Striatal and hypothalamic control of food intake and glucose metabolism

Koekkoek, L.L.

Publication date
2022

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

General Introduction
Obesity and type II diabetes epidemic

We are currently in the midst of a global obesity epidemic. Obesity, defined as having a body mass index (BMI) of over 30 kg/m$^2$, affects over 650 million people around the world \cite{1}. Another 1.9 billion adults are overweight, \textit{i.e.} having a BMI of 25-30 kg/m$^2$ \cite{1}. This obesity epidemic is the result of an increased intake caloric intake, specifically intake of hypercaloric foods, rich in saturated fat and added sugars, and a decrease in physical activity due to a sedentary lifestyle \cite{2}. Overweight and obesity increase the risks for many adverse health events such as cardiovascular disease, chronic pain conditions and several types of cancer, but perhaps the strongest correlation observed, is the one between obesity and type II diabetes mellitus (T2DM) \cite{3, 4}.

T2DM is a progressive disease that is characterized by the presence of insulin resistance, which results in reduced glucose uptake from the blood stream by tissues such as skeletal muscle and adipose tissue, and reduces the suppression of glucose production in the liver \cite{5}, overall resulting in hyperglycaemia. In the early stages of disease, hyperglycaemia can be prevented by increasing insulin release from pancreatic $\beta$-cells, but over time the pancreas cannot keep up with the ever increasing demand for insulin. At this stage, $\beta$-cell failure occurs, reducing insulin secretion, thereby further exacerbating blood glucose levels \cite{6}. While the progression of T2DM is gradual, patients are only diagnosed with T2DM once hyperglycaemia is observed, such as when fasting plasma glucose levels exceed 7.0 mmol/L (or 126 mg/dL) \cite{7}. According to the World Health Organization, approximately 462 million people worldwide suffered from T2DM in 2017 \cite{8}.

To treat T2DM, multiple glucose lowering medications are available \cite{9}, but over 35% of all patients does not accomplish glycaemic goals \cite{10}. Thus, to improve glycaemic control, better treatment strategies are required. As obesity and T2DM are so tightly linked, weight loss is a crucial component of the treatment strategy \cite{11}. Weight loss can be achieved through a combination of lifestyle, diet, physical activity interventions and if these prove to be ineffective, pharmacological treatment and bariatric surgery can be applied \cite{12, 13}. All these treatment options have very low success rates \cite{14}, with the exception of bariatric surgery, but this is highly invasive and has lifelong consequences \cite{15}. Therefore, therapeutic options targeting weight loss and regulation of blood glucose levels need to be improved and to achieve this, a better understanding of the physiology of weight regulation and glucose homeostasis is crucial.

Brain in control of food intake and glucose metabolism

The brain is a key regulator of food intake and glucose metabolism. In order to appropriately respond to changes in energy status or glycaemia, it receives a variety of signals that can be subdivided into three categories: hormonal signals, nutrient signaling and neural input from peripheral sensors. Important hormonal signals include insulin produced by the pancreas, ghrelin produced by the stomach and leptin produced by the adipose tissue \cite{16}. For direct
nutrient signaling, glucose, triglycerides, fatty acids and amino acids travel to the brain via the circulation and are monitored by specialized neurons that contain molecular machinery to sense the presence of nutrients in the extracellular space [17]. Lastly, different sources of neural input inform the brain at various stages of digestion. For example, afferents from the facial nerve convey information from oral taste signaling, whereas vagal afferents inform the brain on the presence of nutrients in the gastrointestinal tract [18].

**Homeostatic brain circuits in control of food intake and glucose metabolism**

Within the brain, a general distinction can be made between homeostatic neural circuits that control food intake by integrating signals concerning the energy balance, to ensure that energy intake meets energy demands, and the reward circuits that deal with the hedonic and pleasurable aspects of food consumption. The homeostatic neural circuit includes areas in the hypothalamus and brain-stem. Within the hypothalamus, the arcuate nucleus has been found to be the most important brain area for the homeostatic control of food intake. The arcuate nucleus resides at the bottom of the hypothalamus next to the third ventricle, where the blood-brain barrier is more leaky, allowing for peripheral nutrients and metabolic signals to convey information about energy status to first-order neurons in the arcuate nucleus [19]. These first-order neurons then project to a number of other hypothalamic brain areas, such as the paraventricular nucleus (PVN) and lateral hypothalamus (LH) [20], but also to neurons in the brain stem [21]. In turn, signals from the gastrointestinal tract that are transferred to the brain stem via different pathways including the vagal nerve, are conveyed to the hypothalamus [21]. Altogether, this homeostatic network allows the brain to appropriately respond to changes in energy status.

Homeostatic neural circuits are not only implicated with the control of food intake, but also regulate glycaemic levels. While multiple hypothalamic nuclei, including the arcuate nucleus, are involved in glycemic control [19], specifically the ventromedial hypothalamus (VMH) plays an important role in the counter-regulatory response to hypoglycaemia [22-24]. To monitor changes in glycaemia, a subset of neurons specialized in monitoring extracellular glucose concentrations is present. These glucose sensing neurons can be either glucose-excited or glucose-inhibited neurons, depending on the change in neural activity that is triggered by a drop in extracellular glucose. Furthermore, glucose sensing neurons contain the molecular machinery necessary to transport glucose into the cell, metabolize glucose and thereby altering the cell’s energy status, and subsequently adjust their activity using ion channels sensitive to changes in energy status [25]. Glucose sensing neurons reside in the VMH, as well as in other hypothalamic areas and in extra-hypothalamic nuclei [26]. Once glucose sensing neurons detect changes in glucose concentrations, the brain can respond directly and indirectly, through its neural connections with peripheral organs, to alter glucose production, glucose uptake, or the release of glucoregulatory hormones in the circulation [27, 28].
Hedonic brain circuits in control of food intake and glucose metabolism

The brain’s reward system is composed of those areas that are activated by a number of pleasurable stimuli, including palatable food intake. Key components are areas such as the striatum, which can be further subdivided into the dorsal and ventral striatum (where the nucleus accumbens (NAC) resides), ventral tegmental area (VTA), amygdala and LH [29-31]. The reward system directs multiple processes concerning the hedonic aspects of food intake, including the motivation to work for certain palatable foods, as well as the experienced pleasantness of different foods [32]. To appropriately respond to food related signals, like the homeostatic system, the reward circuit also receives peripheral signals such as hormones and nutrients [33], and can modulate the homeostatic control of food intake. Interestingly, altering transmission of specific neurotransmitters in the NAC or LH can affect blood glucose levels [34-38], indicating that these areas also play a role in the control of glycaemia. Because of their role in both processing the hedonic aspects of food intake, as well as their ability to modulate blood glucose levels, the NAC and LH are areas of great interest to investigate in relation to obesity and T2DM.

The NAC and control of food intake

The NAC is positioned in the basal forebrain, and is part of the limbic system. It can be subdivided into a core and a shell part that receive input from different brain areas, project to diverse nuclei and have overlapping, as well as completely distinct functions [39-41]. The vast majority of neurons in both the core and shell are medium spiny neurons, whose activity is modulated by cholinergic and GABAergic interneurons [42, 43]. One of the major sources of input the NAC receives, particularly for its role in the reward system, is from dopaminergic neurons in the VTA. Various rewarding stimuli modulate behavior through changes in dopamine signaling in the NAC, mediated by medium spiny neurons that express the dopamine 1 or 2 receptor [29, 44]. In addition to dopamine receptors, NAC neurons also express serotonin, endocannabinoid and opioid receptors [45-47]. It appears that these different neurotransmitter systems modulate different aspects of reward processing: dopamine signaling is crucial for the ‘wanting’ of a reward, whereas endocannabinoids and opioids are more involved in the ‘liking’ of rewards [48]. For example, infusion of dopamine receptor 1 or 2 antagonists reduced the motivation to work for a reward in rodents, without altering taste reactivity (a measure of liking) [49, 50]. Endocannabinoid or opioid infusion into specific parts of the NAC on the other hand enhances the taste reactivity to stimuli [51, 52]. However, NAC-infusion of opioids can also increase ‘wanting’, indicating overlapping functions of opioids in food reward that may depend on the site of stimulation within the NAC [53].

The NAC and control of glucose homeostasis.

Several lines of evidence indicate that the NAC is also involved in the control of glucose homeostasis. Firstly, the NAC contains glucose sensing neurons [54]. Whether glucose neurons in the NAC play a similar role as in the hypothalamus, has to be investigated in future
General introduction

Experiments. Secondly, altering striatal neuronal activity through deep brain stimulation affects blood glucose levels [35, 36]. It appears that different neurotransmitters within the NAC have distinct effects on glucose levels. For example, increasing NAC serotonin levels had hyperglycaemic effects [34], whereas activation of NAC dopamine receptor 1 neurons during a glycaemic challenge lowered blood glucose levels [36]. How these various neurotransmitters within the NAC have interacting effects on the glucoregulatory role of the NAC, and whether other neurotransmitters such as endogenous opioids are involved, remains to be determined.

LH and control of food intake

The LH has long been known to be involved in the control of food intake. Studies dating back to the 1950s showed that lesions of the lateral hypothalamic area reduced food intake [55, 56], whereas electrical stimulation increased feeding [57]. This led to the initial labeling of the LH as the ‘feeding center’ of the brain [56], though it is now evident that the role of the LH in the control of food intake is infinitely more complex. The LH is ideally positioned between the homeostatic and hedonic brain circuits, as it receives input from other homeostatic brain areas within the hypothalamus [58-60], but also received neural projections from reward-related brain areas such as the NAC and the amygdala [40, 61, 62]. Furthermore, activation of LH neurons is rewarding by itself [63], highlighting the role of the LH in reward processing. The two main neuronal populations in the LH are the GABAergic and glutamatergic neurons, that can be further subdivided into 30 different subpopulations [64]. GABAergic and glutamatergic neurons appear to have opposing roles in the control of food intake: ablation of GABAergic LH neurons reduces feeding and motivation to work for a food reward [65], whereas loss of glutamatergic LH neurons stimulates food intake and weight gain [66]. While these opposing roles of the two main populations paint a clear picture of LH control on food intake, studies investigating the role of subpopulations show the reality is much more complex. For example, despite the inhibition on food intake that occurs when glutamatergic LH neurons are activated [67], glutamatergic subpopulations of orexin or melanin-concentrating hormone (MCH) neurons both stimulate food consumption [68-74]. Likewise, stimulation of GABAergic galanin neurons enhances food intake whereas GABAergic neuropeptide Y (NPY) neurons inhibit feeding [75, 76]. Thus, the LH is a highly heterogeneous area where different neuronal subpopulations can have opposing effects on food intake, and a more thorough understanding of how dietary factors influence LH neuronal activity will likely aid in unraveling the complexity of this brain area.

LH and control of glucose metabolism

Like the NAC, the LH contains neurons that are sensitive to fluctuations in extracellular glucose, but the response to glucose differs per subpopulation. Orexin expressing LH neurons and neuropeptide Y (NPY) expressing neurons, are inhibited by glucose [77-79]. MCH neurons on the other hand, are excited by increases in extracellular glucose concentrations [38, 80]. LH neurons are also involved in the control of peripheral glucose concentrations, as
was first shown in rats where LH neuronal stimulation increased blood glucose levels [81]. Moreover, manipulation of orexin or MCH neurons also affects glucose tolerance, again indicating the involvement of specific subpopulations [37, 38].

The NAC and LH also have strong neural projections connecting each other. NAC dopamine receptor 1 neurons project to the LH directly, as well as through the ventral pallidum, whereas dopamine receptor 2 neurons only project through the ventral pallidum [82]. Furthermore, LH neurons can influence NAC functioning through direct projections to the NAC [83], or by affecting dopaminergic neurons in the VTA that project to the NAC [84]. Because of this strong connection between these two areas, a better understanding of how dietary factors influence NAC and LH functioning, will help us to unravel how the brain’s reward system controls food intake. Likewise, by studying the involvement of different neurotransmitter systems within these brain areas in glucoregulation, a better picture of central control of glycaemia can be painted, and possible therapeutic targets can be explored.

Animal models to study obesity and diabetes

A variety of animal models have been employed over the last decades to study the mechanisms that are responsible for the disturbed regulation of food intake and glucose metabolism in obesity and T2DM. To induce obesity, rats or mice can be genetically modified, such as ob/ob mice which are unable to produce leptin, resulting in significant weight gain [85]. Alternatively, feeding of hypercaloric diet might mimic the course of human obesity more closely. While many different hypercaloric diets are available, we have shown that a free choice high-fat high-sucrose (fcHFHS)-diet best mimics human obesity [86].

The fcHFHS diet paradigm consists of rats receiving ad libitum access to regular chow pellets, a bottle of water, a dish of pure beef tallow, and a bottle of a sucrose solution. When rats feed on this diet for five weeks, they become hyperphagic, gain significantly more body weight and show enlarged adipose tissue stores [87]. Interestingly, when rats are given a fcHF or a no-choice HFHS diet, they initially consume more food, but do not continue to overeat and as a consequence, do not gain as much weight [87, 88]. In addition to the increased weight and adiposity, fcHFHS-diet fed rats also have impaired glucose tolerance after 4 weeks of diet access [89], reduced leptin sensitivity [90] and disruptions in both homeostatic as well as reward brain circuitry that are highly similar to those found in individuals with obesity [87, 91].

Because the fcHFHS diet causes overeating and renders animals obese, whereas a fcHF or fcHS diet does not, we have postulated that fat and sugar have interacting effects on food intake. More specifically, we hypothesize that since rats that receive a fcHF diet consume less fat than animals on a fcHFHS diet, sucrose consumption increases fat intake and not vice versa. In line with this hypothesis, fcHS-fed animals drink more sucrose than fcHFHS-fed rats, indicating that fat consumption does not boost sucrose drinking [87]. Which brain areas and neurotransmitters are involved in sucrose-stimulated fat intake remains to be
investigated. However, as sugar is thought to induce the release of endogenous opioids in the NAC [92-94], and opioid stimulation in the NAC causes a sharp increase in fat intake [95-97], we hypothesized that the endogenous opioid system is one of the prime candidates [98].

**Endogenous opioids in the control of food intake and glucose metabolism**

The body produces 4 different endogenous opioids: β-endorphin, leu- and met-enkephalin, dynorphin and endomorphin [99-102]. They are produced in multiple places in the body: primarily in the central and peripheral nervous system, but also in the pituitary gland where they are released into circulation, the gastrointestinal tract, immune cells and in the vasculature. Endogenous opioids bind to three different receptors: the µ-opioid, κ-opioid and δ-opioid receptor [103, 104], which are also expressed throughout the body.

As previously stated, opioids in the NAC are involved in the ‘liking’ as well as the ‘wanting’ of food rewards [53]. It appears that for altering the ‘liking’ of rewards, specific hotspots within the NAC exist that mediate pleasantness, whereas opioid stimulation in a wider area, which encompasses most of the NAC, can increase food intake [53]. While stimulation of all three opioid receptors in the NAC increases food intake [97], the effects of µ-opioid receptor agonism have been studied most extensively. Intra-NAC infusion of the selective µ-opioid receptor agonist [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO), stimulates consumption of a variety of palatable foods such as a high fat diet, a sucrose, saccharin, salt and ethanol solution [95, 105]. However, when a high-carbohydrate and high-fat diet were simultaneously presented after DAMGO infusion into the NAC, rats preferentially increased the high-fat diet, indicating a role for NAC opioid signaling in fat preference [95]. Whether a sucrose-triggered release of NAC opioids also mediates the increased fat intake seen in fcHFHS-fed animals compared to fcHF-fed rats, is yet to be determined.

Studies have attempted to unravel the neural network that is involved in the effects seen upon intra-NAC DAMGO infusion, and revealed a complex network of nuclei and neurotransmitters to be implicated. Firstly, general inhibition of neural activity in the amygdala [106], VTA [107] and LH [107] by infusion of a GABA agonist, largely reduced feeding induced by DAMGO infusion in the NAC. Furthermore, DAMGO’s effects on food intake also require opioid signaling within the amygdala [108] and VTA [109]. Lastly, it appears that orexin neurons within the LH are activated by DAMGO infusion in the NAC, and orexin signaling in the VTA is needed for the effects seen on food intake [110]. Future experiments will have to explore which elements of this complex network are also involved in the sucrose-stimulating effects on fat intake.

In addition to effects of opioid stimulation on food intake, infusion of both endogenous and synthetic opioids, also affects blood glucose levels. The majority of studies found that opioids have hyperglycemic effects [111-129]. In line with these findings, during hypoglycaemia or exercise, when an increase in glucose production is needed, β-endorphin
plasma concentrations are elevated [130-139]. Obesity and diabetes (type I as well as type II) appear to modulate both baseline levels of endogenous opioids [140-146], as well as the effect opioid stimulation has on blood glucose levels [111, 147-154]. The brain, containing high levels of opioid receptors and responsive to doses of opioid stimulation that have no effect when administered peripherally [155], is likely an important mediator of the effects opioids have on glycaemia. Interestingly, obesity also affects brain opioid receptors [156, 157], further underlining the need to better understand how opioid signaling in the brain affects glucose homeostasis.

Outline thesis

The overall aim of this thesis is to study the role of the NAC and LH in the control of food intake and glucose metabolism (Figure 1).

Figure 1. Overview of this thesis. Abbreviations: NAC = nucleus accumbens, LH = lateral hypothalamus.
Previously, it has been shown that the NAC is involved in the control of glucose homeostasis [34-36]. One factor indicating the involvement of a brain area in the control of blood glucose levels, is the presence of glucose sensing neurons. Therefore, in chapter 2 we describe the current state of knowledge in literature about the presence of glucose sensing neurons in the NAC, LH and other areas within the reward system. We outline different glucose transporters, intracellular enzymes and membrane channels that are implicated with glucose sensing neurons, and what is known about their expression within the brain’s reward system. While glucose sensing is known to be present in the NAC, little is known about how they respond to changes in glycaemia. Therefore, in chapter 3, we explore whether the genes outlined in chapter 2 are responsive to an increase in glycaemia, by measuring the mRNA and protein levels of these genes after a sucrose bolus was consumed. Furthermore, as it is known that fat and sucrose feeding have interacting effects on the brain [87], we determine whether consumption of a high fat diet alters how sucrose drinking affects gene expression.

In previous studies, NAC dopaminergic and serotonergic systems were found to be involved in the control of glucose homeostasis [34, 36]. Considering the strong evidence that opioids can modulate glycaemia [111-129], we speculate that NAC opioid transmission also plays a role in the glucoregulatory function of the NAC. Therefore, in chapter 4 we explore whether activation of NAC µ-opioid receptors affects blood glucose levels. While the role of opioid receptors in the central control of glucose homeostasis has not received much attention, a large number of studies investigated the peripheral effects of opioid stimulation on glucose homeostasis. Therefore, in chapter 5, we outline these studies and describe how opioid stimulation has hyperglycemic effects in most situations, but can have complete opposite effects in people suffering from type II diabetes.

We have hypothesized that the opioid system in the NAC may be involved in the stimulatory effects sucrose drinking has on fat intake and in chapter 6 we test this hypothesis. We explore how sucrose drinking alters the increase in fat intake typically seen after intra-NAC infusion of DAMGO, and investigate how sucrose drinking and intra-NAC DAMGO infusion modulate neuronal activity in a network of brain areas downstream of the NAC, including the LH. Lastly, we focus on the effects of diet on the LH specifically, as in chapter 7 we investigate how a high fat diet affects the LH neuronal response to sucrose drinking, as well as drinking of a low-calorie sweetened solution.
Chapter 1

References

1. Organization, W.H. Fact Sheet Obesity and Overweight 2021 06-07-2021].
58. Elias, C.F., et al., Chemically defined projections linking the mediobasal


109. MacDonald, A.F., C.J. Billington, and A.S. Levine, Effects of the opioid antagonist naltrexone on feeding induced by DAMGO in the ventral tegmental area and in the nucleus


