Striatal and hypothalamic control of food intake and glucose metabolism

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Chapter 8

General Discussion
This thesis aimed to explore the role of the nucleus accumbens (NAC) and lateral hypothalamus (LH) in the control of glycaemia and food intake, by exploring three different subjects: i. glucose sensing neurons, ii. the role of opioids in glucoregulation, and iii. the interacting effects of fat and sucrose intake. Firstly, since our group has previously shown that manipulating NAC neural processing can affect glycaemic values [1-3], glucose sensing neurons have been found in the NAC [4] and glucose neurons within the hypothalamus modulate the control of glycaemia [5], we hypothesized that their presence in the NAC points to a glucoregulatory role of the NAC. Therefore, in chapters 2 and 3, we outlined the current knowledge on the molecular mechanisms by which glucose sensing neurons in the NAC (and other brain areas part of the reward system) operate, and whether these molecular mechanisms are responsive to dietary changes. Secondly, we aimed to study how opioid transmission in the NAC influences its glucoregulatory role. We have previously confirmed the glucoregulatory involvement of the serotonin [1] and dopamine [3] system, but the NAC also has extensive opioid receptor expression [6], and central stimulation of the µ-opioid receptor alters peripheral glycaemia [7]. Thus, in chapter 4, we explored how stimulation of NAC µ-opioid receptors affects glycaemia, whereas in chapter 5, we described the general reciprocal relationship that exists between opioids and glucose metabolism. Lastly, our lab has shown that fat and sucrose intake causes persistent hyperphagia [8], and has interacting effects on the brain [8-12]. We hypothesized that sucrose drinking stimulates fat intake, and confirmed this hypothesis in chapter 6. On the other hand, we show in chapter 7 that fat intake disrupts the glutamatergic LH neuronal response to sucrose, as well as to a low-calorie sweetener.

In this discussion, we will first explore the role of the opioid system in glucoregulation more broadly, and speculate about conditions during which the glucoregulatory role of the opioid system is most prominent. Next, we will describe a neural network that could underlie the sucrose-stimulated fat intake that we have explored in chapter 6.

Endogenous opioids and the control of glycaemia.

There are currently over 650 million people around the world that suffer from obesity [13], and another 1.9 billion adults are overweight [13]. Obesity increases the likelihood of developing adverse health conditions, including cardiovascular disease, type II diabetes mellitus (T2DM) and chronic pain conditions [14]. Because of the increased incidence of chronic pain among individuals with obesity, patients with obesity are more likely to receive opioid based medications [15]. This increase in opioid prescriptions forms a great risk for additional adverse health problems in people with obesity, as both exogenous and endogenous opioids have been found to affect glycaemia [7, 16-33], and individuals suffering from opioid addiction exhibit reduced glucose tolerance [34-37]. These effects on glycaemia are therefore particularly problematic for patients with obesity who are already at greater risk of developing insulin resistance and T2DM. A better understanding on how opioids, both exogenous as well as endogenous, affect glycaemia is therefore needed. Thus, in chapter 5, we
provided a review on the relationship between opioids and glucose metabolism. We found that opioids, except when administered in some specific conditions, increase glycaemia. In line with these findings, we reported that hypoglycaemia, as triggered by insulin injection or during exercise, increases the release of the endogenous opioid β-endorphin into the bloodstream, thereby possibly contributing to the counter regulatory response to restore euglycaemia.

The potential role of opioids in the central nervous system in glucoregulation

Opioids are primarily produced in the central nervous system [38], and opioid receptors are expressed throughout the brain [6], suggesting that the brain is an important site for opioid’s effects on glycaemia. Which brain areas mediate opioids’ effects on blood glucose levels has not yet been investigated. Interestingly, while the hypothalamus has traditionally been explored for its role in the control of glucose homeostasis, the brain’s reward system (which contains a significant expression of opioid receptors [6]) also has the necessary molecular tools to monitor glucose levels and modulate glycaemia, as we described in chapter 2. We were particularly interested in the NAC, as our lab has previously shown that manipulation of NAC neuronal activity affects glycaemia [1-3], and the NAC is rich in opioid receptors [6]. Thus, in chapter 4, we studied whether activation of NAC µ-opioid receptor also affects glycaemia. We showed that while infusion of the µ-opioid receptor agonist [D-Ala², N-MePhe⁴, Gly-ol]-enkephalin (DAMGO) into the NAC does not significantly affect basal glycaemia, it enhances the glycemic response to an insulin or glucose tolerance test, indicating that NAC opioid transmission can context-specifically affect glucose homeostasis. Altogether, this raises the question what the physiological role is of NAC endogenous opioid release in glucoregulation. The hypothalamus and in particular the ventromedial hypothalamus (VMH) has been shown to be essential to glucose homeostasis [5], but the role of the brain’s reward system in countering hypoglycaemia is unknown. To address this, a broader picture of opioid system functioning is needed, because while in chapters 4 and 5 we have focused on opioid-induced changes in glycaemia, opioids’ primary role in the body is in modulation of pain signaling.

Opioids as a modulator of analgesia and glucose metabolism during pain

Pain processing involves a large neural network, and opioid receptors are expressed throughout the neurons within this network [39]. Painful stimuli are first registered by thin myelinated or unmyelinated neural fibers, whose cell bodies reside in the dorsal horn of the spine. There, the nociceptive signal is conveyed through the spinal cord to the brain, targeting a network of areas including the somatosensory cortex, anterior cingulate cortex, and reward-related nuclei such as the NAC, amygdala and VTA [39]. This intricate network mediates the highly complex phenomenon of pain perception, which is not merely a reflection of a response to a noxious signal, but rather a combination of sensory, emotional and cognitive signaling. Furthermore, the brain does not passively process peripheral pain
signals, it can also actively modulate neural transmission of pain processing through the descending pain modulatory system [40]. This system, which originates in multiple brain areas and serves to inhibit pain signals at the level of the spinal cord, relies heavily on endogenous opioids to inhibit peripheral pain processing [41]. Moreover, blocking opioid signaling at higher levels of pain processing reduces analgesia [39], indicating that opioids modulate pain signals at various stages of pain signaling.

Interestingly, pain also triggers a number of metabolic effects. For example, general energy requirements are greater, and glucose utilization by the body is increased [42]. To ensure glucose availability, insulin resistance occurs during pain [43, 44]. Because of the important role of opioids in pain modulation, and the known hyperglycemic effects of opioid administration [7, 16-33], it is tempting to speculate that opioids not merely control pain signaling, but also modulate the metabolic changes that occur during pain. Whether this is through the release of endogenous opioids into the blood stream in response to pain [45, 46], indirectly through modulation of glucoregulatory hormones, or through central effects of opioid stimulation is currently unknown and is of interest for future studies.

The potential learned response to pain, mediated by NAC opioid transmission

Due to the strong role for the NAC in motivation, opioid signaling in the NAC has been hypothesized to be part of the mechanism that establishes the correct behavior in response to painful stimuli [47], i.e. learning to avoid behavior that elicits pain and perform behaviors that reduce pain, which is crucial to survival. In line with this hypothesis, NAC neural activity encodes the predictive value of pain relief, further pointing to the potential involvement for the NAC in establishing pain reducing behavior [48]. Furthermore, it could be speculated that the learned response to pain, mediated by the NAC, also includes fine-tuning of the correct metabolic response to pain. Multiple neurotransmitters within the NAC might mediate these metabolic responses to pain. For example, opioid signaling in the NAC plays an important role in nociception: injection of opioids into the NAC induces analgesia [49, 50], whereas blocking of opioid signaling during pain signaling reduces the analgesia [51]. Moreover, we showed in chapter 4 that opioid signaling in the NAC can influence glycaemia. On the other hand, considering the strong role of dopamine in the learned responses to pain [52], and the ability of NAC dopamine signaling to influence glycaemia [3], it would also be highly interesting to explore the role of dopamine, and its possible connection with opioid signaling, in the NAC-mediated response to pain, both with regard to analgesia and to the metabolic response.

One of the overarching aims of this thesis was to explore the role of glucose sensing in the reward system and its relation to glucoregulation. In chapter 2, we outlined what is known about glucose sensing neurons in the reward system. We reported that the NAC contains neurons that have glucose sensing capabilities and expresses different components of the
molecular machinery needed to perform glucose sensing. Next, in chapter 3, the effects of consumption of a fCHF diet and/or a sucrose bolus on the expression of glucose sensing genes was explored. While some relevant effects on specific glucose transporters, as well as the ATP-sensitive potassium channel Kir6.1 were found, the majority of genes measured in this study were not affected by our feeding paradigm. Possibly, diet did not affect glucose sensing machinery at the level of mRNA or protein expression, but alterations in glucose transporter presence in the cell membrane or phosphorylation of enzymes involved in cellular glucose metabolism cannot be ruled out. Alternatively, diet has no effect on NAC glucose sensing machinery, but other conditions such as pain processing, do require NAC glucose sensing. In order to understand the conditions that require NAC glucose sensing, a better understanding of which NAC neuronal subtypes are capable of glucose sensing would be useful. For example, it would be highly insightful to know whether glucose sensing NAC neurons express receptors for neurotransmitters such as dopamine, opioids or serotonin, and whether binding of these neurotransmitters to their NAC receptors not only affects glucose metabolism [1, 3], but also glucose sensing. To conclude, the role of NAC glucose sensing is still to be determined, but it might be particularly relevant during specific conditions that strongly involve NAC functioning, e.g. during motivation-related behaviors or pain.

Future directions

To better understand the reciprocal relationship between the NAC, opioid signaling and glucose metabolism, some simple experiments could provide significant information that is currently lacking. Firstly, to better understand the general relationship between pain, opioids and glycaemia, it would be interesting to determine whether infusions of opioids (either peripherally, or intracerebroventricularly) during pain causes changes in glucose metabolism and the glucoregulatory response. Or alternatively, whether changes in glycaemia during pain can affect pain perception and opioid release in the brain or blood stream. To explore the role of the NAC specifically, it would be insightful to study if opioid antagonist infusion into the NAC, during pain or in baseline conditions, also affects glycaemia. Although these experiments have already been performed to establish the role of the NAC in analgesia [51], effects on metabolism were not investigated. Lastly, while in chapter 5 we reviewed many different studies that explored the role between opioids and glucose metabolism, none of these studies investigated whether the effects seen on glycaemia upon opioid administration are altered by the presence of pain. This seems particularly odd, because opioid medications are prescribed to treat pain, but many studies looking into the effects on glycaemia were performed in healthy volunteers. Therefore, we cannot rule out that during pain processing, when endogenous opioids are released by the brain to mediate pain perception [39, 45, 46], administration of exogenous opioids will have different effects on glycaemia. Overall, these experiments will aid us in better understanding the relationship between pain, opioid signaling (in the NAC) and glucose metabolism and will allow physicians to take the side effects of opioid administration on glycaemia into account when prescribing opioid based medications.
The neural network behind sucrose-stimulated fat intake

Consumption of a diet rich in saturated fats and added sugar is one of the main contributors to the development of overweight and obesity [53]. To study what the effects of these different nutrients are on the body, we developed a free-choice high fat high sugar (fcHFHS) diet, in which rats have access to their regular chow diet and drinking water, as well as a dish of pure beef tallow, and a 30% sucrose solution. When rats are fed this fcHFHS diet for five weeks, they consistently consume more calories than chow-fed animals, and as a consequence, gain significantly more weight [8]. Interestingly, when rats receive a fcHF diet (no sucrose solution available), they start out hyperphagic, but decrease their fat intake over the course of the five weeks, resulting in a similar caloric intake as chow-fed animals, and as a consequence no significant weight gain [8]. Rats consuming a fcHS diet (only the 30% sucrose solution in addition to their regular diet, but no dish of tallow available) are consuming a comparable amount of calories as chow-fed rats, and have similar weight gain throughout the five weeks. Because fcHF-fed rats decrease their fat consumption over time, whereas fcHFHS animals do not [54], we hypothesized that sucrose drinking triggers fat intake.

To study more closely what happens around the consumption of a sucrose bolus, we developed an experimental paradigm in which rats do not have continuous access to a sucrose solution, but only receive a single sucrose bolus at the end of every light period. This paradigm enables us to pinpoint exactly when and how much sucrose rats consume in one bolus, and allows us to visualize the effects sucrose has on the brain, as well as investigate the potential stimulating effects on fat intake. In chapter 3, we measured the expression of several genes involved in glucose handling after sucrose drinking, and investigated how a fcHF diet modulates the effects of sucrose. In chapter 6 we explored the stimulating effects of sucrose on fat intake, the involvement of the opioid system, and neural activity in a network of brain areas including the amygdala and LH. Lastly, in chapter 7, we investigated the direct effects of sucrose drinking on glutamatergic neuronal activity in the LH, in animals on a chow or fcHF diet. By combining the results of these chapters, we can now paint a more detailed picture of how different brain areas respond to sucrose drinking, ultimately resulting in increased fat consumption.

Sucrose stimulates fat intake through the release of endogenous opioids

Firstly, we were able to confirm our hypothesis that consumption of a sucrose bolus triggers a rise in fat intake. This increase is highly comparable to the enhanced fat consumption seen after intra-NAC infusion of a µ-opioid receptor agonist (chapter 6). Furthermore, when NAC µ-opioid receptors are stimulated after a sucrose bolus, it no longer increases fat intake, suggesting a reduced sensitivity to DAMGO infusion after sucrose drinking. We speculated that this reduced sensitivity stems from decreased availability of NAC µ-opioid receptors, due to endogenous opioid release in response to the sucrose consumption. Binding of endogenous opioids such as β-endorphin and met-enkephalin is known to trigger internalization of the µ-opioid receptor [55] and sucrose drinking indeed causes the release of endogenous
opioids in the dorsal striatum [56] and NAC [57]. In line with this hypothesis, we found a comparable increase in NAC c-Fos-expression after sucrose drinking, compared to NAC µ-opioid receptor stimulation. While c-Fos is an immediate early gene, typically used as a proxy for neuronal activity [58], µ-opioid receptor activation also has a direct effect on c-Fos transcription, causing an increase in c-Fos-expression [59]. As the µ-opioid receptor is a Gi/Gq coupled receptor, and opioid binding inhibits neural activity [60, 61], the increase in c-Fos-expression after sucrose drinking or intra-NAC µ-opioid receptor agonist infusion is likely a reflection of µ-opioid receptor activation. Thus, we concluded that sucrose drinking stimulates fat intake, likely through the release of endogenous opioids.

Release of endogenous opioids likely inhibits NAC medium spiny neuron activity

The next question that arises, is how release of endogenous opioids affects NAC neural activity, as well as activity in downstream areas of the NAC, ultimately resulting in an increase in fat intake. While there has been little research on sucrose-triggered release of endogenous opioids, a large number of studies have investigated how NAC µ-opioid receptor stimulation (using the µ-opioid receptor specific agonist DAMGO) induces fat consumption, and which neural network is involved. Thus, we can speculate about the potential downstream effects of sucrose-triggered endogenous opioids release. NAC µ-opioid receptor stimulation affects NAC neural activity in several ways. Firstly, intra-NAC DAMGO infusion increases NAC dopamine release [62], which can affect activity of medium spiny neurons that express dopamine 1 (D1) or dopamine 2 (D2) receptors. However, co-infusion of DAMGO and D1 or D2 receptor antagonists only reduces DAMGO-triggered fat intake when a D1 or D2 receptor antagonist is infused in extremely high concentrations [63], and not when D1 or D2 receptor antagonists are administered in physiologically relevant concentrations [64]. Medium spiny neurons co-express dopamine and µ-opioid receptors [65, 66], and thus it appears that any activation of these neurons triggered by dopamine binding to D1 receptors, is outweighed by the inhibitory effects of µ-opioid receptor stimulation. Indeed, when dopamine and DAMGO are applied simultaneously to a slice preparation, the pathway typically activated by dopamine binding to the D1 receptor is inhibited by DAMGO, causing an overall inhibition of neural activity [67]. Thus, the release of endogenous opioids will likely have an inhibitory effect on medium spiny neurons in the NAC.

Downstream effects of sucrose-induced changes in NAC activity

The inhibition of NAC neuronal activity, has downstream consequences in a number of brain areas. Zhang et al. created an impressive overview of c-Fos-expression in response to intra-NAC infusion of DAMGO, showing that the number of c-Fos-positive cells increases in several brain areas, including the LH [68]. This increase in LH neural activity appears crucial for the effects intra-NAC DAMGO infusion has on fat intake: when a GABA agonist is co-infused in the LH during NAC µ-opioid receptor stimulation, rats no longer increase their fat intake [69]. Furthermore, DAMGO infusion into the NAC increases stimulus-
evoked excitatory signaling in the LH [70]. Within the LH, a subset of neurons called orexin neurons are found to be specifically activated by intra-NAC DAMGO infusion [71], although we were not able to repeat these findings in chapter 6. Whether this is due to experimental differences (our experiment differed from the study by Zheng et al. in timing and diet availability, both of which could affect baseline orexin neuronal activity [72-74]), will have to be determined in future experiments. Overall, the LH appears to be an important downstream target of NAC opioid transmission.

NAC medium spiny neurons have two main pathways through which they project to LH neurons. The first is through direct synapses onto LH neurons, whereas the second pathway consists of projections that go through the ventral pallidum (VP). The first, direct, pathway is comprised solely of D1 receptor-expressing neurons, whereas the indirect pathway that runs through the VP comprises both D1, as well as D2 receptor-expressing medium spiny neurons [75]. Medium spiny neurons are GABAergic [76], and thus opioid-induced inhibition of D1 receptor-expressing neurons that project directly to the LH, will cause LH neuronal activation (as seen upon intra-NAC DAMGO administration [68]). This pathway strongly affects food intake: activation of D1 receptor-expressing medium spiny neuron terminals in the LH rapidly inhibits feeding [77], but whether inhibition of this pathway results in increased fat intake remains to be determined. Involvement of the neuronal populations that comprise the indirect pathway in the control of food intake is less studied. The VP receives GABAergic input from the NAC [78], and sends both GABAergic and glutamatergic projections to the LH [79]. Infusion of a GABA antagonist or DAMGO into the VP triggers food intake [80], and LH neural signaling is needed for this increase in food intake [81], but it is unknown what type of VP neurons projecting to the LH are involved. An increase in LH neural activity is essential for the effects intra-NAC DAMGO has on food intake [69], the most likely scenario would be that in order to stimulate the LH through the VP, an increase in glutamatergic VP neuronal activity occurs. However, optogenetic activation of glutamatergic VP neurons causes behavioral avoidance [82], whereas ablation of these neurons increases sucrose reward seeking [83]. It thus remains to be investigated what the precise role is of the different neural populations that project from the VP to the LH in the control of feeding behavior.

**LH neuronal populations involved in sucrose-stimulated fat intake**

Thus far, it appears that sucrose triggers endogenous opioid release in the NAC, which inhibits NAC neural activity, causing an increase in LH neuronal activity either through direct projections, or through projections that run through the VP (Figure 1). The LH itself consists of two main neuronal populations, GABAergic and glutamatergic neurons, that can be further subdivided into 30 subpopulations [84]. Activation of GABAergic neurons stimulates consummatory behavior [85], whereas stimulation of glutamatergic neuronal inhibits food intake [86]. It therefore may seem likely that GABAergic LH neurons are responsible for triggering the increase in fat intake observed after NAC opioid stimulation, but
because both main populations are so heterogeneous, the answer is not so straightforward. Within glutamatergic LH neurons, important subpopulations for the control of food intake include orexin-expressing, melanin-concentrating hormone (MCH)-expressing, somatostatin-expressing and thyrotropin-releasing hormone (TRH)-expressing neurons [84]. As previously stated, orexin neurons have been implicated with the effects on food intake triggered by intra-NAC DAMGO infusion. In particular, blocking orexin signaling in the ventral tegmental area (VTA) prevents the rise in fat intake upon NAC opioid stimulation [71]. While stimulation of all glutamatergic LH neurons reduces food intake [86], pharmacogenetic activation of orexin neurons stimulates food intake [87]. Likewise, in line with its name, intracerebroventricular infusion of orexin increases feeding [88, 89], whereas administration of an orexin antagonist reduces food consumption [90]. While orexin neurons are thus a very likely candidate to mediate DAMGO’s effects on food intake, we did not observe any differences in orexin neuronal c-Fos expression between vehicle or DAMGO treated rats (chapter 6). Future studies will thus have to determine the exact role of orexin neurons.

Another possible candidate for mediating an increase in food intake upon NAC opioid stimulation, are MCH neurons. While specific activation of this neuronal population does not increase food intake significantly, optogenetic activation of MCH neurons while mice receive a food reward, enhances reward consumption. This points to a role for MCH neurons in reinforcing the value of a reward [91]. Furthermore, mice lacking MCH neurons are hypophagic and weigh less [92], whereas overexpression of MCH causes overeating and subsequent weight gain [93]. A recent study adds another perspective on the role of MCH neurons in the control of food intake, as Noble et al. showed that MCH neurons project to the cerebrospinal fluid (CSF) and release MCH there [94]. When these CSF-projecting MCH neurons are activated, food intake increases [94]. It would be highly interesting to study whether this specific type of MCH neurons is also involved in stimulating effects of intra-NAC DAMGO infusion on food intake.

The recent study describing the various LH subpopulations reported that somatostatin neurons in the LH can be further subdivided into four clusters, one of which is glutamatergic, while the other three are GABAergic [84]. Intracerebroventricular somatostatin infusion increases food intake [95], whereas peripheral administration of somatostatin reduces food intake [96]. Somatostatin-producing neurons are present in multiple brain areas, including several hypothalamic nuclei [97], which could all account for the feeding effects seen upon central infusion of somatostatin. For the glutamatergic somatostatin LH neurons, Mickelsen et al. found that this population is only present in the perifornical area of the LH [84], and sends dense projections to the dorsal lateral septal nuclei, but little is known about their role in food intake regulation.

The last important subpopulation of glutamatergic LH neurons to mention consists of TRH neurons. Administration of TRH into the 3rd ventricle, directly into the LH or peripherally
reduces food intake [98-100]. Because of this, it is unlikely that the LH neurons that are activated upon intra-NAC DAMGO administration are TRH neurons. The reduction in food intake upon general glutamatergic LH neuron activation [86] could possibly be accounted for by stimulating TRH neurons, which will have to be further investigated.

To conclude, the possible involvement of glutamatergic LH neurons in the opioid-induced increase in fat intake is complex (Figure 1). On the one hand, based on the increased LH c-Fos expression after intra-NAC DAMGO infusion [68], and the inhibition of food intake when a GABA agonist is co-infused into the LH during NAC opioid stimulation [69], an increase of LH neuronal activity is expected to stimulate fat intake. On the other hand, it is unlikely that the entire population of glutamatergic LH neurons is activated, as stimulation of this population decreases food intake [86]. However, activation of specific subpopulation of glutamatergic LH neurons can increase food intake, and thus involvement of particular subpopulations cannot be ruled out. Furthermore, it is possible that endogenous opioid release in the NAC stimulates some LH neurons, while inhibiting others, and that the combination of both is required for the increase in fat intake. Future studies investigating the different subpopulations are needed to unravel this.

In chapter 7, the effects of sucrose drinking on glutamatergic LH neuronal activity were studied, in mice fed a chow or fcHF diet. To compare the results from these experiments to findings described in chapter 6, in which we only investigated rats on a fcHF diet, the focus for this discussion will be on the data from fcHF diet-fed mice. In these mice,
sucrose drinking and water drinking elicited a similar response in glutamatergic LH neurons. This does not support a general role for glutamatergic LH neurons in sucrose-triggered fat intake. However, a few cautionary remarks need to be made before drawing a firm conclusion. Firstly, we studied the glutamatergic population as a whole and did not differentiate between different glutamatergic populations. As outlined above, it is possible that a specific subpopulation of glutamatergic LH neurons is involved. Secondly, we do not know whether the data in mice can be extrapolated to rats, as we used rats in chapter 6 to study the opioid system in the NAC, whereas we tested glutamatergic neuronal activity in mice in chapter 7. Lastly, and perhaps most likely, two different components of the response to sucrose are studied in these two chapters. In chapter 7 the direct neural response upon tasting sucrose is studied. This response occurs seconds after mice drink the sucrose solution. In chapter 6 on the other hand, the response induced in the hours after consumption of sucrose drinking, when glycaemic levels have increased, is explored. We cannot conclude from the experiments performed in chapter 6 whether opioids are also released in the NAC upon tasting the sucrose solution. However, it has been shown that in the dorsal striatum, enkephalin is released rapidly upon the start of sugar consumption, which suggests that even prior to fully digesting the sugar endogenous opioids are released [56], but this will have to be investigated in the future.

The other main population of LH neurons, GABAergic neurons, may be a more probable candidate to mediate the increase in fat intake seen upon NAC opioid stimulation, as activation of these neurons increases feeding and consummatory behavior [85]. Like the glutamatergic LH neurons, GABAergic LH neurons can be further divided into many different subpopulations, although the molecular phenotype of these subpopulations was only recently discovered [84]. Two important subpopulations for the control of food intake are the galanin and neurotensin-expressing neurons. Galanin-expressing neurons are not exclusively GABAergic; in fact approximately only half of all galanin neurons produce GABA [101]. Furthermore, unlike GABAergic LH neurons that send projections to the VTA, galanin LH neurons do not innervate the VTA, but instead project to orexin neurons, among others [101]. Activation of galanin LH neurons induces food intake, but not as much as activation of GABAergic LH neurons, suggesting another subpopulation is involved [101]. This subpopulation is unlikely to be the neurotensin neurons, as chemogenetic activation of neurotensin neurons reduces food intake and promotes weight loss [102]. Which other subpopulation of GABAergic LH neurons promotes food intake and contributes to a sucrose-triggered increase in fat intake, remains to be investigated.

In unpublished data (Figure 2), we have explored the direct effects of sucrose drinking on the activity of GABAergic LH neurons, both in chow-fed as well as in fCHF diet-fed mice. These data indicate that in both diet groups, sucrose increases GABAergic LH neuron activation. These data further underline the possibility of a sucrose-induced release of endogenous opioids, which causes disinhibition of GABAergic LH neurons, thereby possibly stimulating fat intake. Future experiments will have to explore this neural network.
Figure 2. Sucrose increases activity in LHVGAT neurons, regardless of diet consumption. A. ΔF/F over time after delivery of water or sucrose at t=0 in chow-fed mice (left) and fcHFD-fed mice (right). B. Average ΔF/F after delivery of water (chow W n=187 neurons, fcHFD W n=221 neurons) or sucrose (chow S n=172 neurons, fcHFD S n=212 neurons).

Other possible brain areas involved in sucrose-stimulated fat intake

While the LH is one of the most important downstream areas of the NAC for the control of food intake [77, 103], it is important to note that other pathways could also be involved. Particularly the connection between the NAC and the VTA, that consists of neurons either directly projecting to the VTA [104] or through the VP [105], is of great interest for the control of food intake. For example, stimulating the neurons projecting from the NAC to the VTA inhibit food-seeking and consumption [106]. Thus, it is possible that the sucrose-induced release of endogenous opioids inhibits NAC neurons that project to the VTA, thereby stimulating fat intake. Furthermore, input from other brain areas could affect the connection between the NAC and LH. For example, the amygdala, an area known to modulate sucrose preference [107], can influence NAC activity through synapses onto neurons in the NAC that project to the LH [108]. In addition, neurons from the basolateral amygdala, can also directly inhibit LH MCH neurons [109]. How the NAC, amygdala and LH interact during the response to sucrose, and in the stimulating effects on fat intake, is yet to be determined.

Future directions

To further explore the neural network that mediates a sucrose-induced increase in fat intake, many aspects still need to be studied. First of all, attempts could be made to better visualize the release of endogenous opioids in the NAC upon sucrose drinking. For this, a similar microdialysis set up as used in the study investigating the dorsal striatum could be employed [56]. Because so far we were mainly interested in the stimulating effects of sucrose on fat intake, experiments investigating c-Fos after sucrose or intra-NAC DAMGO administration
were only performed in rats fed a fcHF diet. However, it would be important to perform experiments (or the proposed microdialysis study) comparing chow-fed and fcHF diet-fed animals, to determine whether endogenous opioid release after sucrose consumption is different in rats on a different dietary background. Secondly, it is also possible to manipulate the different NAC neurons that project directly to the LH, or to the VP during sucrose consumption with opto- or chemogenetics, to explore whether the direct or indirect pathway is involved in a sucrose-stimulated increase in fat consumption. Lastly, to better understand the LH subpopulations involved in the neural response to sucrose drinking, future studies could use the experimental paradigm described in chapter 7, but use a transgenic mouse model to target the different subpopulations of LH neurons, such as the orexin, MCH or galanin neurons. Overall, these experiments will aid in understanding how different dietary components from the Western diet interact to stimulate each other's consumption, causing overeating and significant weight gain.
References

56. DiFeliceantonio, A.G., et al., Enkephalin surges in dorsal neostriatum as a signal to
72. Estabrooke, I.V., et al., Fos expression in orexin neurons varies with behavioral
91. Dilsiz, P., et al., MCH Neuron Activity Is Sufficient for Reward and Reinforces...
108. Kiorouac, G.J. and P.K. Ganguly, Topographical organization in the nucleus accumbens of afferents from the basolateral amygdala and efferents to the lateral