.008

Ritter, S., Bugarith, K., & Dinh, T. T. (2001). Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *The Journal of Comparative Neurology*, *432*(2), 197–216. https://doi.org/10.1002/cne.1097

Rivier, C., & Vale, W. (1983). Interaction of corticotropin-releasing factor and arginine vasopressin on adrenocorticotropin secretion in vivo.

Endocrinology, *113*(3), 939–942. https://doi.org/10.1210/endo-113-3-939

Rossi, M. A., & Stuber, G. D. (2018). Overlapping Brain Circuits for Homeostatic and Hedonic Feeding. *Cell Metabolism*, *27*(1), 42–56. https://doi.org/10.1016/j.cmet.2017.09.021

Sah, R., Ekhator, N. N., Jefferson-Wilson, L., Horn, P. S., & Geracioti, T. D. (2014). Cerebrospinal fluid neuropeptide Y in combat veterans with and without posttraumatic stress disorder. *Psychoneuroendocrinology*, *40*, 277–283. https://doi.org/10.1016/j.psyneuen.2013.10.017

Sajdyk, T. J., Schober, D. A., Smiley, D. L., & Gehlert, D. R. (2002). Neuropeptide Y-Y2 receptors mediate anxiety in the amygdala. *Pharmacology, Biochemistry, and Behavior*, *71*(3), 419–423. https://doi.org/10.1016/s0091-3057(01)00679-7

Salin, P., Kerkerian, L., & Nieoullon, A. (1990). Expression of neuropeptide Y immunoreactivity in the rat nucleus accumbens is under the influence of the dopaminergic mesencephalic pathway. *Experimental Brain Research*, *81*(2), 363–371. https://doi.org/10.1007/BF00228127

Sasaguri, K., Yamada, K., & Yamamoto, T. (2018). Uncovering the neural circuitry involved in the stress-attenuation effects of chewing. *The Japanese Dental Science Review*, *54*(3), 118–126. https://doi.org/10.1016/j.jdsr.2018.03.002

Savontaus, E., Conwell, I. M., & Wardlaw, S. L. (2002). Effects of adrenalectomy on AGRP, POMC, NPY and CART gene expression in the basal hypothalamus of fed and fasted rats. *Brain Research*, *958*(1), 130–138. https://doi.org/10.1016/S0006-8993(02)03674-0

Schwartz, G. J. (2000). The role of gastrointestinal vagal afferents in the control of food intake: Current prospects. *Nutrition (Burbank, Los Angeles County, Calif.)*, *16*(10), 866–873. https://doi.org/10.1016/s0899-9007(00)00464-0

Schwartz, M. W., Marks, J. L., Sipols, A. J., Baskin, D. G., Woods, S. C., Kahn, S. E., & Porte, D. (1991). Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (Fa/Fa) but not obese (fa/fa) Zucker rats. *Endocrinology*, *128*(5), 2645–2647. https://doi.org/10.1210/endo-128-5-2645

Shi, Y.-C., Lau, J., Lin, Z., Zhang, H., Zhai, L., Sperk, G., Heilbronn, R., Mietzsch, M., Weger, S., Huang, X.-F., Enriquez, R. F., Castillo, L., Baldock, P. A., Zhang, L., Sainsbury, A., Herzog, H., & Lin, S. (2013). Arcuate NPY Controls Sympathetic Output and BAT Function via a Relay of Tyrosine Hydroxylase Neurons in the PVN. *Cell Metabolism*, *17*(2), 236–248. https://doi.org/10.1016/j.cmet.2013.01.006

Silveira Villarroel, H., Bompolaki, M., Mackay, J. P., Miranda Tapia, A. P., Michaelson, S. D., Leitermann, R. J., Marr, R. A., Urban, J. H., & Colmers, W. F. (2018). NPY Induces Stress Resilience via Downregulation of Ih in Principal Neurons of Rat Basolateral Amygdala. *The Journal of Neuroscience*, *38*(19), 4505–4520. https://doi.org/10.1523/JNEUROSCI.3528-17.2018

Singh, C., Rihel, J., & Prober, D. A. (2017). Neuropeptide Y regulates sleep by modulating noradrenergic signaling. *Current Biology : CB*, *27*(24), 3796-3811.e5. https://doi.org/10.1016/j.cub.2017.11.018

Sinha, R., & Jastreboff, A. M. (2013). Stress as a common risk factor for obesity and addiction. *Biological Psychiatry*, *73*(9), 827–835. https://doi.org/10.1016/j.biopsych.2013.01.032

Smith, G. P., Jerome, C., & Norgren, R. (1985). Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *The American Journal of Physiology*, *249*(5 Pt 2), R638-641. https://doi.org/10.1152/ajpregu.1985.249.5.R638

Smith, S. M., & Vale, W. W. (2006). The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*, *8*(4), 383–395.

Sominsky, L., & Spencer, S. J. (2014). Eating behavior and stress: A pathway to obesity. *Frontiers in Psychology*, *5*, 434. https://doi.org/10.3389/fpsyg.2014.00434

Sørensen, G., Jensen, M., Weikop, P., Dencker, D., Christiansen, S. H., Loland, C. J., Bengtsen, C. H., Petersen, J. H., Fink-Jensen, A., Wörtwein, G., & Woldbye, D. P. D. (2012). Neuropeptide Y Y5 receptor antagonism attenuates cocaine-induced effects in mice. *Psychopharmacology*, *222*(4), 565–577. https://doi.org/10.1007/s00213-012-2651-y

Sørensen, G., Wegener, G., Hasselstrøm, J., Hansen, T. V. O., Wörtwein, G., Fink-Jensen, A., & Woldbye, D. P. D. (2009). Neuropeptide Y infusion into the shell region of the rat nucleus accumbens increases extracellular levels of dopamine. *NeuroReport*, *20*(11), 1023–1026. https://doi.org/10.1097/WNR.0b013e32832d4848

Stanley, B. G., Chin, A. S., & Leibowitz, S. F. (1985). Feeding and drinking elicited by central injection of neuropeptide Y: Evidence for a hypothalamic site(s) of action. *Brain Research Bulletin*, *14*(6), 521–524. https://doi.org/10.1016/0361-9230(85)90100-5

Stratford, T. R., Swanson, C. J., & Kelley, A. (1998). Specific changes in food intake elicited by blockade or activation of glutamate receptors in the nucleus accumbens shell. *Behavioural Brain Research*, *93*(1), 43–50. https://doi.org/10.1016/S0166-4328(97)00140-X

Stratford, T. R., & Wirtshafter, D. (2004). NPY mediates the feeding elicited by muscimol injections into the nucleus accumbens shell. *Neuroreport*, *15*(17), 2673–2676.

https://doi.org/10.1097/00001756-200412030-00024

Stuber, G. D., & Wise, R. A. (2016). Lateral hypothalamic circuits for feeding and reward. *Nature Neuroscience*, *19*(2), 198–205. https://doi.org/10.1038/nn.4220

Suda, T., Tozawa, F., Iwai, I., Sato, Y., Sumitomo, T., Nakano, Y., Yamada, M., & Demura, H. (1993). Neuropeptide Y increases the corticotropin-releasing factor messenger ribonucleic acid level in the rat hypothalamus. *Brain Research. Molecular Brain Research*, *18*(4), 311–315. https://doi.org/10.1016/0169-328x(93)90094-6

Sweerts, B. W., Jarrott, B., & Lawrence, A. J. (2001). The effect of acute and chronic restraint on the central expression of prepro-neuropeptide Y mRNA in normotensive and hypertensive rats. *Journal of Neuroendocrinology*, *13*(7), 608–617. https://doi.org/10.1046/j.1365-2826.2001.00674.x

Tanaka, M., Yamada, S., & Watanabe, Y. (2021). The Role of Neuropeptide Y in the Nucleus Accumbens. *International Journal of Molecular Sciences*, *22*(14), 7287. https://doi.org/10.3390/ijms22147287

Tataranni, P. A., Larson, D. E., Snitker, S., Young, J. B., Flatt, J. P., & Ravussin, E. (1996). Effects of glucocorticoids on energy metabolism and food intake in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *271*(2), E317–E325. https://doi.org/10.1152/ajpendo.1996.271.2.E317

Taylor, K., Lester, E., Hudson, B., & Ritter, S. (2007). Hypothalamic and Hindbrain NPY, AGRP and NE Increase Consummatory Feeding Responses. *Physiology & Behavior*, *90*(5), 744–750. https://doi.org/10.1016/j.physbeh.2006.12.014

Tempel, D. L., & Leibowitz, S. F. (1993). Glucocorticoid receptors in PVN: Interactions with NE, NPY, and Gal in relation to feeding. *American Journal of Physiology-Endocrinology and Metabolism*, *265*(5), E794–E800. https://doi.org/10.1152/ajpendo.1993.265.5.E794

Tiesjema, B., Adan, R. A. H., Luijendijk, M. C. M., Kalsbeek, A., & la Fleur, S. E. (2007). Differential Effects of Recombinant Adeno-Associated Virus-Mediated Neuropeptide Y Overexpression in the Hypothalamic Paraventricular Nucleus and Lateral Hypothalamus on Feeding Behavior. *Journal of Neuroscience*, *27*(51), 14139–14146. https://doi.org/10.1523/JNEUROSCI.3280-07.2007

Treece, B. R., Covasa, M., Ritter, R. C., & Burns, G. A. (1998). Delay in meal termination follows blockade of N-methyl-d-aspartate receptors in the dorsal hindbrain. *Brain Research*, *810*(1), 34–40. https://doi.org/10.1016/S0006-8993(98)00867-1

Trotta, M., Bello, E. P., Alsina, R., Tavella, M. B., Ferrán, J. L., Rubinstein, M., & Bumaschny, V. F. (2020). Hypothalamic Pomc expression restricted to GABAergic neurons suppresses Npy overexpression and restores food intake in obese mice. *Molecular Metabolism*, *37*, 100985. https://doi.org/10.1016/j.molmet.2020.100985

Turner, A., Keating, C., & Tilbrook, A. J. (2012). *Sex Differences and the Role of Sex Steroids in Sympatho-Adrenal Medullary System and Hypothalamo-Pituitary Adrenal Axis Responses to Stress*. https://doi.org/10.5772/25611

van den Heuvel, J. K., Furman, K., Gumbs, M. C. R., Eggels, L., Opland, D. M., Land, B. B., Kolk, S. M., S. Narayanan, N., Fliers, E., Kalsbeek, A., DiLeone, R. J., & la Fleur, S. E. (2015). Neuropeptide Y Activity in the Nucleus Accumbens Modulates Feeding Behavior and Neuronal Activity. *Biological Psychiatry*, *77*(7), 633–641. https://doi.org/10.1016/j.biopsych.2014.06.008

van Dijk, G., & Strubbe, J. H. (2003). Time-dependent effects of neuropeptide Y infusion in the paraventricular hypothalamus on ingestive and associated behaviors in rats. *Physiology & Behavior*, *79*(4), 575–580. https://doi.org/10.1016/S0031-9384(03)00125-2

Vanzhula, I. A., Calebs, B., Fewell, L., & Levinson, C. A. (2019). Illness pathways between eating disorder and post-traumatic stress disorder symptoms: Understanding comorbidity with network analysis. *European Eating Disorders Review*, *27*(2), 147–160. https://doi.org/10.1002/erv.2634

Wahlestedt, C., & Reis, D. J. (1993). Neuropeptide Y-related peptides and their receptors—Are the receptors potential therapeutic drug targets? *Annual Review of Pharmacology and Toxicology*, *33*, 309–352. https://doi.org/10.1146/annurev.pa.33.040193.001521

Wahlestedt, C., Skagerberg, G., Ekman, R., Heilig, M., Sundler, F., & Håkanson, R. (1987). Neuropeptide Y (NPY) in the area of the hypothalamic paraventricular nucleus activates the pituitary-adrenocortical axis in the rat. *Brain Research*, *417*(1), 33–38. https://doi.org/10.1016/0006-8993(87)90176-4

Wang, L., Goebel-Stengel, M., Yuan, P.-Q., Stengel, A., & Taché, Y. (2017). Corticotropin-releasing factor overexpression in mice abrogates sex differences in body weight, visceral fat, and food intake response to a fast and alters levels of feeding regulatory hormones. *Biology of Sex Differences*, *8*(1), 2. https://doi.org/10.1186/s13293-016-0122-6

Wang, S., Chen, J., Yue, G., Bai, M., Kou, M., & Jin, Z. (2013). Neuropeptide Y and leptin receptor expression in the hypothalamus of rats with chronic immobilization stress. *Neural Regeneration Research*, *8*(18), 1721–1726. https://doi.org/10.3969/j.issn.1673-5374.2013.18.010

Wang, S.-X., Chen, J.-X., Yue, G.-X., Bai, M.-H., Kou, M.-J., & Jin, Z.-Y. (2012). Xiaoyaosan Decoction Regulates Changes in Neuropeptide Y and Leptin Receptor in the Rat Arcuate Nucleus after Chronic Immobilization Stress. *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–16. https://doi.org/10.1155/2012/381278

Werdermann, M., Berger, I., Scriba, L. D., Santambrogio, A., Schlinkert, P., Brendel, H., Morawietz, H., Schedl, A., Peitzsch, M., King, A. J. F., Andoniadou, C. L., Bornstein, S. R., & Steenblock, C. (2020). Insulin and obesity transform hypothalamic-pituitary-adrenal axis stemness and function in a hyperactive state. *Molecular Metabolism*, *43*, 101112. https://doi.org/10.1016/j.molmet.2020.101112

West, K. S., & Roseberry, A. G. (2017). Neuropeptide-Y alters VTA dopamine neuron activity through both pre- and postsynaptic mechanisms. *Journal of Neurophysiology*, *118*(1), 625–633. https://doi.org/10.1152/jn.00879.2016

Williams, K. W., Margatho, L. O., Lee, C. E., Choi, M., Lee, S., Scott, M. M., Elias, C. F., & Elmquist, J. K. (2010). Segregation of Acute Leptin and Insulin Effects in Distinct Populations of Arcuate Proopiomelanocortin Neurons. *Journal of Neuroscience*, *30*(7), 2472–2479. https://doi.org/10.1523/JNEUROSCI.3118-09.2010

Wood, J., Verma, D., Lach, G., Bonaventure, P., Herzog, H., Sperk, G., & Tasan, R. O. (2016). Structure and function of the amygdaloid NPY system: NPY Y2 receptors regulate excitatory and inhibitory synaptic transmission in the centromedial amygdala. *Brain Structure & Function*, *221*(7), 3373–3391. https://doi.org/10.1007/s00429-015-1107-7

World Health Organisation, (WHO). (2021, June 9). *Obesity and overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wu, Z., Kim, E. R., Sun, H., Xu, Y., Mangieri, L. R., Li, D.-P., Pan, H.-L., Xu, Y., Arenkiel, B. R., & Tong, Q. (2015). GABAergic Projections from Lateral Hypothalamus to Paraventricular Hypothalamic Nucleus Promote Feeding. *Journal of Neuroscience*, *35*(8), 3312–3318. https://doi.org/10.1523/JNEUROSCI.3720-14.2015

Xu, A. W., Kaelin, C. B., Morton, G. J., Ogimoto, K., Stanhope, K., Graham, J., Baskin, D. G., Havel, P., Schwartz, M. W., & Barsh, G. S. (2005). Effects of Hypothalamic Neurodegeneration on Energy Balance. *PLOS Biology*, *3*(12), e415. https://doi.org/10.1371/journal.pbio.0030415

Yamada, S., Islam, M. S., van Kooten, N., Bovee, S., Oh, Y.-M., Tsujimura, A., Watanabe, Y., & Tanaka, M. (2020). Neuropeptide Y neurons in the nucleus accumbens modulate anxiety-like behavior. *Experimental Neurology*, *327*, 113216. https://doi.org/10.1016/j.expneurol.2020.113216

Yamada, S., van Kooten, N., Mori, T., Taguchi, K., Tsujimura, A., & Tanaka, M. (2021). Efferent and Afferent Connections of Neuropeptide Y Neurons in the Nucleus Accumbens of Mice. *Frontiers in Neuroanatomy*, *15*, 741868. https://doi.org/10.3389/fnana.2021.741868

Yang, L., Scott, K. A., Hyun, J., Tamashiro, K. L., Tray, N., Moran, T. H., & Bi, S. (2009). Role of dorsomedial hypothalamic neuropeptide Y in modulating food intake and energy balance. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *29*(1), 179–190. https://doi.org/10.1523/JNEUROSCI.4379-08.2009

Yau, Y. H. C., & Potenza, M. N. (2013). Stress and Eating Behaviors. *Minerva Endocrinologica*, *38*(3), 255–267.

Yi, M., Li, H., Wu, Z., Yan, J., Liu, Q., Ou, C., & Chen, M. (2018). A Promising Therapeutic Target for Metabolic Diseases: Neuropeptide Y Receptors in Humans. *Cellular Physiology and Biochemistry*, *45*(1), 88–107. https://doi.org/10.1159/000486225