Central regulation of glucose metabolism

Effects of nutrients, serotonin and dopamine

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Summary

General discussion

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The overall aim of this thesis was to study the role of nutrients in the central regulation of body weight and peripheral glucose metabolism. To this aim, we studied the effects of nutrients, either derived from a free-choice high-fat high-sugar (fcHFHS) diet or directly infused towards the brain, on nutrient sensing pathways in the hypothalamus and on glucose metabolism. Secondly, we aimed to study a nucleus accumbens-lateral hypothalamus-liver axis in the regulation of glucose metabolism and studied neurotransmitter input from the nucleus accumbens (NAc) to the hypothalamus in relation to glucoregulation.

Obesity is a serious health condition, characterized by overconsumption of (calorie dense) nutrients, and is turning into epidemic numbers. Since body weight regulation is orchestrated by the brain, the understanding of the interaction between nutrients and the brain is essential to unravel the pathophysiology underlying the development of obesity. The hypothalamus has been firmly established as a master regulator of glucose homeostasis, and since insulin resistance is a hallmark of the obese condition, studying neuronal circuits in the control of glucose metabolism may provide new therapeutic targets. Experimental models, in which animals are provided with diets enriched in fat and sugar, provide a way to study obesity and mimic a Western style diet. However, most of these diets lack the component of choice, that is, all the nutrients, including the additional fat and sugar, are processed in one pellet. To incorporate the aspect of choice and to study the contribution of individual dietary components of the diet in the development of obesity and insulin resistance, we use a free-choice high-fat high-sugar diet (fcHFHS). This consists of a dish of saturated fat and a bottle of 30% sugar water in addition to regular chow and tap water. Rats on a fcHFHS diet for a short period of time become hyperphagic and leptin and insulin resistant, while these effects are less apparent when providing rats with only fat (fcHF) or sugar (fcHS) in addition to regular chow and tap water. Thus, the combined consumption of fat and sugar results in sustained hyperphagia, obesity and insulin resistance. Animals on the fcHFHS diet also show altered neuropeptide expression in the arcuate nucleus (Arc) of the hypothalamus, that is, neuropeptide Y (NPY) expression is increased and pro-opiomelanocortin (POMC) expression is decreased. These alterations could drive the observed hyperphagia. Interestingly, neurons within the hypothalamus, including those containing POMC and NPY, can sense glucose and fatty acids, but it remains to be determined how nutrient sensing is affected by consumption of a fcHFHS diet, and how these expected central changes contribute to peripheral insulin resistance in these conditions.

In **chapter 1**, we summarized the literature on the effects of high-fat diets (HFD) on gene expression in nutrient sensing pathways in the rodent hypothalamus. In addition, we measured expression of genes involved in hypothalamic nutrient sensing and showed that rats on a one-week fcHFHS diet have an increased mRNA expression of fatty acid synthase (FAS), acetyl CoA carboxylase (ACC) and the brain specific homologue carnitine palmitoyl transferase (CPT)1c in whole hypothalamic lysates, suggesting an alteration in hypothalamic lipid metabolism.

Hormones and nutrients that enter the brain have to cross the blood-brain-barrier (BBB), except when going through the median eminence (ME) near the Arc, which has a leaky structure. The cerebrospinal fluid (CSF)-brain barrier at the third ventricle, next to the hypothalamus, consists of tanycytic cells, which are linked together with tight junctions. Substances can pass these barriers either via diffusion, (active) transport or paracellular

passage, where in the latter case the expression of tight junctions determines the passage over the BBB and CSF-brain barrier. In addition, glucose transporter 1 (GLUT-1) controls influx of glucose over the BBB and it has been shown that some HFDs can alter BBB permeability and tight junction and GLUT-1 protein expression. Furthermore, triglycerides from a HFD cause leptin resistance at the BBB. We hypothesized that the hypothalamic changes in Arc neuropeptide expression and the central leptin resistance we observed after exposing rats to the fcHFHS diet, might be due to altered permeability of the BBB. We investigated this in **chapter 2**, by studying the BBB in rats exposed to the fcHFHS diet for one week. We measured diffusion of Evans blue dye over the ME and measured the protein expression of tight junctions and GLUT-1. As acute fasting has been reported to alter BBB function, we assessed this under both feeding and fasting conditions. We observed no changes in BBB permeability, nor changes in expression of the tight junction proteins occludin and claudin-5, as well as intracellular zonula occludens protein 1 and GLUT-1, irrespective of whether animals were fed or fasted.

The main aim of this thesis was to unravel the direct effects of nutrients on the brain and glucose metabolism. Exposure to the fcHFHS diet has been shown to rapidly increase adiposity, circulating glucose and FA levels, and cause insulin resistance. In order to assess the acute effects of nutrients on the brain without these peripheral events, we directly infused nutrients into the carotid artery to activate nutrient sensing neurons in the hypothalamus and determined effects on glucose metabolism, in particular endogenous glucose production (EGP). In **chapter 3** we show that the experiments using two catheters in the carotid artery and one catheter in the jugular vein are feasible and can be performed in metabolic cages without disturbing basal glucose metabolism, corticosterone levels and energy expenditure.

Using this technique, we assessed the direct effects of intracarotically infused nutrients on hypothalamic gene expression and EGP. We infused Intralipid®, an emulsion of triglycerides, glucose or the combination of both and show in **chapter 4** that the combination of Intralipid® with glucose increases EGP without changing plasma concentrations of insulin and corticosterone. The observed change in EGP was specific to the combination of fat and sugar, as we did not observe effects when Intralipid® and glucose were infused separately. Since we previously showed changes in expression of hypothalamic genes involved in lipid metabolism after a short-term exposure to the fcHFHS diet and observed changes in genes involved in β-oxidation after infusion of Intralipid® and glucose, we hypothesize that this increase in EGP could be caused by increased β-oxidation in the hypothalamus. It remains to be determined whether this association between altered gene expression and glucose metabolism is causal.

In addition to the hypothalamus, we previously showed that the nucleus accumbens (NAc) was also affected by overconsumption of fat and sugar. Due to its dense neuronal input to the hypothalamus, the NAc was of interest to study with respect to glucose metabolism. In the second part of this thesis, we therefore aimed to determine the role of the NAc and its projection to the hypothalamus, in the regulation of peripheral glucose metabolism. The NAc has dense projections to the lateral hypothalamus (LH) and, earlier, we showed that deep brain stimulation (DBS) of the shell region of the NAc (sNAc) increases circulating levels of glucose and glucagon with concomitant changes in c-fos expression in the LH. Others showed that DBS in the sNAc increases serotonin and dopamine release in this

area. We therefore hypothesized that the alterations in blood glucose levels and other glucoregulatory hormones are a direct consequence of the modulation of neurotransmitters in the sNAc. To test this, we infused either fluoxetine (a serotonin reuptake inhibitor; **chapter 5**) or vanoxerine (a dopamine reuptake inhibitor; **chapter 6**) in the sNAc, via bilateral reverse microdialysis, to determine the role of local endogenous serotonin and dopamine changes in the sNAc on peripheral glucose metabolism.
In **chapter 5**, we show that increasing endogenous serotonin in the sNAc significantly enhances blood glucose levels partly explained by increases in EGP, suggesting an additional effect on glucose uptake. The glucoregulatory hormones insulin and glucagon did not change. In **chapter 6,** we studied the effect of dopamine modulation in the sNAc on peripheral glucose metabolism. We first show that in mice, blood glucose levels and liver phosphoenolpyruvate carboxykinase (PEPCK) mRNA expression were lower after sNAc injection of the dopamine reuptake inihibitor vanoxerine compared to vehicle injection. We next assessed EGP in rats after bilateral microdialysis of vanoxerine and observed a marked decrease in EGP together with a decrease in plasma glucagon concentrations over the first 10 minutes after infusion. Since the sNAc projects to the LH, we hypothesized that the observed effects were through projections of the sNAc to the LH and subsequently, from the LH to the liver. Since projections from the sNAc to the LH are mainly GABAergic, an inhibitory neurotransmitter, we measured the ratio of gene expression of excitatory glutamate transporter (vGLUT) and GABA transporter (vGAT) in the hypothalamus as an indirect measure of GABA transport. We observed an increased vGAT/vGLUT ratio in the hypothalami of vanoxerine infused animals and hypothesized that blocking GABAergic signaling in the LH would prevent the observed effects of vanoxerine on blood glucose concentrations. We therefore injected a GABA antagonist in the LH before injecting vanoxerine in the sNAc and indeed were able to abolish the effects of vanoxerine in the sNAc on blood glucose concentrations. In line with the change in GABAergic tone, we found that vanoxerine increases the probability to excite dopamine receptor 1 (D1) expressing neurons and showed that these neurons terminate in the LH and provide inhibitory GABAergic terminals onto LH neurons, confirming that there is a direct GABAergic projection from the sNAc via D1 neurons to the LH. The LH innervates the liver via vagal efferents and in the final experiment, we show that parasympathetic denervation of the liver prevents the effects of vanoxerine infusion in the sNAc on glucose and EGP. In **chapter 7** the results summarized above are discussed in the context of existing literature.

Taken together, in this thesis we show that short-term overconsumption of a Western style diet high in saturated fat and sugar leads to alterations in the nutrient sensing pathway of the hypothalamus, where the intracarotid infusion of both fat and sugar has direct effects on expression of genes involved in hypothalamic lipid metabolism. The short-term access to this Western style diet did not affect blood-brain-barrier permeability or expression of tight junction proteins nor the glucose 1 transporter in the hypothalamus. Secondly, we show that the sNAc, a brain area important in the rewarding and motivational aspects of feeding behavior, acts as a regulator of glucose metabolism, amongst others via serotonin and dopamine neurotransmitters. The sNAc-dopamine-LH-liver axis is GABAergic, involves activation of D1 receptor expressing neurons in the sNAc, and depends on parasympathetic innervation of the liver. These novel insights provide the background for more in depth research on the role of the reward system in the control of glucose metabolism and body weight regulation.

General Discussion

The brain is crucial in the regulation of glucose metabolism and changes in the brain are associated with obesity and diabetes [1]. As the prevalence of obesity and diabetes is rising, together with the increased intake of foods and beverages high in fat and sugar, it is of importance to:

1. Gain more insight into the role of nutrients acting in the brain and how changes in the brain affect glucose metabolism.
2. Expand our knowledge on the central regulation of glucose metabolism, beyond the classical pathways.

Together this will add to a better understanding of how glucose homeostasis is disturbed under conditions of excessive intake of energy-dense food and/or obesity.
The hypothalamus is known to be the main homeostatic glucose regulator. It contains neurons that sense and respond to alterations in levels of metabolic substrates including circulating glucose and fatty acids (FA's) [2]. We, therefore, first studied the effect of a hypercaloric diet, the free-choice high-fat high-sugar (fcHFHS) diet and the effect of direct infusion of nutrients towards the brain, on nutrient sensing pathways in the hypothalamus and the central actions of nutrients on glucose metabolism. Given the important role of the blood-brain-barrier (BBB) as gateway of nutrients and signaling molecules to enter the hypothalamus, we also studied whether the BBB at the level of the hypothalamus, is altered after short-term consumption of a fcHFHS diet.
The homeostatic actions within the hypothalamus do not stand on their own. The hypothalamus receives input from several areas throughout the brain, including the nucleus accumbens (NAc), part of the mesolimbic system. The NAc is clearly involved in the rewarding and motivational aspects of feeding behavior and consumption of a fcHFHS diet has been shown to affect the mesolimbic circuitry [3, 4]. The NAc, and specifically the shell region of the NAc (sNAc), has been shown to densely project to the lateral hypothalamus (LH) [5], which in turn contains pre-autonomic neurons that project to the pancreas and liver to regulate glucose homeostasis [6-9]. The sNAc is therefore an interesting target to study with regards to glucose metabolism. In line, we have shown earlier that deep brain stimulation (DBS) of the sNAc increased blood glucose levels, together with increased neuronal activation in the LH [10]. However, the mechanisms behind this sNAc effect on glucose metabolism remain to be determined.

The overall aims of this thesis were:

1. Study the effects of lipids and sugar on the BBB, on hypothalamic nutrient sensing pathways and on glucose metabolism (**part 1**).
2. Study the role of neurotransmitters in the sNAc in the regulation of glucose metabolism (**part 2**).

PART 1

The hypothalamic nutrient sensing pathway

The hypothalamus has an important role in the regulation of glucose metabolism. It is in direct contact with the blood stream through its fenestrated BBB, which allows for paracellular diffusion or (active) transport of small molecules, nutrients and hormones to sample peripheral energy needs. When energy demand changes, the hypothalamus orchestrates an appropriate response and modulates glucose metabolism through autonomic innervation of organs involved in glucose control as well as through release of humoral signals [11, 12]. This mechanism to maintain homeostasis can be disturbed due to overconsumption. To understand how this response is altered by overconsumption of specific nutrients and to study this paradigm in a human-like setting, we used the animal fcHFHS diet model [13].

Sensing in the hypothalamus

In **chapter 1** we showed that a one-week exposure to a fcHFHS diet increases expression of several genes in the glucose and FA sensing pathways in the hypothalamus. We found that the fcHFHS diet increased levels of acetyl-CoA carboxylase (ACC) mRNA. ACC converts acetyl-CoA to malonyl-CoA, which is shown, in the hypothalamus, to decrease food intake via inhibition of β-oxidation by inhibiting CPT1a, the rate limiting enzyme for transport of fatty acids FA into the mitochondrion and therefore β-oxidation [14]. In addition, we observed a small increase in fatty acid synthase (FAS) mRNA expression. Despite the increase in ACC, animals on a fcHFHS diet remain hyperphagic. Of note, increased mRNA ACC levels do not necessarily imply increased protein levels nor activity, i.e. decreased phosphorylation of the protein. ACC is expressed in two isoforms, α and β, both expressed in the hypothalamus, and leptin specifically activates the α isoform in the Arc [15]. Given the changed leptin concentrations and alterations in leptin sensitivity on the fcHFHS diet [16], it would be important to also assess, in future, phosphorylation of both isoforms. We did not find changes in CPT1a, but several lines of evidence support the view that CPT1a might not be the (only) downstream target of malonyl-CoA in the Arc to alter food intake [15, 17]. It was shown that leptin enhances ACC activity in the Arc and increases malonyl-CoA levels, both leading to decreased food intake [15], however these effects were independent of CTP1a [18]. In contrast, it was shown that in the VMH, CPT1a is regulated by malonyl-CoA [19], and that chronic overexpression of CPT1a in the VMH leads to hyperphagia and obesity via alterations in neurotransmitter vesicular transporters and NPY receptor NPY5R in the Arc [20]. These nuclei specific pathways underscore the importance of studying expression patterns in separate nuclei instead of the hypothalamus as a whole.

Interestingly, we did observe an increase in the expression of CPT1c in the hypothalamus after a one-week fcHFHS diet. CPT1c is an isoform of CTP1 exclusively expressed in the brain. It has little acetyltransferase activity and is found in the endoplasmic reticulum and not in the mitochondrion like the other isoforms, and thus seems not to be involved in β-oxidation of long-chain fatty acids (LCFA's). It is expressed widely throughout the brain, including the cortex, hippocampus, striatum and hypothalamus and is involved in cognition, motor activity and energy homeostasis [21]. Its actions on food intake regulation are hypothesized to be regulated via ceramide. Overexpression of CTP1c in

the Arc of rats increased ceramide levels and increased NPY expression and food intake in animals being refed after an overnight fast [22]. Its overexpression further blocked leptin's ability to downregulate NPY and attenuated leptin's inhibition of food intake when leptin was injected in the Arc [22]. These findings show that in the Arc, leptin's actions on NPY might be modulated by ceramide, downstream of CPT1c and malonyl-CoA. Others showed opposite results with ghrelin, an orexigenic hormone; ICV ghrelin increases ceramide levels and fails to increase food intake and NPY when CPT1c is knocked out in mice, or when blocking ceramide de novo synthesis [23].
This led us to propose a new hypothesis for a mechanism underlying the sustained hyperphagia during a fcHFHS diet, *i.e.* an increase in CPT1c and ceramide levels in the Arc, downstream of malonyl-CoA (**figure 1**). We have shown before that ICV leptin injections in fcHFHS-fed animals lower food intake, however leptin did not decrease NPY expression (or increase POMC expression), compared to chow control animals, pointing to leptin resistance of the Arc [4]. Leptin resistance in the Arc would lead to less ACC activation followed by less increase in levels of malonyl-CoA, a disinhibition of CPT1c, increased ceramide levels and subsequently increased NPY expression and food intake.

The fcHFHS diet and permeability of the BBB

The hypothalamus and particularly the Arc, are in close contact with the median eminence (ME) part of the BBB with a leaky structure. Since it was shown that rats on a fcHFHS diet are leptin resistant at the Arc level and elicit altered NPY and POMC gene expression [24], we hypothesized that these effects could be partly explained by a disrupted permeability of the BBB. We, therefore, subjected rats to one week of a fcHFHS diet, and studied the ME-Arc complex using 2 methods; 1) using Evans blue diffusion over the ME and 2) assessing protein expression of tight junction (TJ) proteins in the ME-Arc area (**chapter 2**). Previous studies have shown that a short-term HFD caused increased expression of occludin, a tight junction protein involved in the paracellular passage of small molecules over the BBB [25], and that a chronic HFD diet is associated with increased passage of molecules over the BBB [26, 27]. However, results were contradictory [26, 28] and seem to be dependent on duration and composition of the diet. Our results show that a one-week fcHFHS diet, known to induce hyperphagia, leptin resistance and alterations in NPY and POMC expression in the Arc in rats [24], did not affect permeability of the ME. Feeding status is an important determinant of tight junction protein expression with fasting rapidly increasing TJ protein expression in the ME [29], suggesting that experimental conditions with regard to fasting duration might impact the results. Since we hypothesized an increase in protein expression, we studied our rats in the non-fasting state. However, we also added a group of animals on a fcHFHS diet that were fasted overnight and compared Evans blue diffusion and protein expression of these rats with those from fasted or non-fasted chow rats. Interestingly, we did not observe increased diffusion or alterations in protein expression of zonula occludens protein 1 (ZO-1), claudin-5 and occludin, i.e. BBB permeability, in the fasting condition. This is in contrast with what has been found by others [29]. The discrepancies might be explained by the use of a different animal (mice vs. rats) and the longer fasting period that was used (24 hr vs. 16-18 hr).
In addition to tight junction proteins, we also studied the glucose transporter 1 (GLUT-1), which transports glucose from the blood into the brain. Fasting has been shown to time-dependently increase GLUT-1 protein expression in the hypothalamus [30]. An increase

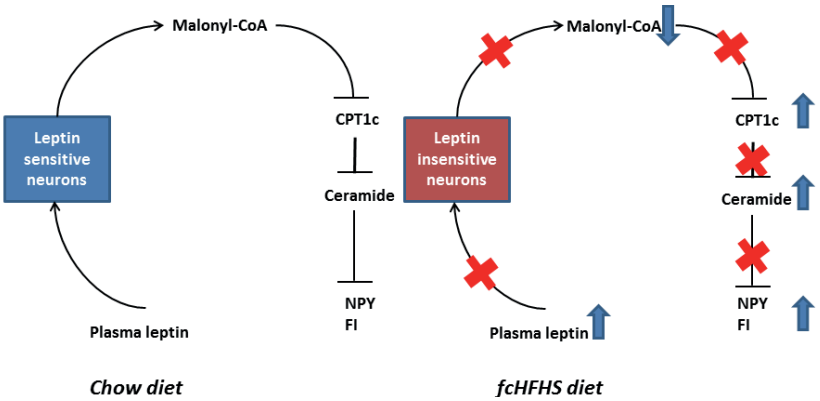

Figure 1. Proposed pathway by which altered CPT1c after a fcHFHS diet (right) contributes to increased NPY expression and hyperphagia, in contrast to chow control conditions (left). fcHFHS-fed animals are leptin insensitive at the level of the arcuate nucleus and thus increased plasma leptin fails to increase malonyl-CoA levels. This leads to a disinhibition of CPT1c, increases ceramide levels leading to increased NPY and food intake (FI).

in circulating glucose levels due to the fcHFHS diet-induced insulin resistance might alter the expression or sensitivity of GLUT-1 and thus affect the passage of glucose over the BBB. It has recently been shown that HFD exposure for 3 days downregulated GLUT-1 protein expression in mice, as measured in isolated vascular endothelial cells. Strikingly, prolonged HFD (28 days), restored GLUT-1 protein expression, facilitated by an increase in vascular endothelial growth factor (VEGF) in macrophages, suggesting a compensatory mechanism [31]. GLUT-1 is detected in the brain as two forms with different molecular weights, the 55 kDa form, responsible for the passage of glucose over the BBB, and the 45 kDa form, hypothesized to be primarily located in astrocytes and responsible for glucose transport into glia cells [32]. We did not find changes in GLUT1 protein expression upon our dietary intervention. This does not necessarily mean that translocation of GLUT-1 (which defines the amount of glucose that can be transported into the cell) or GLUT-1 protein did not change. This should be assessed in future studies. In addition, we observed bands of 45 kDa, and therefore assessing GLUT-1 expression specifically in astrocytes either *in vitro* or with immunofluorescent stainings in the ME barrier, that surrounds the vascular wall, might be focus of future studies. Finally, GLUT-1 and TJ protein expression can undergo posttranscriptional regulation and are not evenly distributed over the 3rd ventricle, Arc and ME and thus assessing phosphorylation of the proteins and conducting localization studies using an immunofluorescent approach, or in vitro BBB models [33] are necessary to fully exclude effects of the fcHFHS diet on the BBB.
In conclusion, we show that short-term exposure to a fcHFHS diet did not alter ME BBB permeability and thus the alterations in Arc neuropeptide expression after a fcHFHS diet are likely explained by other factors than changes in the BBB.

Peripheral versus central nutrients
To explore the direct effect of nutrients on the expression of metabolic genes within the hypothalamus, we infused lipids, glucose or the combination of both, directly towards the brain via a carotid cannula (**chapter 4**).

In line with what we observed in rats exposed to the fcHFHS diet, infusing lipid and glucose concomitantly increased expression of ACC and to a lesser extent FAS, whereas lipids or glucose infused separately did not. These preliminary results deserve further study. As discussed earlier, an increase in ACC increases malonyl-CoA and inhibits β-oxidation through inhibition of CPT1a. Studies focusing on hypothalamic lipid metabolism and energy sensing through AMPK upon exposure to excessive amounts of fat and sugar would be necessary to unravel the underlying pathophysiological pathways of hypothalamic control of glucose metabolism and food intake on a Western diet. Studying isolated effects of macronutrients on these pathway provide important insights in the direct role of nutrients in the brain without the bias of differences in body weight and metabolic state, which occur when animals consume a fcHFHS diet. It is, however, important to note, that during a meal many hormonal changes occur that may affect the brain as well, which could be important to add to the infusion regimes in future experiments.

In addition to the ACC-malonyl-CoA-CPT1a pathway, we also studied expression of the peroxisome proliferator-activated receptors (PPARs). The PPARs are ligand activated transcription factors that regulate expression of genes involved in cell differentiation and various metabolic processes, especially lipid and glucose homeostasis. The PPAR family comprises three isoforms: PPARγ, PPARα and PPARβ/δ and it has been shown that unsaturated fatty acids serve as their natural ligands. All three isoforms are expressed in the brain but not much is known about their central function in the regulation of energy homeostasis. As shown in **chapters 1 and 3**, a fcHFHS diet did not affect PPARγ gene expression while the infusion of the combination of lipids and glucose increased PPARγ mRNA expression. A possible explanation lies in the difference between the used fat sources in both experiments. Fatty acids serve as natural ligands for PPARs and PPARβ/δ interacts with both saturated fatty acids (SFA) and unsaturated fatty acids, which could explain the increase of this gene in both models. In contrast, PPARγ predominantly binds polyunsaturated FA's (PUFA)[34]. In the fcHFHS diet, the dish of fat contains around 40% SFA and 8% PUFA, whereas the Intralipid® infused via the carotid artery is composed of 16% SFA and 60% PUFA. The direct infusion of a lipid emulsion composed of high amounts of PUFA might be able to activate hypothalamic PPARγ whereas the fcHFHS diet, with a higher amount of SFA, does not. The exact involvement of PPARγ in central glucose regulation is currently under investigation in our lab. PPARγ agonist ICV infusion has shown to lead to a positive energy balance in rats [35] and neuronal PPARγ k.o. mice show increased fasting glucose levels and increased insulin levels compared to their littermate controls when on HFD [36]. An overview of the differences in hypothalamic gene expression after a fcHFHS diet and after infusion of lipids and glucose is provided in **table 1**.

Central nutrients and glucose metabolism

As we show in **chapters 2 and 3**, providing rats with a fcHFHS diet or infusing nutrients directly towards the brain, alters metabolic gene expression in the hypothalamus. Peripherally, rats on a fcHFHS diet show increased leptin levels, hyperglycemia and glucose intolerance after one week [37]. Following up on this study, it was shown that fcHFHS diet resulted in both hepatic and peripheral insulin insensitivity whereas fcHF rats were only resistant at the level of the liver, despite being equally obese [38]. These data suggest that the palatable component consumed plays a role in the development of site-specific insulin

sensitivity. The peripheral alterations in rats on the fcHFHS diet were accompanied by an increase in NPY and a decrease in POMC mRNA expression in the hypothalamus [24], whereas the fcHF diet animals showed an opposite hypothalamic responses compared to the fcHFHS diet rats; a decreased NPY and increased POMC gene expression. This led us to hypothesize that the combined nutrients from this diet could directly and differentially affect hypothalamic nutrient sensing areas to affect peripheral glucose metabolism. To test this, we combined the intracarotid infusion of nutrients with an infusion of a glucose tracer to assess EGP to study the direct effect of these nutrients on the hypothalamus and glucose metabolism in freely moving rats (**chapter 3**). In support of our hypothesis, we found an increase in EGP when infusing the combination of Intralipid® and glucose, while EGP did not change upon infusing these compounds separately. Previously it has been shown that ICV infusion of oleic acid decreased EGP [39] and although this approach is not comparable to intracarotid infusion of a lipid emulsion, the addition of glucose to fat might exert opposite effects on EGP. Alternatively, the lipid emulsion we used contained a mix of saturated and unsaturated fatty acids which may have differential effects on EGP compared to infusion of oleic acid alone [40, 41]. ICV infusions of different fatty acids with or without glucose will be informative to test this hypothesis. The observed increase in EGP in our study requires further investigation and we propose to focus on nutrient responsive neurons in the hypothalamus since our results show a clear effect on metabolic gene expression after intracarotid infusion of fat and glucose. In line, it has been shown that fatty acid sensing cells in the hypothalamus alter their firing rate dependent on extracellular glucose concentrations [42], but the direct involvement of these nutrient sensing genes in affecting EGP remains to be determined.

The changes in hypothalamic metabolic gene expression and EGP were independent of changes in plasma concentrations of corticosterone and insulin, which points to a direct effect of the infused nutrients on the liver probably via the autonomic nervous system. Others have shown that intracarotid infusion of Intralipid® led to increased liver acetylcholinesterase activity, which increases breakdown of acetylcholine and reduces activity at the muscarinic receptor and thus could reflect decreased parasympathetic activity in the liver [43]. Furthermore, depriving energy in the hypothalamus results in increased sympathetic activity, increased EGP, and mobilization of substrates restoring peripheral energy [44]. Whether the combination of lipids and glucose leads to increased sympathetic innervation of the liver is subject of future research.

Gene	fcHFHS	Intralipid® + glucose	Intralipid®	Glucose
ACC	↑	↑	=	=
FAS	0.08	=	=	=
CPT1a	=	=	=	=
CPT1c	↑	=	=	=
PPARγ	=	↑	=	↓
PPARα	=	=	=	=
PPARβ/δ	↑	↑	=	=
PGC1α	↑	=	=	=

Table 1. Effects of a fcHFHS diet or intracarotid nutrient infusion on hypothalamic expression of genes involved in central lipid metabolism in rodents (fcHFHS: compared to chow; infusions: compared to saline infusion).

Altogether, we showed that nutrients, either directly infused towards the brain or consumed during a fcHFHS diet affect glucose metabolism associated with alterations in hypothalamic metabolic gene expression. Whether these effects are solely a direct manipulation of nutrients on the hypothalamus or whether other brain areas are involved in these observations remains to be determined. In this regard, the mesolimbic system is of specific interest. The nucleus accumbens (NAc) contains glucose and lipid responsive cells and the fcHFHS diet has been shown to affect the mesolimbic system including the NAc. Here, rats on a fcHFHS diet were more motivated to work for sucrose pellets [3], and NPY injections in the NAc selectively increased fat intake in rats on a fcHFHS diet [45]. Furthermore, the fcHFHS diet significantly increased c-fos expression, indicative for neuronal activation, in the NAc of fasted fcHFHS rats compared to chow animals (unpublished observation). The effect of nutrients on the NAc and glucose metabolism are of interest since it has been shown that the shell of the NAc (sNAc) projects to the LH [5, 46, 47], an important area for regulating glucose homeostasis via autonomic innervation of the liver and pancreas [9, 48, 49].

PART 2

Corticolimbic input to the hypothalamus in the regulation of glucose metabolism

The NAc is known for its role in reward related behavior, including evaluating rewarding aspects of food and food motivation. Given its major role in food intake and the tight relation between regulation of food intake and metabolism in general, a role for the NAc in glucose metabolism seems evident. In line, recent data show overlapping neuronal circuits regulating feeding behavior and glucose metabolism outside of the hypothalamus. First proof for regulation of glucose metabolism by the mesolimbic system was provided by DBS experiments in the shell of the NAc in rats. We showed that DBS of the sNAc increases blood glucose and plasma glucagon concentrations. It also increased neuronal activation in the LH, an output area of the sNAc, and known to regulate glucose metabolism [10]. Since DBS in the sNAC increases serotonin and dopamine levels [50], we specifically studied the role of serotonin and dopamine in the sNAc on glucose metabolism by administering a serotonin reuptake inhibitor (SSRI) or a dopamine reuptake inhibitor (DRI) to enhance local endogenous levels of these neurotransmitters to understand their physiological role in regulating glucose metabolism.

The results of these studies confirm an important role of the mesolimbic system in the regulation of glucose metabolism, and we identified a novel pathway by which dopamine in the sNAc affects glucose metabolism via a sNAc->LH->liver axis.

Neurotransmitters in the sNAc to control glucose metabolism

We first studied the effects of sNAc serotonin on glucose metabolism by infusing the serotonin reuptake inhibitor fluoxetine, which has been shown to increase serotonin levels when infused in the NAc [51, 52]. In the study described in **chapter 5**, we infused fluoxetine via reverse microdialysis in the sNAc and observed a significant increase in blood glucose levels. We combined the sNAc infusion of fluoxetine with the infusion of a stable glucose isotope to assess EGP and to determine possible mechanisms by which fluoxetine

influenced blood glucose levels. We found a modest increase in EGP upon increasing sNAc serotonin suggesting an additional effect on peripheral glucose uptake. The earlier reported DBS-induced increase in blood glucose concentrations was accompanied by an increase in plasma glucagon concentrations, however during fluoxetine infusions in the sNAc plasma glucagon concentrations did not change. To further explore the role of the sNAc in glucose metabolism, we next studied the effect of the dopamine reuptake inhibitor, vanoxerine on glucose metabolism (**chapter 6**). Vanoxerine has been shown to enhance extracellular dopamine concentrations when infused in the NAc [53]. In contrast to serotonin, we observed a decrease in EGP, blood glucose levels and plasma glucagon concentrations compared to saline infusions. In addition, infusion of vanoxerine in the NAc area in mice resulted in lower PEPCK mRNA expression in the liver compared to saline infusion. Together, these data suggest that increasing dopamine concentrations in the sNAc decreases EGP most likely by a decrease in gluconeogenesis. Taken together, dopamine and serotonin within the sNAc have differential roles in regulating glucose metabolism. Below we discuss possible mechanisms by which these sNAc neurotransmitters affect glucose metabolism.

sNAc neuronal populations and their projections

The sNAc mainly contains medium spiny neurons (MSNs) providing dense GABAergic projections to the ventral pallidum (VP) and the LH [46]. The sNAc is innervated by many areas and neurotransmitters which could possibly all be involved in regulating glucose metabolism. However, dense serotonin and dopamine input is provided by the raphe nuclei [54] and ventral tegmental area (VTA), respectively [55]. In addition, the sNAc receives norepinephrine afferents from the locus coeruleus and nucleus of the solitary tract (NTS) and sparse afferents from other brainstem regions, including the pedunculopontine tegmentum, parabrachial nucleus, and periaqueductal gray [54].

The serotonergic projections from the raphe nuclei contact cholinergic interneurons in the sNAc [56]. Next to the MSNs, the striatum contains three groups of interneurons, two groups that are GABAergic, and the cholinergic interneurons. Cholinergic neurons provide the sole source of acetylcholine (ACh) in the striatum, where the levels of ACh are exceptionally high [57]. The cholinergic neurons regulate the duration, strength and spatial pattern of striatal MSNs output by binding to pre- and postsynaptic receptors, thereby influencing neurotransmitter outflow. The effects of ACh on MSNs are mediated by the muscarinic (M) receptors, present on all cell types in the striatum, making ACh an important modulator of MSN activity and thus neurotransmitter release [58]. Previous research has shown that fluoxetine or serotonin infusion in the posterior medial NAc, dose dependently decreased acetylcholine (ACh) outflow [51, 59] and thus, the cholinergic interneuron could be a direct target of extracellular serotonin. ACh increases excitability of GABAergic MSNs via M1 receptors [57] thus, a reduction of ACh outflow by serotonin may lead to decreased GABAergic release to the LH by the MSNs, eventually increasing blood glucose. In line, it has previously been shown that a GABA antagonist (bicuculline) administered to the LH increased blood glucose concentrations and EGP [60]. As DBS of the NAc increased blood glucose concentrations and led to an increase in neuronal activation in the LH, we hypothesize that increasing serotonin in the sNAc reduces GABAergic output to the LH via ACh activation of M1 receptors, which, in turn, increases EGP by the liver through sympathetic innervation. Alternatively, M2/3 receptor

activation by ACh reduces glutamatergic transmission on presynaptic terminals of MSNs [57], and thus decreased ACh outflow after fluoxetine in the sNAc might increase glutamatergic release towards the LH and further increase sympathetic output.

Regarding the actions of dopamine, sNAc dopamine receptor neurons can be roughly divided in MSNs expressing the D1 receptor (D1-MSNs) and substance P and dynorphin and MSNs expressing the D2 receptor (D2-MSNs) and enkephalin. In addition, a small population of enkephalin neurons in the sNAc express both, D1 and D2 receptors. MSNs produce both GABA and glutamate, allowing them to potentially modulate the basal ganglia network bidirectionally [61-63]. sNAc MSNs have been shown to project to the LH [46], with D1 neurons providing the dominant source of LH innervation [64].
In **chapter 6**, we showed that infusing vanoxerine in the sNAc led to an increase in the GABAergic tone in the hypothalamus as assessed by the vGAT/vGLUT mRNA ratio. As the sNAc has been shown to project densely to the LH, we hypothesized the effects of vanoxerine on glucose metabolism to be mediated by GABAergic input to the LH. More specifically, it was recently shown that stimulation of the sNAc (with stimulation of the cNAc as a control), increased GABA transmission in the LH, and not glutamate transmission [65]. We next showed that injecting a GABA antagonist (bicuculline) in the LH, to inhibit GABA transmission, blocked the effects of vanoxerine infused in the sNAc on glucose metabolism.
As mentioned, the sNAc D1-MSNs provide the dominant source of sNAc GABAergic inhibition to LH and provide rapid control over feeding via innervation of LH neurons [64]. In line with the change in GABAergic tone we observed in the hypothalamus, and the role that hypothalamic GABA plays in the vanoxerine-induced effects on glucose metabolism, we found that vanoxerine increased frequency of EPSCs of D1-MSNs without alterations in amplitude. This suggests increased sensitivity for glutamatergic input. Thus, vanoxerine increases the probability to excite D1-MSNs. We furthermore showed, by ChR2:eYFP fluorescence expressing $sNAc^{PDYN/D1}$ neurons, that these neurons provide inhibitory GABAergic terminals onto LH neurons, confirming that there is a direct GABAergic projection to the LH from the sNAc via D1 neurons. We propose that this is the functional pathway by which vanoxerine affects glucose metabolism.
Although we focused on the GABAergic input, which we hypothesize to be a direct innervation from the sNAc to the LH, D1-MSNs also project to the medial VP. Single-axon tracings in rats suggest that sNAc neurons with terminal fields in the LH may also provide collaterals to the VP, thus one and the same axon can project to both the VP and the LH [46]. If, as shown by [66], the D1 MNS project to the LH via the VP, activation of these neurons would lead to a disinhibition, *i.e.*, decreased GABAergic innervation of the LH, since VP → LH innervation is GABAergic [67]. As we observe increased GABAergic innervation in the LH from D1-MSNs and we did not observe clear neuronal activation occurring in the LH after vanoxerine infusion, the sNAc-VP-LH pathway cannot explain the observed effect on EGP. Selectively inhibiting GABAergic transmission in the VP together with the assessment of glucose kinetics could provide more insight in the involvement of this indirect pathway. In addition, there remains a possibility that the reuptake inhibitors infused in the sNAc reach the VP and that the activation of dopamine neurons, present in the VP [68], interfered with the effects in the sNAc.

Vanoxerine increases dopamine in the synaptic cleft and dopamine has a high affinity for both D1 and D2 receptors, thus it is logical to assume that D2 receptors are also activated after vanoxerine application. Therefore, the D2-MSNs might also play a role in the GABAergic inhibition of the LH, even though it has been shown that D1-MSNs provide the dominant source of GABAergic inhibition of the LH [64]. Nevertheless, to fully establish that our effects on glucose metabolism via the LH are regulated specifically by the D1 neurons, or, that projections from D2-MSNs or neurons expressing both receptors, also contribute, experiments with specific D1 and D2 agonists combined with measurement of peripheral glucose kinetics would help to resolve these questions. Alternatively, selective inhibition or activation of the D1-MSN to LH pathway using D1-CRE rats and designer receptor exclusively activated by designer drugs (DREADD, for review see [69]) and measure glucose kinetics while manipulating this D1-MSN to LH pathway provide another elegant way of testing these hypotheses.
To understand the role of the sNAc in glucose control, our first approach was to target endogenous serotonin and dopamine levels separately to study their individual effects on glucose metabolism. It is, however, clear that there is interaction between the neurotransmitter systems. For instance, serotonin can affect dopamine outflow, via acting on 5-HT2c receptors which are abundantly expressed in the sNAc [70]. However, fluoxetine itself does not seem to affect dopamine release as it has limited affinity for the dopamine transporter (DAT) [71]. Although DBS has been described to influence both neurotransmitter systems [50], it is most likely that the serotonergic effect mediated the effects on glucose metabolism.

Targeted LH cell populations after sNAc serotonin and dopamine elevations
It is clear from our data that the vanoxerine effects on glucose metabolism are mediated by the LH. We have shown earlier that DBS of the sNAc, that increases dopamine and serotonin levels [50], increased c-fos expression in the LH [10]. In line with this, it was recently reported that DBS of the NAc increased cerebral blood flow in the LH as measured by fMRI [72]. Also [D-Ala^2, MePhe^4, Gly-ol^5]-enkephalin (DAMGO), a μ-opiod receptor agonist (with high affinity for enkephalin and thus D2 or D1/D2 neurons) injected in sNAc has been shown to induce c-fos expression in the LH [73], and increased signaling in LH neurons [74]. It is thus clear that manipulations in the sNAc affect LH neurons either directly or via the VP [5]. The question, however, remains which cell populations in the LH are the target of the GABAergic MSNs in the sNAc, mediating the effects on glucose metabolism.
Cell populations in the LH, implicated in glucose metabolism, are the orexin and melanin-concentrating hormone (MCH) neurons. Orexin neurons have been shown to be involved in glucose homeostasis including the counter regulatory response to hypoglycemia [75]. Their excitability is modulated by peripheral signals including glucose, leptin and insulin [76]. In addition, orexin expressing neurons have been shown to be activated by infusing the GABA antagonist bicuculline in the LH, which increased EGP and blood glucose levels. The increase in EGP observed after orexin infusion in the LH could be abolished by hepatic sympathetic denervation [60]. Furthermore, orexin knock-out (k.o.) mice show impaired glucose tolerance, insulin resistance and impaired insulin signaling [77]. The role of MCH neurons in regulation of glucose homeostasis is less clear. MCH neurons have been shown to be glucose exciting cells, and some studies showed that they are involved in

the regulation of glucose homeostasis [78, 79]. However, altering MCH in experimental settings does not always have effects; MCH infusion in the LH does not alter plasma glucose levels or EGP [60] and k.o. of the MCH precursor pMCH decreased basal EGP levels but did not affect endocrine dynamics under glucose or insulin challenges [80].
We showed that increased GABAergic transmission in the LH is associated with a decrease in EGP, which could be abolished by administration of bicuculline in the LH. This might suggest decreased orexin activity after sNAc vanoxerine. However, immunohistochemical analysis of the LH did not reveal a vanoxerine-sNAc-induced decrease in neuronal activation of orexin neurons or decreased orexin immunoreactivity. However, it can be difficult to observe a decrease in orexin activation when using a control where baseline orexin activity is already very low. In line with this, it was shown that subcutaneous injection of D1 and/or D2 agonists increased c-fos expression in orexin neurons, however this could not be abolished by lesioning the NAc, pointing to a dopamine effect on orexin beyond NAc dopamine [81]. We also correlated the vGLUT/vGAT ratio to orexin mRNA expression and found a negative correlation, which points to an increased GABAergic tone with increasing orexin expression, suggesting that there could be an intermediate neuron to disinhibit orexin. However, with increased orexin, one would expect an increase in EGP while we observed a clear decrease. Interestingly, orexin peptide and orexin mRNA expression show opposite patterns in their daily rhythm [82], therefore it is possible that the negative correlation of GABA and orexin is a reflection of lower orexin peptide signaling. If orexin is mediating the effect, it will not involve a direct projection from D1-MSN neurons onto orexin cells since we and others, [64] showed that the sNAc-MSNs inhibited cells are orexin and MCH negative and the neurons that are inhibited by D1-sNAc-MSNs are GABAergic. In addition, Stuber & Wise [83] showed that vGAT expressing neurons, do not co-localize with orexin or MCH. Interestingly, others showed that the MSNs from the NAc to the LH innervate glutamatergic neurons and that these neurons are separate from orexin- or MCH-containing neurons [84]. Taken together, our observations and other literature strongly suggests that NAc MSNs do not directly innervate orexin or MCH-containing neurons, however, whether the yet unidentified LH (inter)neurons are GABAergic or glutamatergic, remains to be determined.
Other cells in the LH, hypothesized to be involved in energy metabolism, are neurotensin producing neurons. Peripheral and ICV administration of neurotensin suppresses feeding [85], and a leptin receptor k.o. specifically in neurotensin containing neurons in the LH, induces obesity [86]. In addition, this k.o. model is associated with altered DA transmission in the sNAc, suggesting a reciprocal connection between the sNAc and LH which, next to food intake, might also be involved in the regulation of glucose metabolism. Neurotensin neurons highly co-localize with galanin neurons, but not with MCH and orexin [87]. It will be interesting to investigate the alterations in neurotensin or galanin expression in the LH after sNAc-vanoxerine infusions.

From lateral hypothalamus to the liver

Earlier work has shown that hypothalamic modulation of glucose metabolism involves the autonomic nervous system [48, 60, 88]. We determined whether sNAc vanoxerine affected vagal efferent nerve activity and observed increased vagal activity during vanoxerine infusion. The involvement of the vagal nerve was further supported by our observation that parasympathetic denervation of the liver prevented the sNAc

vanoxerine-induced EGP decrease in rats. The parasympathetic nerves that innervate the liver originate from the dorsal motor nucleus of the vagus (DMV), located in the brainstem, and a projection area of the LH [9, 89]. Insulin in the DMV has been shown to decrease EGP via phosphorylation of ERK1/2 [90], kinases that participate in the regulation of a large variety of processes including cell metabolism, proliferation, and transcription [91]. However, we did not find increases in insulin levels (**chapter 6**) nor differences between vanoxerine and vehicle infused rats in ERK1/2 phosphorylation in the DMV (data not shown).
In conclusion, our results imply that enhanced dopamine and serotonin levels in the sNAc have opposite effects on activity of the LH; dopamine stimulates GABAergic input to the LH and we hypothesize that serotonin decreases GABAergic input, although the latter needs to be confirmed. In turn, dopamine and serotonin in the sNAc led to opposing outcomes on glucose metabolism; serotonin increased blood glucose levels, partly explained by an increase in EGP while dopamine in the sNAc decreased glucose levels and EGP via vagal activation of the liver. It could well be, that sNAc serotonin increases sympathetic nervous output to the liver. The neuronal populations targeted in the LH by serotonin and dopamine remain to be determined. The possible routes by which dopamine and serotonin in the sNAc affect hepatic glucose metabolism is depicted in **figure 2**.

A role for nutrients in the regulation of glucose metabolism by the ventral striatum?
In part 1 of my thesis I described the effects of palatable food components on the hypothalamus and glucose metabolism, which points to a role for direct nutrient effects in diet-induced metabolic dysregulation. In part 2 we showed a new role for

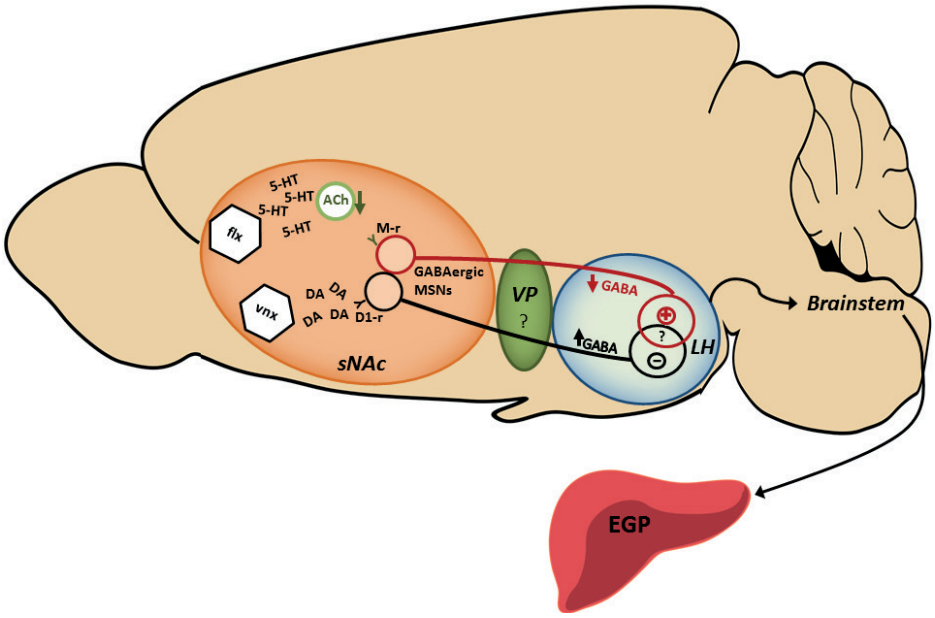

Figure 2. Possible route by which fluoxetine (flx) and vanoxerine (vnx) in the sNAc affect hepatic glucose metabolism. Serotonin, 5-HT; dopamine, DA; D1-r, dopamine receptor type 1; M-r, muscarinic receptor.

serotonin and dopamine in the sNAc, in a sNAc-LH-liver axis in the regulation of glucose metabolism, and it is of interest that changes in these neurotransmitters especially dopamine, have also been shown to occur with diet-induced obesity in rodents, and in humans with obesity. Regarding serotonin, the literature is sparse; a positive association between body mass index (BMI) and 5-HT(4)R density has been found in the NAc in healthy individuals [92], however, in animals, no differences in, for instance, 5-HT2A/2C receptor densities were found in the NAc in DIO mice [93]. For dopamine, several studies show that (morbidly) obese individuals have reduced striatal D2 receptor and D2/D3 receptor availability, as measured with positron emission tomography (PET) [94, 95] and single photon emission computed tomography (SPECT) [96].

In animal models, it has become clear that intake of macronutrients, particularly those high in fat and sugar, and/or their metabolic consequences, affect the mesolimbic system. We have observed an increase in c-fos expression in the NAc of rats after one week of fcHFHS diet (unpublished results). Furthermore, ICV leptin administration in rats after a fcHFHS diet decreased enkephalin expression in the NAc, and increased tyrosine hydroxylase (TH) expression (the rate limiting enzyme for dopamine production) in the VTA compared to vehicle controls. Under basal conditions (without leptin injection), fcHFHS-fed rats show lower TH expression in the VTA compared to chow [4], which was also confirmed by other studies that show decreased TH in the VTA after 6 weeks HFD [97]. It has been shown that under physiological circumstances, insulin in the VTA suppresses excitatory synaptic transmission onto VTA dopamine neurons and reduce aspects of palatable feeding behavior [98]. However, in a hyperinsulinemic mouse model, insulin showed reduced capacity to cause a synaptic depression of VTA dopamine neurons, which may lead to increased food intake [99]. The above findings all point to altered dopamine output of VTA neurons to their target areas including the NAc, either caused by increased nutrient intake or resulting from the consequences of a fcHFHS diet or HFD, *i.e.* increased circulating leptin or insulin levels. Obese rats have decreased extracellular dopamine levels in the NAc [100] and rats on a cafeteria diet for 15 weeks have decreased dopamine release measured *ex vivo* in coronal slices of the NAc [101]. In line with what is found in human obesity, rats consuming a fcHFHS diet for 28 days, show reduced striatal D2/3 receptor availability, specifically in animals that consumed a lot of fat [102]. Interestingly it was recently shown that saturated and not unsaturated FA's suppress dopamine function and signaling in the NAc, independent of peripheral parameters such as body weight gain and glucose, leptin and insulin concentrations [103]. These data point to a direct effect of nutrients on this neurotransmitter system. A more direct role of nutrients in control of the NAc dopamine system has also been established. Cansell *et al.* (2014) show that intracarotid infusion of lipids directly decreased spontaneous and amphetamine-induced locomotor activity in mice. Brain lipid delivery also decreased reward seeking, whereas NAc-selective knockdown of LpL, the enzyme that hydrolyzes triglycerides, increased reward seeking. Finally, brain triglycerides delivery abolished preference for palatable food, whereas NAc-specific LpL knockdown increased palatable food intake [104]. Thus, lipids in the NAc are involved in dopamine related behavior and since we have shown that NAc dopamine affects glucose metabolism, lipid actions in the NAc might be linked to glucose metabolism via dopamine. In addition, glucose has also been shown to affect the NAc; Delaere *et al.*

(2013) showed that hepatic portal vein infusion of glucose increases c-fos expression in the NAc, and triggers dopamine release [105]. Since the NAc has been shown to contain glucose monitoring neurons, peripheral glucose may also act directly at the NAc to control energy metabolism [106].

Lipids and glucose have to cross the BBB, which has the property to dynamically adjust permeability upon fasting or nutrient challenges [29]. Although we did not find evidence that a short-term fcHFHS diet alters BBB permeability at the median eminence (**chapter 2**), BBB permeability and/or nutrient uptake in other regions of the brain including the NAc, might be altered upon short-term or chronic high nutrient intake. This is supported by recent findings showing that 3 days of HFD feeding in mice reduced glucose uptake in the striatum, including the NAc, accompanied by reduced GLUT-1 expression [31]. Prolonged HFD (28 days) feeding, however, restored GLUT-1 expression levels and glucose uptake. Another study showed that feeding rats a Western style diet for 90, but not 10 or 40 days, increased sodium fluorescein diffusion over the dorsal striatum, which suggests increased permeability, however glucose and lipid transport over the striatum was not measured. The discrepancies between these studies, and our observations might be explained by the use of either different animal species, different composition of diets, different time points and/or different methods to assess permeability. Western style diet-induced disruptions of the permeability of the BBB might lead to altered nutrient or hormone entrance to specific brain areas, which then could result in altered food intake behavior, or (regulation of) glucose metabolism. As mentioned before, the NAc contains glucose sensing neurons [106] and insulin receptors [107]. Interestingly, Woods *et al.* (2016) show that DIO rats have decreased insulin receptor activity *i.e.* receptor phosphorylation in the ventral striatum after increases in systemic insulin levels, resulting in an altered food preference. The authors propose that this could be partly due to decreased insulin transport over the BBB [107]. The combination of altered transport over and/or permeability of the BBB after a high palatable diet with elevated systemic levels of glucose and insulin could have detrimental effects on NAc metabolic sensing and behavioral and metabolic outcomes. It would be very interesting for future studies to look at the role of the BBB in the NAc with regard to the nutrient-sNAc-glucose metabolism interaction.

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