Thesis

The effects of intranasal neuropeptide Y on high-fat diet-induced anhedonia of mice

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Graz, 08.11.2023

Declaration of Academic Integrity

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Graz, 08.11.2023 *Maike Haag m.p.*

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Zusammenfassung

Die westliche Ernährung enthält einen hohen Anteil an gesättigten Fettsäuren und ist sehr energiedicht, was zu übermäßigem Essen und der Entwicklung von ernährungsbedingter Fettleibigkeit führt. Es gibt immer mehr Belege dafür, dass Fettleibigkeit bei der Entwicklung von psychischen Erkrankungen wie schweren Depressionen eine Rolle spielt. Zu den Veränderungen im Körper, die bei der Entwicklung von depressiven Verstimmungen aufgrund von ernährungsbedingter Fettleibigkeit eine Rolle spielen, gehören die Dysregulation der Hypothalamus-Hypophysen-Nebennieren-Achse, die Freisetzung von proinflammatorischen Zytokinen, sowie eine Leptin- und Insulinresistenz. Darüber hinaus zeigen sowohl Patienten mit leichter depressiver Störung als auch Mausmodelle nach fettreicher Ernährung eine geringere Expression von Neuropeptid Y (NPY), einem Peptid mit antidepressiven, anxiolytischen und orexigenen (appetitanregenden) Eigenschaften. Ziel dieser Studie war es daher, die Wirkung von intranasalem NPY auf durch fettreiche Ernährung induzierte Anhedonie und sein Potenzial als einen neuen therapeutischen Ansatz zu untersuchen. In dieser Studie fütterten wir 48 männliche Mäuse über einen Zeitraum von 8 Wochen mit einer fettreichen Nahrung mit 48 kJ% Fett oder einer Kontrolldiät mit 12 kJ% Fett. Die Mäuse wurden zu zweit in Käfigen unter Standard-Laborbedingungen untergebracht. Anschließend wurden die Mäuse in Einzelkäfigen untergebracht, um den Saccharose-Präferenz-Test zur Beurteilung einer Anhedonie durchzuführen. An Tag 2 und Tag 4 des Saccharose-Präferenz-Test wurde entweder NPY oder steriles destilliertes Wasser in einer Dosis von 100 µg intranasal verabreicht. 3 Stunden nach der zweiten NPY-Applikation wurden Gehirn- und Blutplasmaproben entnommen. Die Gehirne wurden mikrodissektiert, um anschließend den Hypothalamus auf verschiedene relevante Marker zu analysieren. Das gleiche Experiment wurde mit Y2-Rezeptor-Knock-out-Mäusen wiederholt.

Fettreiche Nahrung verringerte die NPY-Genexpression und die Proteinmenge im Hypothalamus. Intranasales NPY reduzierte die Saccharose-Präferenz in der Gruppe mit fettreicher Nahrung 3 Stunden nach der Applikation, was auf eine Anhedonie hinweisen könnte. Dieser Effekt ging mit einer geringeren Nahrungsaufnahme, einem größeren Gewichtsverlust und erhöhten Corticosteronwerten im Plasma einher. Die Saccharose-Präferenz, die Nahrungsaufnahme und das Körpergewicht blieben in

dem Experiment mit Y2-Knock-out-Mäusen unverändert. Unsere Ergebnisse stehen im Widerspruch zum derzeitigen Wissen über NPY als appetitanregendes und antidepressives Peptid. Die Tatsache, dass NPY die Nahrungsaufnahme hemmte und Anhedonie im Saccharose-Präferenz-Test auslöste, könnte auf die sedierende Wirkung von NPY zurückzuführen sein, die bei höheren Dosen auftritt. Eine andere mögliche Erklärung könnte eine Desensibilisierung gegenüber NPY durch fettreiche Nahrung sein. Da in der Y2-Knock-out-Gruppe kein Effekt beobachtet wurde, könnte der Y2-Rezeptor auch eine Rolle bei unseren Beobachtungen spielen.

Abstract

Western diet contains a high percentage of saturated fatty acids and is dense in energy, which leads to overeating and the development of diet-induced obesity. There is growing evidence about the role of obesity in the development of mental health disorders such as major depression. Metabolic disturbances implicated in the development of depressive mood due to diet-induced obesity include dysregulation of the hypothalamic-pituitary-adrenal axis, release of proinflammatory cytokines, leptin and insulin resistance. In addition, both patients with depressive disorder and mouse models of high-fat diet (HFD)-induced depression show lower expression of neuropeptide Y (NPY), a peptide with antidepressant, anxiolytic and orexigenic properties. Therefore, the aim of this study was to evaluate the effect of intranasal (i.n.) NPY on HFD-induced anhedonia and its potential as a new therapeutic approach. In this study, we fed 48 male mice a HFD containing 48 kJ% of fat or control diet containing 12 kJ% of fat for a period of 8 weeks. Mice were housed in cages of two under standard laboratory conditions. Subsequently, the mice were placed in single cages in order to perform the sucrose preference test (SPT) to evaluate anhedonia. On day 2 and day 4 of SPT either NPY or sterile distilled water was applied i.n. at a dose of 100 µg. Mice were sacrificed 3 hours after the second NPY application in order to collect the brains and blood plasma samples. Brains were microdissected and the hypothalamus was used for analysis of markers of relevance. The same experiment was repeated in Y2 receptor knock-out mice.

HFD reduced NPY gene expression and protein levels in the hypothalamus. Contrary to expectations, i.n. NPY reduced sucrose preference in the HFD group 3 hours after application, which is indicative of anhedonia. This effect was accompanied by lower food intake, greater weight loss and increased corticosterone levels in plasma. Sucrose preference, food intake and body weight remained unchanged in the experiment with Y2 knock-out mice.

Our results are contrary to current evidence about NPY as an orexigenic and antidepressant peptide. The fact that NPY inhibited food intake and induced anhedonia in the SPT may be due to the sedative effect of NPY, which occurs at higher doses. Another possible explanation might be a desensitization to NPY by HFD. Since no effect was seen in the Y2 knock-out group, the Y2 receptor may also play a role in our observations.

Previous publications

The effect of intranasal neuropeptide Y on high-fat diet-induced anhedonia

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Abbreviations

5-HT	serotonin
ACTH	adrenocorticotropic hormone
AgRP	Agouti-related protein
ANS	autonomic nervous system
ARC	arcuate nucleus of the hypothalamus
BBB	blood-brain-barrier
Bdnf	brain-derived neurotrophic factor
BMI	body mass index
cAMP	cyclic adenosine monophosphate
CNS	central nervous system
CRH	corticotropin-releasing hormone
DA	dopamine
fMRI	functional magnetic resonance imaging
FST	forced swim test
GABA	gamma-aminobutyric acid
GLP-1	glucagon-like peptide-1
GR	glucocorticoid receptor
HFD	high-fat diet
HPA	hypothalamic-pituitary-adrenal
i.c.v.	intracerebroventricular
ICD-10	International Classification of Diseases-10
IFN-α	interferon-alpha
IL-1b	interleukin-1 beta
IL-2	interleukin-2
IL-6	interleukin-6
i.n.	intranasal
KO	knock-out
LDL	low density lipoprotein
LPS	lipopolysaccharide
MAO	monoamine oxidase
MCP-1	monocyte chemoattractant protein-1
mPFC	medial prefrontal cortex

mRNA	messenger ribonucleic acid
NE	norepinephrine
NF-kB	nuclear factor kB
NIRKO	neuronal insulin receptor knock-out
NPY	neuropeptide Y
PKC-θ	protein kinase C - θ
PTSD	post-traumatic stress disorder
PVN	paraventricular nucleus
PYY	peptide YY
SCFAs	short-chain fatty acids
SES	socioeconomic status
SPT	sucrose preference test
SSRI	selective serotonin reuptake inhibitors
TLR-4	Toll-like-receptor 4
TNF-alpha	tumor-necrosis-factor-alpha
TST	tail suspension test
WT	wild type

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1 Introduction

1.1 Depression

1.1.1 Definition & Epidemiology

Depression is an affective disorder characterized by depressed mood, lethargia, listlessness and somatic symptoms appearing during the most time of the day on most days since more than 2 weeks. Compared to the bipolar disorder or mania, depression is a unipolar affective disorder with only depressive episodes (1). Depression is more common in the female population than in the male and usually gets clinically apparent between the age of 30 and 45. The risk of suffering from depression once in your lifetime is 16-20% in the overall German population, 12,3% in the male and 25% in the female subpopulation (2).

1.1.2 Pathogenesis

The pathogenesis of depression is a multifactorial process affected by genetic, neurobiological and psychosocial factors that affect the individual's ability to cope with stressful life events, which could possibly lead to depression (1). This ability to adapt and resist to those life events without developing depression or other psychological disorders is called resilience (3). As the pathophysiology of depression is complex and not completely explored yet, the following mechanisms are only examples for the various processes, whose interaction and mutual stimulation leads to depression (4).

1.1.2.1 Genetic polymorphisms

Studies showed that 50% of monozygotic twins and 20% of dizygotic twins develop depression when one of the siblings shows a unipolar affective disorder. This incidence shows that genetic alterations have an impact on the predisposition to depression. Genetic alterations on Chromosome 8, 11, 12, 21 and 22 have been identified to change the genetic predisposition to depression as well as epigenetic factors such as DNA-methylation (1). Although the neurobiological mechanisms of depression remain unclear, antidepressant drugs, which increase levels of serotonin and/or noradrenaline, improve the symptoms of depression. That's why the monoamine hypothesis assumes that lower levels of the monoamines serotonin and noradrenaline can lead to depression in individuals with other predispositions (5).

There are different polymorphisms in genes that have been identified to affect serotonin homeostasis and contribute to the development of depression. For example, the gene of the presynaptic serotonin transporter can be coded by a short or a long allele. People with the short allele have a slower synthesis of the serotonin transporter, which is responsible for serotonin reuptake into the presynaptic neuron. When stress occurs, serotonin neurons take longer to adapt to the new stimulation and it has been suggested that this mechanism could render people with the short allele more sensitive to stress (5). However, not every person with the short allele experiencing stress will develop depression. There are other gene polymorphisms such as polymorphisms of the brain-derived neurotropic factor (Bdnf) that increases the likelihood to develop depression and can coexist with the short allele of the serotonin transporter. Bdnf enhances synaptic plasticity by stimulating the production of the serotonin transporter and the enzyme that synthesizes serotonin. One Bdnf allele has been identified to be associated with decreased Bdnf expression and thus synaptic plasticity. Impaired synaptic plasticity in turn reduces the ability to adapt to changes in stimulation of the neurons caused by environmental stimuli such as stress. These genetic polymorphisms are only examples for many genetic and neurobiological factors that can contribute to the pathophysiology of depression (5).

1.1.2.2 Inflammation

In addition to the neurobiological factors altered by genetic polymorphisms, increased inflammation and changes in the hypothalamic-pituitary-adrenal axis (HPA) contribute to the development of depression. Chronic inflammation associated with diseases such as the metabolic syndrome, psoriasis vulgaris, or atherosclerosis causes a constant release of proinflammatory cytokines like tumor-necrosis-factor-alpha (TNF-alpha), interleukin-1 beta (IL-1b), interleukin-2 (IL-2) and interleukin-6 (IL-6) (4). Some of those cytokines are able to pass the blood-brain-barrier (BBB) themselves and act directly in the central nervous system (CNS) while others are not able to pass the BBB but stimulate astrocytes at the BBB to produce and release cytokines into the CNS (6, 7). It has been hypothesize that those cytokines activate microglia in cortical areas and decrease synaptic plasticity of serotonin neurons and glutamatergic neurons, which increases the sensitivity to stress as described above. In addition to inflammation-associated physical disorders, chronic psychological stress leads to

increased levels of cytokines. It is assumed that a dysregulation of the innate immune response to stress exists that leads to a higher release of proinflammatory cytokines and makes people with that dysregulation more likely to develop depression under chronic stress. This finding shows that a vicious circle between chronic inflammation that increases sensitivity to stress, and psychological stress that leads to higher levels of proinflammatory cytokines could be relevant for the pathophysiology of major depression (7). This hypothesis is underpinned by studies that show a higher incidence of depression in female patients with chronic inflammatory diseases and patients who receive interferon-γ as a hepatitis C treatment (8).

1.1.2.3 Hypothalamic-pituitary-adrenal axis

Chronic stress does not only alter levels of proinflammatory cytokines but also cortisol by activating the hypothalamus in order to release corticotropin-releasing factor. Corticotropin-releasing factor stimulates the pituitary to release corticotropin, which subsequently initiates cortisol release by the adrenal glands (5). High levels of cortisol can act on glucocorticoid receptors (GR) on peripheral tissues and several brain areas but relevant for the explanation of depressive symptoms are the medial prefrontal cortex (mPFC), the amygdala and the hippocampus (4).

Cortisol binds to glucocorticoid receptors in the hippocampus, which inhibit corticotropin-releasing factor release in the hypothalamus and thus further release of cortisol (5). This negative feedback mechanism works inadequately in depressed patients who experienced early childhood trauma. This implicates that childhood trauma causes epigenetic changes in the activity of the HPA axis and the inability to counteract increased cortisol release under chronic stress via negative feedback (9).

Elevated cortisol levels, caused either by chronic stress or hyperactive HPA axis, affect the assessment of situations and processing of emotions in the amygdala by enhancing the activity of the amygdala as a reaction to negative stimuli. If the amygdala is activated, situations are more likely to be classified as dangerous even if they are not dangerous. This results in increased fear and anxiety and a stimulation of the HPA axis to release even more cortisol (4, 10, 11). In addition, chronic stress and enhanced activity of the amygdala both decrease the activity of mPFC. As the mPFC is also involved in processing of emotions, this results in a state of poor self-concept because of the inadequate processing of negative emotions such as failure (4).

1.1.2.4 Psychosocial factors

Apart from genetic and neurobiological predispositions, psychosocial circumstances can also promote or trigger depression. Life events as described in the following table, can contribute to a single or recurrent depressive episodes (4).

Table 1: Psychosocial factors contributing to the development of depression. Adapted from (6) and (12)

Category	Risk factor
Demographic factors	- Advanced age - Female sex - Ethnicity
Socioeconomic status	- Low Income / poverty - Unemployment - Low educational status
Socioenvironmental events	- Natural disasters - War - Discrimination - Physical and/or psychological abuse - Low social support - Emotional trauma - Poor housing conditions - Family history of depression - Bad health condition of a family member - Loneliness
Lifestyle	- Poor physical health - Physical inactivity - Alcohol abuse - High-fat or high-sugar diet

1.1.3 Symptoms

Beside the mentioned leading symptoms, a depressive episode can clinically manifest in many different ways (1). The most common symptoms of a depressive episode are sleep disturbances, depressed mood, difficulty concentrating, suicidal thoughts, fatigue, appetite disturbances, hopelessness, delusions, and suicide attempts. The severity of the disease can range from mild depressed mood with listlessness to feelings of numbness and depressive stupor. Depressive stupor is a state in which patients remain apathetic and almost motionless. Based on the constellation of symptoms, different manifestations of depression can be distinguished. Inhibited depression is characterized by a reduction in psychomotor function and activity. The maximum form of inhibited depression is depressive stupor. In contrast, anxious

agitated depression is manifested by anxious drivenness, akathisia, hectic behavior, and whining. Somatized depression manifests itself primarily through somatic complaints such as loss of appetite, constipation, sleep disturbances, lack of libido, pain, and other discomforts such as a feeling of pressure in the chest. Finally, patients with anankastic depression tend to exhibit obsessive behaviors such as increased conscientiousness and orderliness (1).

1.1.4 Diagnosis

Screening of suspected depression begins with specific questions about symptoms. If depression is suspected during screening, the severity as well as the course and duration of the episode should be evaluated (13). In addition, risk factors and possible triggers should be inquired in the medical history (1). Based on the criteria specified by the International Classification of Diseases-10 (ICD-10), depression is eventually divided into 5 levels of severity. This requires at least two major criteria and at least two minor criteria being present for at least 2 weeks. The main criteria are depressed mood, loss of interest and increased fatigue or decreased drive. Minor criteria include other symptoms such as loss of self-confidence, suicidal thoughts, concentration disorders, psychomotor agitation, sleep disorders, loss of appetite and weight. Based on the severity of these criteria, there may be a mild depressive episode, a moderate depressive episode or a major depressive episode with or without psychotic symptoms (13).

1.1.5 Treatment

The treatment of depression depends on the severity of the episode and can be divided into acute treatment, maintenance therapy and the prevention of recurrence. Acute mild depressive episodes can initially be treated with a watchful waiting approach and, if persistent for more than 14 days, can be supported by psychotherapy. Pharmacotherapy is not worthwhile because of the side effect profile. Moderate depression, on the other hand, requires therapeutic intervention in all cases. Depending on the trigger of the episode and the patient's wishes, a decision can be made between pharmacotherapy with antidepressants and psychotherapy. In the case of a major depressive episode, combination therapy of antidepressant and psychotherapy should be initiated (2, 13).

The choice of antidepressant used to start therapy depends on what symptoms the patient is experiencing and what side effects are manageable. Anxious agitated patients or patients with sleep disorders should take sedating antidepressants such as mirtazapine or tricyclic antidepressants such as amitriptyline. However, because of their anticholinergic side effects (e.g., dry mouth, constipation) and blood pressure lowering effects, tricyclic antidepressants are not suitable for the elderly because this can lead to hypotension with increased risk of falling, among others. Alternatively, selective serotonin reuptake inhibitors (SSRI) such as citalopram can be prescribed to these patients as they have no sedative and no anticholinergic side effects (2, 13).
The treatment is started with a minimal dose, which is then slowly increased day by day. It should also be noted that the onset of beneficial effects can be expected with a latency of 1-2 weeks. The treatment should be continued as maintenance therapy for another four to nine months after the symptoms of the depressive episode have subsided.
In recurrent depressive episodes (two or more), it is recommended to take the acute therapy medications for at least 2 years as prevention of recurrency. Psychotherapy should be continued for the same length of time (1, 13).

1.2 Association between obesity and depression

The association between obesity and depression is a bidirectional connection in which obesity (body mass index >= 30) increases the risk of developing depression and depressed patients are at higher risk of becoming obese. On the one hand, there are socioeconomic circumstances that have a demonstrable influence on the obesity-depression relationship and, on the other hand, there are biological mechanisms that attempt to explain some of the socioeconomic influences (14).

1.2.1 Socioeconomic influences and risk factors

The systematic review of K. Preiss, L. Brennan and D. Clarke identified several environmental circumstances that influence the interaction between obesity and depression (14). Within the 46 included studies, eight different variables that increase the risk of developing depression were identified. First of all, people with a body mass index (BMI) of 40 or higher have the highest probability to develop depressive symptoms. As severe obesity impairs physical performance, it leads to a reduction in

quality of life and thus is more likely to lead to depression than is the case for individuals with lower levels of obesity (14).

Contrary to expectations, overweight patients with high socioeconomic status (SES) have a higher risk of depression as well. This may be because overweight patients with high SES are more likely to experience discrimination based on their body weight. Discrimination based on body weight and body image dissatisfaction also has a negative impact on the relationship between obesity and depression independent of SES (14). This can be explained by the fact that discrimination lowers the self-esteem of the person affected, which in turn is both a symptom and a cause of depression. In addition, the discrimination and lowered self-esteem makes it difficult for these people to interact interpersonally and thus to establish social contacts that could provide social support. Since little social support has been shown to increase the risk of developing depression, this fact explains why obesity also increases depression risk because it often results in less social support.

Lack of drive as the main symptom of depression leads depressed people to engage in less physical activity and exercise. As a result, they burn fewer calories and their BMI increases. This is one reason why physical activity is proven to modulate the association between obesity and depression (14).

The last identified factor that has an impact on the association between obesity and depression is binge eating disorder. However, binge eating increases the likelihood of developing depression only in patients who are trying to reduce their weight, not in people who do not want to change anything about their obesity (14). This is probably because people who actually want to lose weight feel discouraged by binge eating episodes.

1.2.2 Biological explanations

1.2.2.1 Food choices and palatability of food

Just as with socioeconomic influences, the link between obesity and depression operates in a cycle at the biological level. On the one hand, eating high-sugar and high-fat food in particular makes you feel better and gives you a sense of reward. This leads to overeating of such food, resulting in weight gain. This explains why depressed patients tend to eat palatable food more often to uplift their mood (15). However, frequent consumption of palatable, energy-dense food leads to obesity and thus again

promotes a depressed mood. On the other hand, acute and chronic stress can lead to decreased food intake in some people and increased food intake in others. Regardless of the amount consumed, the composition of the diet in both groups changes toward a higher proportion of palatable and energy-dense foods (16).

Palatable foods usually contain a large amount of saturated fat. Compared to unsaturated fats, saturated fats mainly lead to the aggregation of visceral fat. The amount of abdominal fat increases the risk of developing depression much more than the body fat percentage in general. This conclusion was derived from the finding in depressed individuals that the higher the abdominal fat mass, the higher cortisol levels, insulin and leptin resistance, and proinflammatory cytokine levels (15). How these substances promote depression is explained in the following paragraphs.

1.2.2.2 Glucocorticoids

As already described, chronic stress leads to permanently elevated cortisol levels and, due to the central effect of cortisol, to depressive symptoms (5). In addition to its effect in the CNS, high cortisol levels in depressed patients lead to an increase in visceral fat mass. People with high visceral fat mass were shown to have a stronger HPA axis response to stress stimuli, which in turn explains why people under chronic stress are more likely to accumulate fat on the abdomen and this in turn has been shown to promote progression of depression (15). In contrast, although people with abdominal obesity without depression do not show consistent elevated cortisol levels, they equally show increased reactivity of the HPA axis to stress stimuli (17). As already known, hyperactivity of the HPA axis leads to an increased activation of the amygdala, thus to a reduced ability to evaluate situations emotionally and to low self-confidence (15).

1.2.2.3 Leptin

Leptin is a proteohormone produced by fat cells proportional to their quantity. This means that the higher the body fat mass, the higher the leptin levels. Leptin has an anxiolytic and antidepressant effect, but conversely, studies have not continuously demonstrated that low leptin levels lead more often to depression or that high leptin levels lead to a lower prevalence of depression. However, in a rat model, chronic mild

stress could induce depressive symptoms. These changes in the behavior of the rats were accompanied by elevated leptin levels (15).

One assumption as to why a correlation with depressive episodes can be observed in obese people with high leptin levels, contrary to expectation, is that leptin resistance develops after long-term elevated leptin levels. This occurs mainly in people with abdominal obesity. Leptin resistance would explain why, despite high levels of leptin, there is no antidepressant effect due to binding of leptin to its receptor in the hippocampus (15).

Under physiological conditions, leptin inhibits the activity of the HPA axis and thus also cortisol secretion. In the case of leptin insensitivity, this effect of leptin is absent, contributing to further deterioration of HPA axis dysregulation and visceral fat mass accumulation due to exacerbated cortisol levels (15, 18).

Furthermore, the levels of fatty acids such as palmitate rise in the blood in response to palatable food intake. Palmitate is able to cross the blood-brain barrier and exert its effect directly in the brain. One way palmitate contributes to depression is by preventing leptin from binding to its receptor and exerting its antidepressant and anxiolytic effects (15).

1.2.2.4 Inflammation

In addition to leptin, adipose tissue produces many other mediators like antiinflammatory (e.g., adiponectin) and proinflammatory cytokines (e.g. IL-6, TNF-α) (19). With increasing body fat, especially visceral fat, the production of antiinflammatory cytokines decreases and the production of proinflammatory cytokines increases (20, 21). These attract macrophages to the adipose tissue, which are responsible for further release of cytokines such as IL-6 (20, 22).

However, the range of action of proinflammatory cytokines is not limited to adipose tissue. They can be transported via the bloodstream across the blood-brain barrier into the brain (23). In the brain, proinflammatory cytokines can bind to receptors and thus exert direct effects, as well as influencing the synthesis of neurotransmitters and their receptors (20).

As described earlier, proinflammatory cytokines can bind to receptors of brain cells, which promotes them to produce even more cytokines and promote further inflammation (6, 7). Those cytokines, especially IL-2 and Interferon-alpha (IFN-α)

stimulate the enzymatic degradation of tryptophan. However, tryptophan is needed for the synthesis of serotonin. The absence of sufficient tryptophan thus results in lower serotonin production. However, IFN-α, which inhibits the expression of a serotonin receptor, and IL-1b and TNF-α, which decrease the amount of serotonin in the synaptic cleft by increasing reuptake in neurons, also affect serotonergic transmission in the brain (20). Considering the monoamine hypothesis, these findings explain how obesity-induced inflammation causes depressive symptoms (5). In addition, the HPA axis is stimulated by cytokines (e.g. IL-1b, IL-6, TNF-a) (20). For further mechanisms of how HPA activation and inflammation contributes to depression, see Chapter 1.1.2.2 and 1.1.2.3.

1.2.2.5 Insulin

The aforementioned cytokines, especially TNF-a, may activate intracellular mechanisms that drive the development of insulin resistance in peripheral tissues as well as in brain areas that express the insulin receptor (e.g. hypothalamus) (15, 24).
In addition to that, as already mentioned, a diet high in saturated fatty acids increases circulating palmitate levels in the blood. Palmitate does not only impair central leptin signaling but also insulin signaling (15). Benoit et al. proved that palmitate drives the translocation of protein kinase C - θ (PKC-θ) to the inside of the cell membrane in the hypothalamus of rats. PKC-θ impedes the binding of insulin to the insulin receptor and the activation of intracellular effector pathways of insulin that are usually activated by the binding of insulin to the insulin receptor on hypothalamic cells. This process is called central insulin resistance (25).
The molecular mechanisms of how central insulin resistance leads to depressive symptoms in humans have not yet been adequately elucidated. However, there are already findings in animal models that are a possible explanation (26). For example, neuronal insulin receptor knock-out (NIRKO) mice showed higher levels of brain monoamine oxidase (MAO) A and B in the striatum and nucleus accumbens. This results in more monoamines being degraded. Considering the monoamine hypothesis, it is not surprising that NIRKO mice show anxiety and depressive-like behavior (5, 26).
Further, HPA axis activity is influenced by insulin. In the case of obesity-induced insulin resistance, the inhibitory effect of insulin on the hypothalamus is disturbed, resulting in HPA axis hyperactivity and an inadequate stress response of the HPA axis. On the

one hand, cortisol itself influences mood, and on the other hand, high cortisol levels in turn lead to the redistribution of adipose tissue to the abdomen, driving the vicious circle between visceral obesity and depression (26, 27).
In addition to stimulating cortisol production, the hypothalamus also regulates food intake and body weight homeostasis. Under physiological conditions, insulin inhibits appetite and food intake by acting in the hypothalamus. Central insulin resistance prevents insulin from exerting its effects at the hypothalamus and thus leads to increased food intake and body weight gain (26, 27).
In summary, insulin resistance affects mood both directly and via further increase in visceral body fat, contributing to the co-occurrence of diet-induced obesity and mood disorders (15).

1.2.2.6 Gut microbiome

The gut microbiome comprises the totality of all bacteria and microorganisms of the gut. The two phyla Firmicutes and Bacteroidetes represent the most abundant taxa of the intestinal microbiome.(20) Although each person has a unique composition of the microbiome, a certain ratio between Firmicutes and Bacteroidetes has been suggested to be important for the homeostasis of the whole organism (20, 28).
The microbiota can influence physiological processes by producing various metabolites and neurotransmitters. These include gamma-aminobutyric acid (GABA), norepinephrine (NE), serotonin (5-HT), dopamine (DA), and acetylcholine. In addition, although dietary fiber cannot be digested, they can be fermented by bacteria in the intestinal lumen. This produces SCFAs, which in turn influence the immune system and prevent the release of proinflammatory cytokines and promote the release of antiinflammatory cytokines (20). Furthermore, short-chain fatty acids (SCFAs) stimulate the production of peptide YY (PYY), glucagon-like peptide-1 (GLP-1) and GLP-2 from the hormone-producing enteroendocrine L cells of the intestinal epithelium. Said molecules have innumerable sites of action in the human body, which include the gut-brain axis (29).
The gut-brain axis is a bidirectional connection between gut and brain through the nervous system, the immune system, and endocrine signaling. Communication through the nervous system involves the CNS, the autonomic nervous system (ANS), the enteric nervous system and the HPA axis (28, 29). Thus, the brain can

communicate with enteric effector cells and signals can be sent from said cells to the brain via the ANS. Effector cells involved are free nerve endings of visceral afferents, immune cells, epithelial cells and enteroendocrine cells, smooth muscle cells of the intestinal wall and interstitial cells of Cajal, which are electrical pacemakers and mediators of enteric neurotransmission. Intestinal effector cell activity is one part of the gut-brain axis that is under the influence of the gut microbiome (28).

Various studies showed that a HFD, obesity and depression are associated with changes in the composition of the gut microbiome (29, 30). In response to HFD, the ratio of Firmicutes to Bacteroidetes increases, the diversity of the microbiome decreases in humans and animal models (20, 30) and the numbers of the probiotic bacteria (live, beneficial bacteria) Bifidobacterium and Lactobacillus decrease (20). Therefore, it can be assumed that changes in the gut microbiome lead to changes of metabolites produced by the bacteria, and that these metabolites modify mood and emotions via the gut-brain axis (31).

In agreement with this hypothesis, the behavior of gut-microbiota-depleted control-diet-fed mice changed after they were transplanted with cecal and colonic feces from mice fed with HFD. They showed anxiety-like behavior and poorer cognitive performance compared to the group whose feces were derived from control diet fed mice. These observations were accompanied by changes in gut microbiome composition, increased intestinal permeability, increased systemic inflammation and inflammation in the medial prefrontal cortex, a brain region that plays a role in anxiety and cognitive behavior (32). Similarly, transplantation of fecal microbiome from patients with major depression to germ-free rats (raised under sterile conditions) triggered major symptoms of depression such as anhedonia and anxiety (30).

In patients with HFD-induced obesity, the diet often contains very little dietary fiber. This may result in less fiber being fermented to SCFAs by the gut microbiota and consequently more proinflammatory and less antiinflammatory cytokines being released (20). How proinflammatory cytokines contribute to depressive-like behavior can be found in chapter 1.2.2.4. Furthermore, decreased levels of SCFAs may decrease levels of the satiety hormones GLP-1 and PYY. GLP-1 normally stimulates insulin secretion and improves insulin sensitivity. Decreased SCFA-induced GLP-1 release may lead to lower insulin release, and thus lower satiety and promoted weight gain, and lower insulin sensitivity (29). Besides, rats with depressive-like behavior showed fewer symptoms of depression, higher brain serotonin concentration, and

increased brain-derived neurotrophic factor (BDNF) expression in the hippocampus after an injection of the SCFA sodium butyrate. Therefore, it can be hypothesized that lower levels of butyrate, as a result of changes in the microbiome, may diminish the antidepressant effects of butyrate and thus contribute to the development of depressive behavior (33). It is also worth noting that decreased SCFAs are associated with higher permeability of the intestinal wall (29), which fits with the findings that HFD fed mice express fewer tight-junction proteins in the intestinal wall. A disturbed intestinal barrier leads to lipopolysaccharides (LPS) from the intestinal microbiome being able to pass into the blood and trigger systemic inflammation (20, 29). In addition to GLP-1 and PYY, the microbiome affects the secretion of other peptide hormones from enteroendocrine cells such as galanin and ghrelin. Both initiate the release of adrenocorticotropic hormone (ACTH) and cortisol (28).
These findings are supported by experiments with probiotics in rodents. Thus, rats exposed to early-life stress by separating them from their motherwere administered the antidepressant citalopram or the probiotic Bifidobacterium infantis. The control group showed increased levels of the inflammatory marker IL-6, lower levels of norepinephrine in the brain, and increased expression of corticotropin-releasing factor in the amygdala. Interestingly, these parameters were normalized in both the citalopram and probiotics groups, suggesting that the probiotic Bifidobacterium infantis has antidepressant effects (34). Similar results were obtained in a study with mice administered Lactobacillus rhamnosus JB-1. These mice showed a lower increase in cortisol, and thus lower HPA axis activity, in response to stress compared to the control group (35). In addition, male rats showed significantly higher plasma tryptophan levels after 14 days of Bifidobacterium infantis administration than the control group. This finding was accompanied by lower levels of proinflammatory cytokines (TNF-a, IL-6, IFN-y) in the group treated with the probiotic (36). In summary, the fact that alterations of the gut microbiome have been found in obese individuals and that the gut microbiome communicates with the brain via the gut-brain axis suggests that the gut microbiome contributes to the development of depression in obese individuals, although the exact relationship has only begun to be explored.

1.3 High-fat diet-induced depression in mouse models

As mentioned earlier, mouse studies were conducted to further explore the link between Western diet, obesity, and depression (37). Ying-Yiu Lam et al. (2021) fed male mice a HFD (60% of energy from fat) for 12 weeks. At the end of the feeding period, the mice showed depressive-like behavior in the SPT, forced swimming test (FST), and tail suspension test (TST) compared to the control group. Apart from behavioral changes, HFD lead to decreased insulin sensitivity, higher levels of triglycerides, free fatty acids, and low density lipoprotein (LDL) cholesterol in plasma, and activation and alteration of astrocyte morphology in the hippocampus(38), which is typically associated with depression (39). All of these findings were reversible by therapy with pioglitazone, an insulin sensitizer used in the treatment of diabetes mellitus type 2 (38). The changes in lipid metabolism in HFD induced depression in mouse models were reproduced in several other studies (40, 41). Pereira Braga et al. (2021) also observed weight gain, insulin resistance, and depressive-like-behavior in female mice after 12 weeks of HFD feeding. When switched from HFD to standard chow for an additional 4 weeks following the 12 weeks, insulin sensitivity, emotional-cognitive behavior, and body weight improved (42). In another study, Kai-Jing Yin et al. (2020) examined the neurotransmitters DA, 5-HT, and NE in the hippocampus of mice with HFD induced depression. For this purpose, male mice were fed with HFD for 8 weeks to induce depression-like behavior. All three neurotransmitters were significantly lower in the hippocampus of the HFD group than in the control group fed with standard chow (41). In addition, Hassan et al. (2019) also conducted an 8-week experiment in which male mice were fed HFD. Compared to the control group, the HFD group exhibited several aspects of depressive-like behavior like lower self-care, sociability and anhedonia. In further analyses, the hippocampus and hypothalamus of HFD mice showed lower expression of NPY and higher plasma levels of NPY (43).

In conclusion, depression could be induced by HFD in several experiments in mouse models, while the exact mechanisms are not clear.

1.4 Anhedonia as an aspect of depression

Anhedonia is one of the main criteria to diagnose depression. It is defined as the loss of the ability to feel pleasure in situations that used to bring pleasure, leading to decreased reward-directed behavior (44). The definition has been expanded over time to include the whole reward system from desire and motivation to anticipation and reward after consumption. Disruptions at any point can result in no reward being sensed and thus anhedonia (44, 45). This newer definition also already hints at the pathophysiology of anhedonia, namely a disturbance of the dopaminergic/mesolimbic reward system. The task of this system is to detect potentially rewarding stimuli, test them for hedonic valence, and induce the emotion of pleasure. Dopamine is involved in initiating reward-directed behavior after a positive experience is attributed to a stimulus and, as a result, results in motivation to pursue the stimulus in order to feel reward. Studies with rodents showed that they exhibited less reward-directed behavior when less dopamine was released from the nucleus accumbens. The results of a study with humans who were asked to put themselves in a happy or sad mood with their own stimuli also fit in with this concept. In functional magnetic resonance imaging (fMRI), the activation in the nucleus accumbens was lower, the more severe anhedonia of the depressed patients was (46). As food, especially sweet food, activates this system, it is used to test the functioning of hedonic responses (47).

To measure depressive-like behavior in mice in order to explore pathogenesis and treatment options, behavioral tests have been developed for each aspect of a depressive phenotype. To quantify anhedonia as an aspect of depression, the sucrose preference test has been established. For this, mice are offered two bottles in their home cage. One contains water and the other a sucrose solution. Healthy mice usually prefer the sucrose solution because it activates their reward system. If a mouse is anhedonic, it does not feel this pleasure after drinking sucrose and therefore has no internal motivation to drink more of the sucrose solution. Therefore, an anhedonic mouse drinks less sucrose solution relative to water than a healthy mouse. The translation of the sucrose preference test from mice to humans has been confirmed by similar experiments with depressed humans, which yielded comparable results (48).

1.5 NPY and its receptors

NPY is a peptide consisting of 36 amino acids (49) and is mainly found in the central and peripheral nervous system (50). It is produced in the CNS by GABAergic neurons in many brain regions. NPY is most abundantly expressed in the hypothalamus, hippocampus, amygdala, nucleus accumbens and cerebral cortex (51). NPY is also produced in the periphery by certain neurons such as enteric neurons, as well as by platelets and immune cells (50). At this time, 5 NPY receptors are known to occur in mammals, the Y1, Y2, Y4, Y5, and Y6 receptor but the Y6 receptor is not functional in humans (52). All are postsynaptic pertussis toxin-sensitive G protein-coupled receptors that exert their effects by different mechanisms depending on the cell type. All receptors except Y2 are postsynaptic receptors, while Y2 occurs predominantly presynaptically. Binding of NPY to a Y-receptor leads to a reduction of cyclic adenosine monophosphate (cAMP) via inhibition of adenylate cyclase and this in turn leads to activation or inactivation of calcium and potassium channels or changes in nitric oxide (53). The effects that NPY exerts through Y-receptor binding are diverse. In the following, only the modes of action of NPY that are relevant for the experiment will be explained.

NPY is an important regulator of appetite and acts orexigenic in the hypothalamus, thereby influencing body weight and obesity. This effect is clearly demonstrated in a wide variety of experiments (53). For example, a marked increase in food intake was observed in female mice after injection of NPY into the paraventricular nucleus (PVN) of the hypothalamus (54). In line with this, an upregulation of NPY in the PVN could be measured in rats after a fasting period of 4 days (55). It is assumed that leptin and insulin in particular influence the expression of NPY. This is exemplified by the fact that intracerebroventricular administration of leptin during food deprivation markedly increases NPY messenger ribonucleic acid (mRNA) in the hypothalamus (56). In addition, rats with absence of insulin show higher NPY expression in the hypothalamus and hyperphagia, which return to normal after administration of insulin (57). The appetite-enhancing effect of NPY is most likely mediated by Y1 and Y5 as agonists of the receptors lead to increased food intake, while antagonists reduce food intake (53). This also explains the fact, that NPY is downregulated in the hypothalamus of mice after feeding a HFD (43, 58, 59). Since HFDs lead to an overconsumption of calories, it is logical that the body tries to maintain energy homeostasis and counteract this by reducing the release of appetite-stimulating hormones.

Furthermore, NPY plays a role in an individual's resilience and response to stress. The physiological sequence of the stress response is that a stressor activates the HPA axis by leading to the release of corticotropin-releasing hormone (CRH) in the hypothalamus and, via the HPA axis, ultimately to the release of cortisol (50). NPY in turn counters this cascade by being released in the PVN when CRF levels rise (60). NPY prevents an excessive stress response (61) and is important for the homeostasis of the reaction of the HPA axis. The ability of NPY to counteract the reactivity of the HPA axis to a stress stimulus is stronger in males than in females. This finding emerges from the observation that male NPY knock-out (KO) mice had higher corticosterone levels in response to a stress stimulus compared with wild type (WT), whereas in the same experiment with female mice, the difference was less pronounced (50). Furthermore, in a mouse model of post-traumatic stress disorder (PTSD), low expression of NPY in hippocampus, amygdala, and the periaqueductal gray correlated with disturbed behavior after stress exposure, whereas significantly higher NPY expression was measurable in mice with less disturbed behavior (62). Consistent with this, intrahippocampal injection of NPY one hour after exposure to stress resulted in less disturbed behavior (62).

In addition, NPY has an anxiolytic effect in brain regions responsible for the regulation of mood. These include the cerebral cortex, the amygdala and the hippocampus (50). The anxiolytic effect of NPY is demonstrated in various experiments. Sevora et al. applied NPY in a rat model of stress induced anxiety and depressive-like behavior and observed a reduction in anxiety-like and depressive-like behavior by NPY (63). I.n. treatment with 100 µg NPY could also prevent the development of stress-induced behavioral impairments like anxiety-like and depression-like behavior in rats (64). Broqua et al. applied NPY, a Y1 agonist and Y2 agonist intracerebroventricular (i.c.v.) in an anxiety model of rats. NPY and the Y1 agonist both reduced signs of anxiety, while the Y2 agonist had no effect. This also implicates the involvement of the Y1 receptor in mediating the anxiolytic effect of NPY (65). There is evidence that not only the Y1 receptor but also Y5 is involved in the anxiolytic effect of NPY (66). Y2 and Y4 KO mice both display a reduction of anxiety and depressive-like behavior, which indicates that stimulation of these receptors has the opposite effect of Y1, namely promotion of anxiety- and depressive-like behaviors (67, 68).

Lastly, NPY exerts an antiinflammatory effect in adipose tissue (50). The Y1 receptor is the Y receptor most expressed on immune cells and through which the majority of

the antiinflammatory effects of NPY are mediated. In adipose tissue, NPY inhibits the release of proinflammatory molecules like IL-6 and TNF-a and increases the release of antiinflammatory cytokines such as IL-10 from macrophages (69). Since addition of NPY to macrophages in vitro leads to decreased secretion of proinflammatory monocyte chemoattractant protein-1 (MCP-1) and TNF-a, and knockout of Y1 receptors on macrophages leads to inflammation and increased expression of MCP-1, it can be assumed that the Y1 receptor is responsible for the antiinflammatory effects of NPY (70).

1.6 Aim of this study

I.n. drug administration has recently been established in the treatment of humans with major depression and treatment-refractory bipolar disorder, which demonstrates that i.n. drug administration could be a promising route of administration of drugs that should act within the CNS. Especially when applying peptides, i.n. application has the advantage of quick delivery to the brain via the olfactory nerve and trigeminal nerve and a better bioavailability given that peptides would be digested if taken orally.

The aim of this study was to determine whether a HFD with 48 kJ% from fat, which reflects the fat content of a typical Western diet better, is also able to reduce SP compared to a control diet. Given that NPY expression is reduced in the hypothalamus of mice with reduced sucrose preference, we wanted to test the potential of i.n. drug administration of NPY as a therapeutic option for HFD-induced anhedonia. In addition, we wanted to investigate the effects of i.n. NPY on feeding behavior, HPA axis and central proinflammatory cytokine expression, as these are all factors associated with obesity and depression and are influenced by NPY. The last thing to aim for was to determine the role of the Y receptors in the effect of i.n. NPY.

2 Materials and methods

2.1 Ethics statement and experimental animals

48 male 8-week old C57BL/6J mice were purchased from Charles River Laboratories (Sulzfeld, Germany) and habituated to the new environment for at least 1 week before the beginning of the experiment. During habituation the animals were kept in groups of 2 per cage in an environment with 22°C of room temperature, a relative air humidity of 50% and a 12h light/dark cycle. Standard laboratory control diet and tab water was provided at libitum. Experiments were approved by an ethical committee at the Federal Ministry of Science, Research and Economy of the Republic of Austria (permit BMWF-66.010/0146-V/3b/2018). All procedures were conducted according to the Directive of the European Parliament and of the Council of 22 September 2010 (2010/63/EU).

2.2 Study design

Mice were divided in 2 groups of which one group was fed a high-fat-diet (HFD, S5745-E712, 48 kJ% fat (mainly palm oil), 34 kJ% carbohydrates, 18 kJ% proteins) and the other group was fed a control diet (S5745-E702, 12 kJ% fat, 65 kJ% carbohydrates, 23 kJ% proteins), as used by others (71), over 8 weeks. The diets were purchased from ssniff Spezialdiäten GmbH (Soest, Germany). The body weight of each mouse as well as the diet was weighed once a week, in order to calculate the weekly food intake of each pair of mice.

2.3 Intranasal neuropeptide Y application

After the 8-week feeding period, the animals were randomly divided into 4 groups (Co, HFD, i.n. NPY, HFD + i.n. NPY). Mice were lightly sedated with isoflurane and a pipette was used to apply either 100 µg NPY dissolved in 10 µL of sterile distilled water, or sterile distilled water only, equally divided between both nostrils of the sedated mice. After the application, the mice were held in a tilted backward position until they started to wake up to help NPY uptake from the nasal cavity to the brain (72). This procedure was performed on day 2 and day 4 (Figure 1).

2.4 Sucrose preference test

Sucrose preference test was initiated 3 days before NPY application. The mice were single housed, transferred to the test room and provided with one bottle filled with tab water and one filled with 1% sucrose solution. The daily water and sucrose intake during the 5 days of the sucrose preference (= percentage of sucrose solution intake relative to total fluid intake) test was calculated by the change in bottle weight (73). The daily food intake was calculated by the difference in the weight of the food pellets. Body weight of each mouse was weighed on day 0, day 2 before i.n. NPY application, day 3 and day 4. Measurements of body weight on day 4 were taken before and 3 hours after i.n. NPY application (Figure 1).

The feeding scheme stayed the same during sucrose preference test.

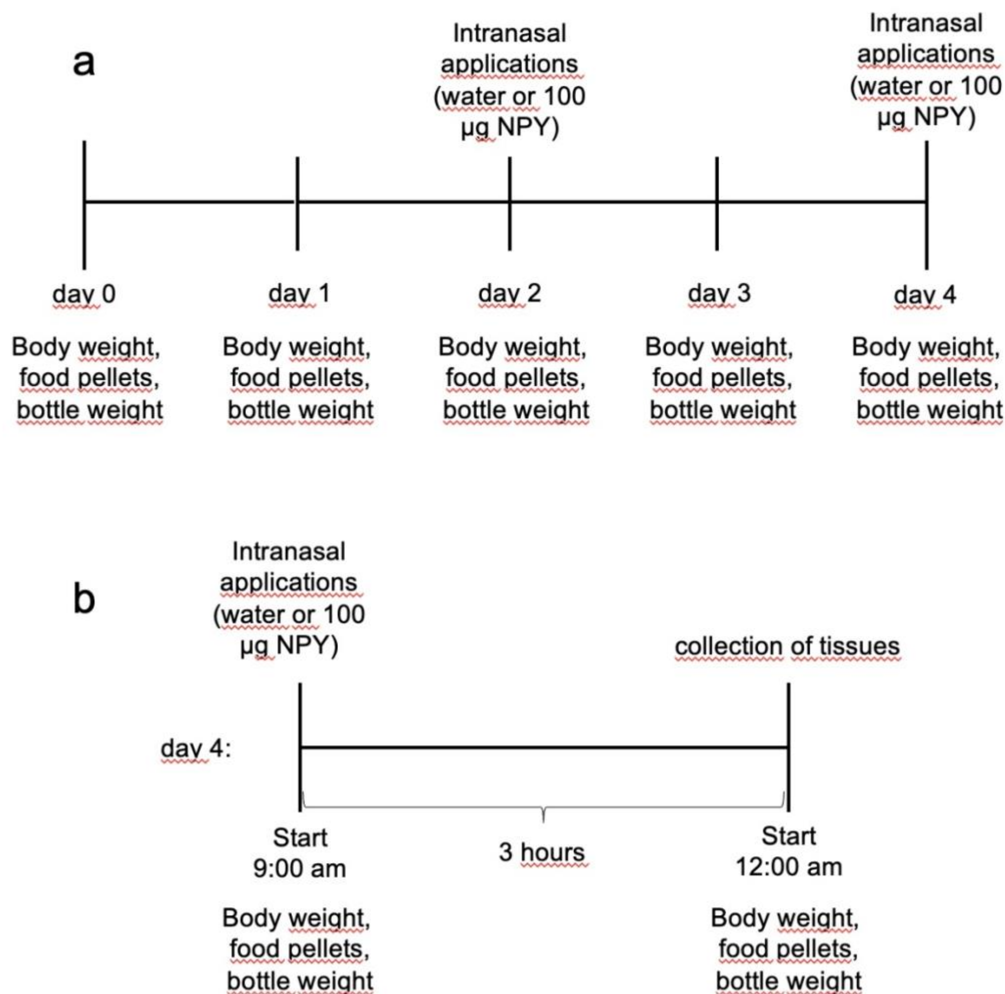

***Figure 1: Sucrose preference test and i.n. NPY application**, a) on each day of the SPT body weight, food pellets and bottle weights were measured to calculate food intake and SP, b) on day 4 of SPT body weight, food pellets and bottle weight was measured at 9:00 am directly before the i.n. treatment started. After 3 hours, body weight, food pellets and bottle weight was measured again to calculate short-term weight gain, food intake and SP. Mice were sacrificed directly afterwards and blood plasma and brains were collected for further analyses.*

2.5 Collection of tissues

The mice were deeply anaesthetized by intraperitoneal pentobarbital injection (150 mg/kg) before the abdomen and thorax were opened to aspirate cardiac blood from the right atrium. After centrifugation the plasma was separated from the cells with a pipette into separate tubes to immediately freeze them on dry ice and store at -70°C until further use. For collection of the brain, the skull was opened cranial with a scissor, starting at foramen magnum. The skull bones were removed in small pieces until the cranial nerves could be cut in order to release the brain from the cranial cavity. After that, the brains were immediately frozen in 2-methylbutane on dry ice. Once they were frozen, they were wrapped in aluminum foil and stored at -70°C until the microdissection.

2.6 Brain microdissection

Prior to microdissection, brains were transferred to a cryostat and brought to a temperature of -20°C. Brains were manually cut into 1-millimeter-thick slices and transferred to a cold plate (-20°C). Hypothalamus, hippocampus, amygdala, striatum, and prefrontal cortex were dissected under a microscope and immediately put in tubes on dry ice. The dissected brain tissue was stored at -70°C until further analysis by quantitative polymerase chain reaction. The required equipment for the microdissection was wiped with RNase AWAY (Carl Roth, Karlsruhe, Germany) before and between each brain to prevent contamination of the tissue by RNases from the working environment.

2.7 Ribonucleic acid (RNA) extraction for quantitative polymerase chain reaction (qPCR)

RNA was extracted from the collected hypothalamus of each mouse with the RNeasy Lipid Tissue Mini Kit (QIAGEN). First, each tissue sample was homogenized in 1 ml QIAzol Lysis Reagent by using the Precellys evolution Homogenizer from Bertin Technologies SAS (Montigny-le-Bretonneux, France). After that, the samples were left to incubate at room temperature for 5 minutes. To separate the RNA from the cell fragments, 200 µL chloroform was added to the sample. In the next step, samples got shaken for 15s, incubated at room temperature for 3 min and centrifuged at 12.000 x g for 15 min at 4°. After that, the organic phase with the cell fragments was on the

bottom of the eprovette, the interphase was in the middle and the aqueous phase with the RNA on top. It is important to avoid the interface while separating the aqueous phase from the remaining inter- and organic phase with a pipette. The aqueous phase got mixed and vortexed with 1 volume of 70% ethanol in a separate eprovette to precipitate the RNA from the aqueous phase. After that, the sample was transferred to a 2 ml collection tube of the RNeasy Mini spin column to centrifuge it at 8000 x g for 15 s. The remaining aqueous phase could now be separated from the visible RNA pellet. 700 µL of Buffer RW1 was added to the pellet. Then it was centrifuged at 8000 x g for 15 s and the supernatant was separated from the pellet to discharge it. This procedure was repeated with 500 µL of Buffer PRE two times. Finally, the pellet could be transferred to a new 1.5 ml eprovette, mixed with 30 µL RNase-free water depending on the required RNA concentration, centrifuged at 8000 x g for 1 min and was ready for further use.

2.8 Reverse transcription of RNA to complementary desoxyribonucleic acid (cDNA)

The High-Capacity cDNA Reverse Transcription Kit (by ThermoFisher Scientific) was used for reverse transcription of the extracted RNA to cDNA. To obtain accurate results, it is important that our prepared RNA pellet is free of RNase activity. We ensured this by dissolving the RNA pellet in RNase free water.

First of all, the kit, stored at -20°C should be thawed on ice. To prepare the 2X RT master mix, the following reagents were mixed on ice for each 20µL-reaction: 2.0 µL RT Buffer, 0.8 µL 25X dNTP Mix, 2.0 µL 10X Random Primers, 1.0 µL MultiScribe Reverse Transcriptase. After that, 5.8 µL 2X RT master mix was pipetted into each tube. Then, 1 µg of RNA samples were added to each tube and topped up with nuclease-free H2O in order to give a total amount of 20µL. The tubes were closed to centrifuge them shortly in order to spin down the added liquids. Finally, for cDNA synthesis, the tubes were placed in the thermal cycler for 10 minutes at 25°C, then for 120 minutes at 37°C, for 5 minutes at 85°C and at 4°C until further use.

2.9 Quantitative polymerase chain reaction (qPCR)

In contrast to conventional PCR, qPCR can be used to measure gene expression already at low levels. In this study, several markers of relevance such as

proinflammatory cytokines, NPY and Crh were analyzed by qPCR. To prepare the 96 well plates for the qPCR, the following was pipetted in each well: 5 µl Taqman Master Mix, 0.5 µl specific primer, 0.5 µl distilled water, 4 µl cDNA. TaqMan gene expression assays were used as primers (Table 2). All samples were measured as triplicates. GAPDH and PPIL3 were used as reference housekeeping genes for quantification of target gene expression. Relative quantification was performed according to the 2-ΔΔCt method (74) using the mean value of the housekeeping genes as reference. Group differences were expressed as fold changes.

Table 2: TaqMan gene expression assays for qPCR

Gene	Primer details
Npy	Mm 03048253_m1
IL-1b	Mm 00434228_m1
Crh	Mm 01293920_s1
Bdnf	Mm 01334043_m1
Gapdh	Mm 9999915_g1
Ppil3	Mm 00510343_m1

2.10 NPY and corticosterone EIA

NPY assay was performed to evaluate hypothalamic NPY peptide levels as described (43). First, NPY peptide had to be extracted from the hypothalamic tissue samples. For this purpose, the samples were homogenized in lysis buffer (50 mM Tris-HCl pH 8, 150 mMNaCl, 1% (v/v) Triton X-100, 0.5% (v/v) sodiumdeoxycholate and 10 mM PMSF) in the Peqlab Precellys 24 homogeniser. After that, the samples were centrifuged (10,000 rpm, 4°C, 10 min) to separate the pellet debris from the supernatant, which contains the protein. The protein content of the supernatant was measured with the BCA protein assay kit (Pierce Biotechnology, Rockford, IL, USA). 200 µg of protein from each sample was added to 0.5 ml 2 N acetic acid each and centrifuged (2400 rpm, 4°C, 10 min). After that, the supernatants were transferred to separate tubes, freeze-dryed and stored at −70°C until further use. To perform the NPY assay by using the fluorescence immunoassay (Phoenix Pharmaceuticals,

Burlingame,CA, USA), the frozen samples were diluted in assay buffer and the assay was run according to the manufacturer's instructions (43).
Corticosterone plasma levels were determined with an enzyme immunoassay kit (Enzo Life Sciences, Catalog No. ADI-900-097) according to manufacturer's instructions. First, the assay buffer (10 ml assay buffer/100 ml H_2O), wash buffer (7.5 ml wash buffer/150 ml H_2O) and plasma samples (diluted in assay buffer in a ratio of 1:200) had to be prepared. Then the standards were prepared according to the following dilution scheme: 5 tubes were labeled #1 - #5. Then 900 µl of assay buffer was pipetted into tube #1 and 800 µl of assay buffer into tube #2 - #5. After that, 100 µl of the 200 000 pg/ml standard was pipetted into tube #1 and vortexed. Then 200 µl of tube #1 was pipetted into #2 and was mixed by vortexing. Then 200 µl of tube #2 was pipetted into #3 and was mixed. This process was repeated until tube #5 to create a standard with increasing dilution. After that, the assay procedure can start when all reagents are at room temperature for at least 30 min. 100 µl of assay buffer and 100 µl of standards #1 - #5 was pipetted into each appropriate well according to the assay layout sheet followed by 100 µl of the plasma samples. Then 50 µl of blue conjugate an 50 µl of yellow antibody was pipetted into the appropriate wells. The plate was incubated at room temperature on a plate shaker for 2 h at 500 rpm. After the incubation time, the wells were emptied and washed 3 times by adding 400 µl of wash solution to each well. Again, 5 µl of the blue conjugate had to be pipetted into the appropriate wells. Then 200 µl of the pNpp substrate was pipetted into the wells in order to incubate the plate for 1h. To stop the reaction, 50 µl of stop solution was pipetted into each well and the read out of optical density at 405 nm started immediately afterwards with a microplate reader.

2.11 Statistical analysis

GraphPad Prism5 (GraphPad Software Inc., La Jolla, CA, United States) was used for statistical analysis and creation of the graphs for the data generated by qPCR, ELISA and behavioral tests. T-test and two-way ANOVA followed by Tukey's and Sidak's post-hoc test was performed for analysis as appropriate. Compared factors were HFD or control diet and i.n. water or i.n. NPY. A P-value ≤ 0.05 was considered as statistically significant. N refers to the number of mice in each group.

3 Results

3.1 Mice on HFD gain more weight

Mice fed a HFD over 8 weeks constantly gain significantly more weight than on control diet with a significant interaction between time and treatment groups ($F(24,352)=8.869$, $p<0.0001$) (Figure 2). Although the food intake in grams did not differ between the groups, the HFD group consumed significantly more calories over the 8 weeks than the group on control diet (main effect of treatment: $F(3,2)=4.026$, $p=0.0216$) .

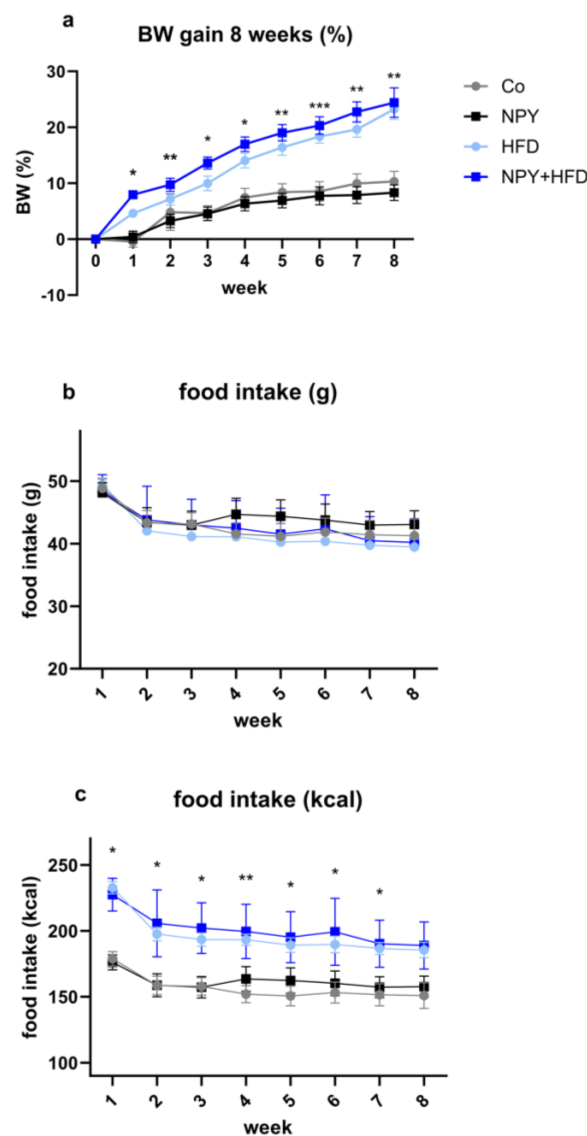

Figure 2: Body weight* gain *in* % (a) and food intake in grams (b) and calories (c) *during 8 weeks of feeding either* control *diet** *(12 kJ% fat, 65 kJ% carbohydrates, 23 kJ% proteins)* ***or HFD *(48 kJ% fat (mainly palm oil), 34 kJ% carbohydrates, 18 kJ% proteins)*. Mice were housed in cages of two and supplied with fresh diet once a week and body weight was measured once a week in order to calculate weekly body weight gain. Values represent means, n= 12 *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ *vs. control diet* ***(two-way RM ANOVA)***

3.2 Mice on HFD show no signs of anhedonia

After 8 weeks of HFD-feeding, mice on HFD did not show reduced SP compared to mice on control diet (Figure 3). Unpaired t-test showed no significant effect of diet on sucrose preference. The absolute sucrose intake and total fluid intake was statistically lower in HFD-fed mice than in control-diet-fed mice after 8 weeks of feeding. Unpaired t-test showed a significant effect of diet on sucrose intake ($t(40)=2.965$, $p=0.0051$) and a significant effect of diet on total fluid intake ($t(40)=2.939$, $p=0.0054$). There was no statistically significant change in absolute water intake after 8 weeks of feeding.

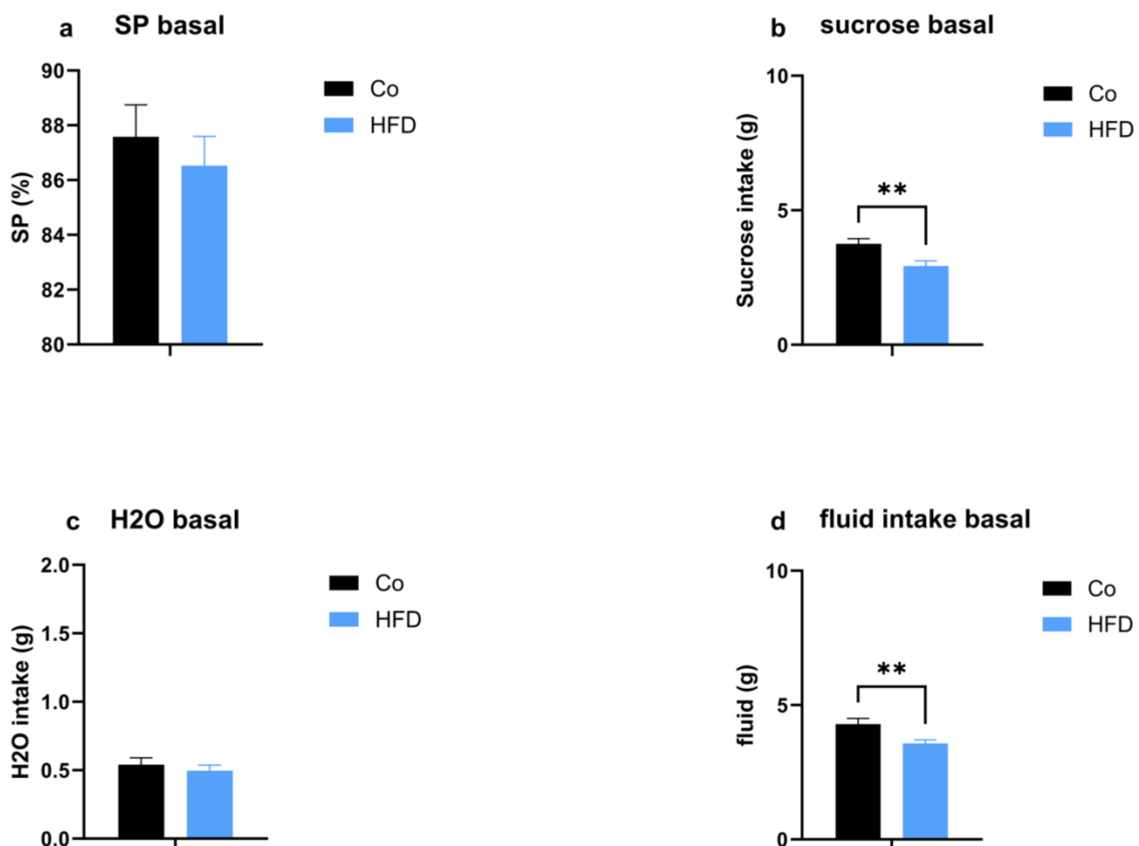

Figure 3: Sucrose preference, absolute sucrose intake, absolute water intake and fluid intake of mice after feeding a HFD or control diet for 8 weeks. Mice were fed either a HFD or control diet for 8 weeks and housed in single cages afterwards to perform the SP test. Each mouse was given 2 bottles, one each filled with water and one with 1% sucrose solution. Liquid consumption of each bottle was measured on day 1 and day 2 in order to calculate SP within these 24 hours. a) no statistically significant difference in SP in the HFD group compared to the control group was found, b) sucrose intake is statistically lower in HFD-fed mice, c) there is no statistically significant difference in water intake between HFD-fed or control-diet-fed mice, d) total fluid intake is statistically lower in HFD-fed mice. Values represent means ± SEM, n=24, $^{**}p \leq 0.01$ vs. control diet group (**unpaired t-test**)

3.3 Intranasal NPY reduces short-term sucrose preference in mice on HFD

I.n. treatment with NPY decreased SP in mice on HFD 3 h after NPY application (Figure 4). Two-way ANOVA showed a significant interaction between the effects of HFD and i.n. treatment (F(1,36)=9.693, p=0.0036) in the SP test. This effect disappeared after 24 and 48 h, when no significant differences in SP were visible.

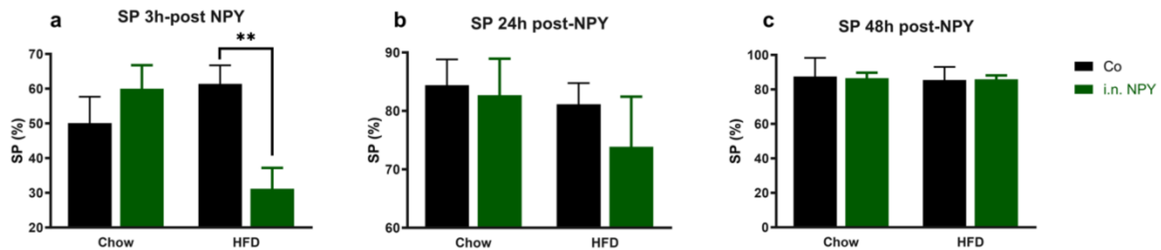

Figure 4: Effects of HFD and NPY on sucrose preference as assessed 3h, 24h and 48h after intranasal NPY treatment. I.n. NPY reduces SP in HFD fed mice 3h after application (a) but not after 24h (b) and 48h (c). Each mouse was given 2 bottles, one filled with water and one with 1% sucrose solution. Liquid consumption of each bottle was measured in order to calculate SP 3h, 24h and 48h after treatment with either i.n. NPY (10 µg) or sterile distilled water. Values represent means ± SEM, n= 12, **p ≤ 0.01, vs. HFD-fed mice receiving i.n. water (**two-way ANOVA, Tukey's multiple comparisons test**)

3.4 Intranasal NPY induces weight loss 3h post-NPY

Mice on HFD lost less weight 3 h after i.n. treatment compared to mice on control diet. In addition, i.n. NPY induced weight loss (Figure 5). Two-way ANOVA showed a significant effect of i.n. treatment (F(1,37)=17.59, p=0.0002) and diet (F(1,37)=11.92, p=0.0014) on weight loss.

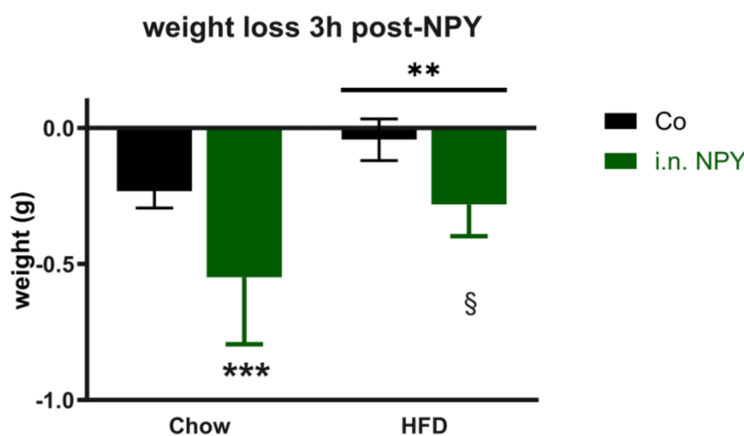

Figure 5: ***Weight loss 3h after intranasal NPY treatment.*** *I.n. NPY induces weight loss in control diet and HFD fed mice 3h after application. Each mouse was weighed before and 3h after i.n. treatment to calculate weight loss.* Values represent means ± SEM, n=12, **p ≤ 0.01, ***p ≤ 0.001, § = 0.0641 vs. control (water)/control diet ***(two-way ANOVA, Tukey's multiple comparisons test)***

3.5 Intranasal NPY reduces food intake 3h post-NPY

Food intake 3 h after i.n. NPY application was significantly lower in experimental groups treated with i.n. NPY compared to the vehicle control (water). Food intake in kcal was significantly higher in experimental groups fed HFD than with control diet (Figure 6). Two-way ANOVA revealed a significant interaction between the effects of HFD and i.n. treatment ($F(1,35)=4.180$, $p=0.0485$). Tukey's multiple comparisons test showed that the HFD:Co group had significantly higher caloric intake than the control diet:Co group, but i.n. treatment only had a statistically significant effect on caloric intake within the HFD groups, but not in the control diet groups. This effect disappeared after 24 and 48 h, when no significant differences between groups were visible.

Similar trends were observed for food intake in grams 3 h after i.n. treatment. Two-way ANOVA showed a significant reduction of food intake in grams after i.n. treatment, whereas Tukey's multiple comparisons test showed only a strong trend of the effect of i.n. NPY within the HFD groups ($p=0.0660$).

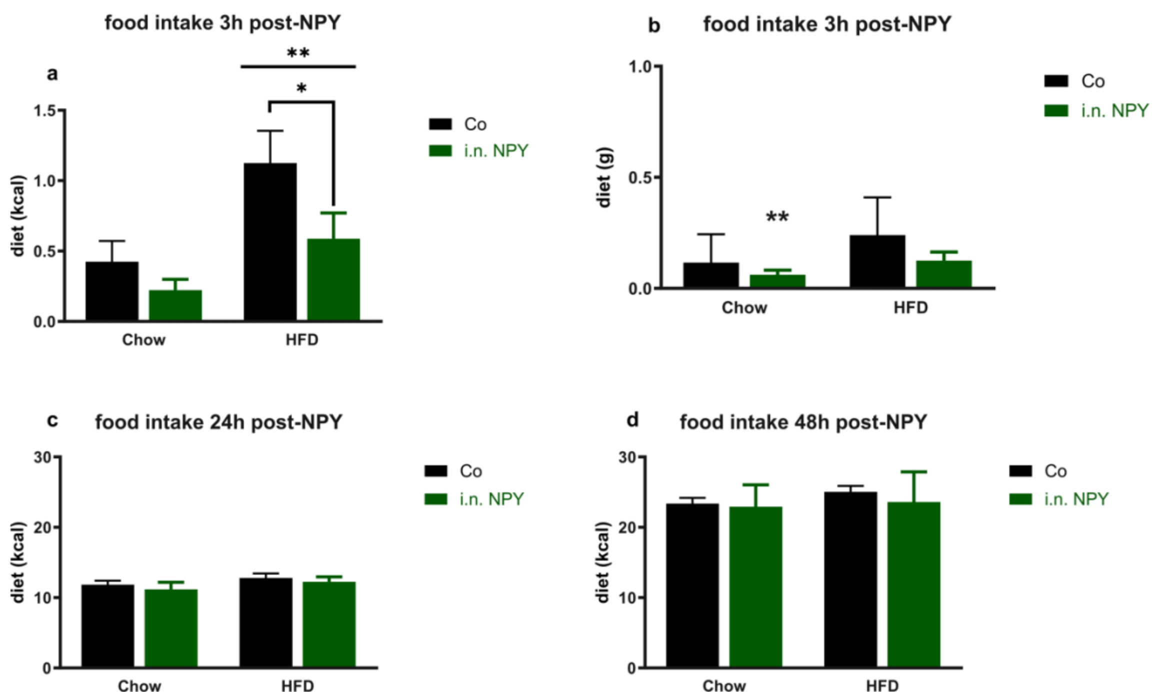

Figure 6: Food intake 3h after intranasal NPY treatment. *I.n. NPY reduces food intake (kcal) of HFD fed mice 3h (a) but not 24h (c) and 48h (d) after i.n. treatment.. Mice on HFD consume more food compared to mice on control diet (a) I.n. NPY reduces food intake (g) of control diet fed mice 3h after i.n. treatment (b). Diet pellets were weighed before i.n. treatment, 3h, 24h and 48h after treatment.* Values represent means ± SEM, n=12, $*p \leq 0.05$, $**p \leq 0.01$, vs. control (water)/control diet **(two-way ANOVA, Tukey's multiple comparisons test)**

3.6 HFD reduces NPY expression in hypothalamus

NPY expression in hypothalamus was significantly lower in experimental groups fed HFD (Figure 7). Two-way ANOVA revealed a significant effect of HFD ($F(1,38)=29.48$, $p<0.0001$) on the expression of NPY mRNA in hypothalamus. This effect was also reflected in the measurement of NPY concentration at the peptide level in the hypothalamus. Two-way ANOVA showed a main effect of HFD ($F(1,36)=9{,}713$, $p=0.0036$) on NPY in hypothalamus.

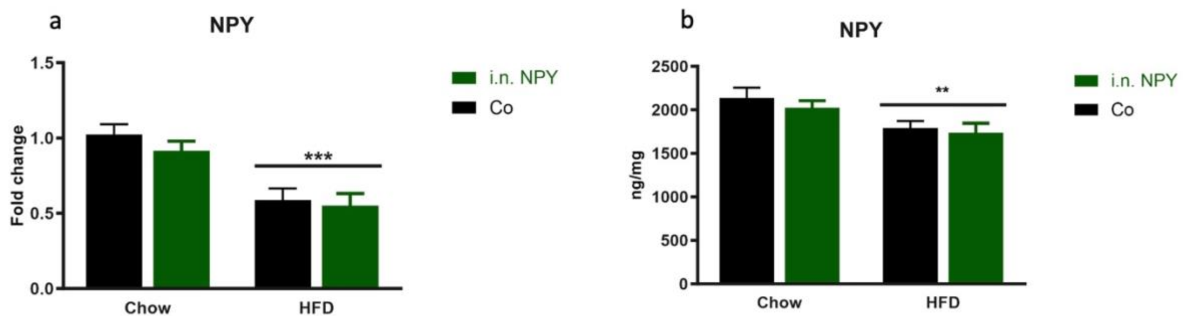

Figure 7: NPY expression and protein levels after intranasal NPY treatment. *HFD decreases NPY mRNA expression (a) and protein levels (b) in hypothalamus of HFD fed mice. Mice were treated with either i.n. NPY or sterile distilled water in order to collect the brains 3h afterwards. Hypothalamic tissue was analyzed for NPY mRNA expression and NPY protein levels.* Values represent means ± SEM, n=12, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ vs. control (water)/control diet **(two-way ANOVA, Tukey's multiple comparisons test)**

3.7 Intranasal NPY alters hypothalamic Bdnf and Crh expression and blood plasma levels of corticosterone

Although not statistically significant, i.n. NPY reduced Bdnf expression in hypothalamus compared to water in mice on control diet (Figure 8). Two-way ANOVA revealed a strong tendency in interaction between diet and i.n. treatment ($F(1,35)=3.737$, $p=0.0613$). Turkey's multiple comparisons test reflects this trend in control diet fed mice after i.n. NPY ($p=0.1977$).

Crh expression differs significantly between mice on control diet after i.n. treatment with water or NPY (Figure 8). Two-way ANOVA revealed a significant interaction between diet and i.n. treatment ($F(1,37)=8.009$, $p=0.0075$). Sidak's multiple comparisons test showed a significant effect of i.n. NPY on mice fed control diet.

Two-way ANOVA revealed a tendency in interaction between diet and i.n. treatment ($F(1,33)=3.137$, $p=0.0858$) (Figure 8). Sidak's multiple comparisons test showed a significant effect of i.n. NPY on corticosterone levels of mice on HFD ($p=0.0485$).

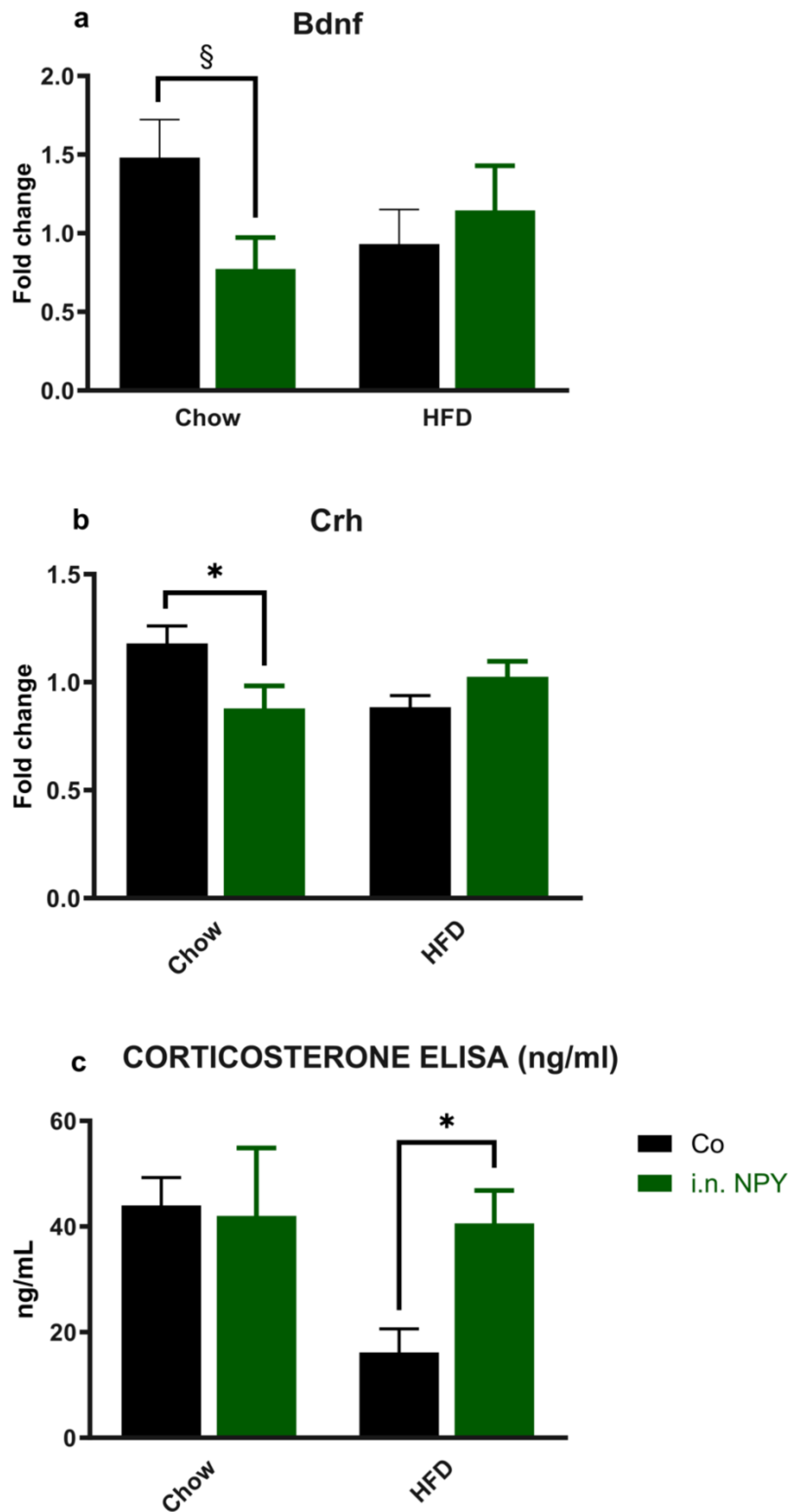

Figure 8: Changes of Bdnf and Crh expression in hypothalamus and corticosterone levels in blood plasma *(a) Although not statistically significant, Bdnf expression of control diet fed mice shows a trend to a decrease after i.n. NPY (b) I.n. NPY reduces CRH expression in hypothalamus of control diet fed mice (c) I.n. NPY increased plasma corticosterone levels of HFD fed mice compared to treatment with water.Mice were treated with either i.n. NPY or sterile distilled water in order to collect the brains and blood plasma 3h afterwards. Hypothalamic tissue was analysized for CRH and Bdnf expression.* Values represent means ± SEM, n=12, *p ≤ 0.05, §=0.1977 vs. control (water)/control diet **(two-way ANOVA, Tukey's multiple comparisons test)**

3.8 HFD increases Il-1b expression in hypothalamus

Although not statistically significant, there was a strong tendency of higher Il-1b expression in hypothalamus of mice fed HFD (Figure 9). Two-way ANOVA revealed a tendency of the effect of HFD ($F(1,37)=3.69$, $p=0.0624$) on Il-1b expression.

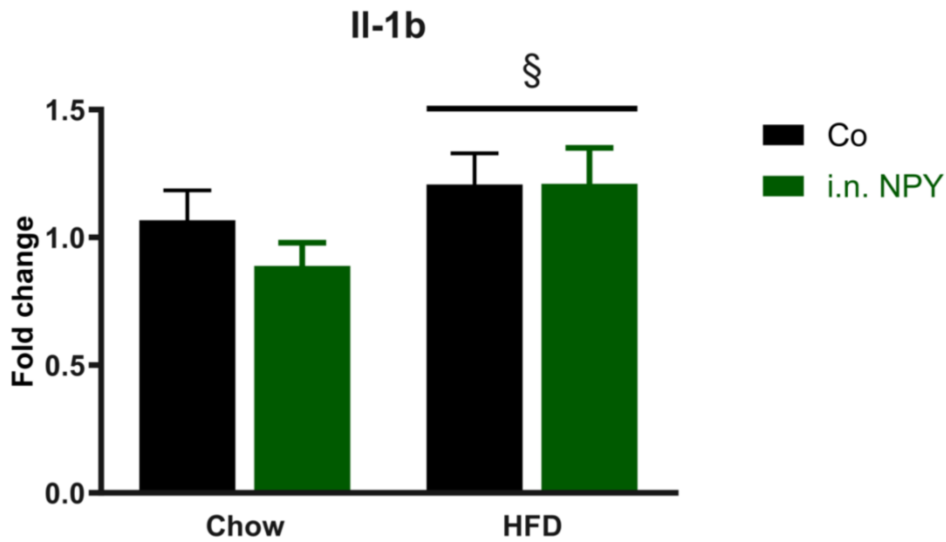

***Figure 9: IL-1b expression of HFD fed mice is increased compared to control diet fed mice, although not statistically significant**. Mice were treated with either i.n. NPY or sterile distilled water in order to collect the brains 3h afterwards. Hypothalamic tissue was analyzed for IL-1b mRNA expression.* Values represent means ± SEM, §=0.0624 vs. control diet **(two-way ANOVA, Tukey's multiple comparisons test)**

3.9 Intranasal NPY doesn't change SP in HFD Y2 knockout mice 3 h post-NPY

SP did not change in Y2 KO mice on HFD after i.n. NPY treatment. I.n. NPY also had no significant effect on body weight and food intake in Y2 KO mice on HFD 3 h after i.n. treatment (Figure 10).

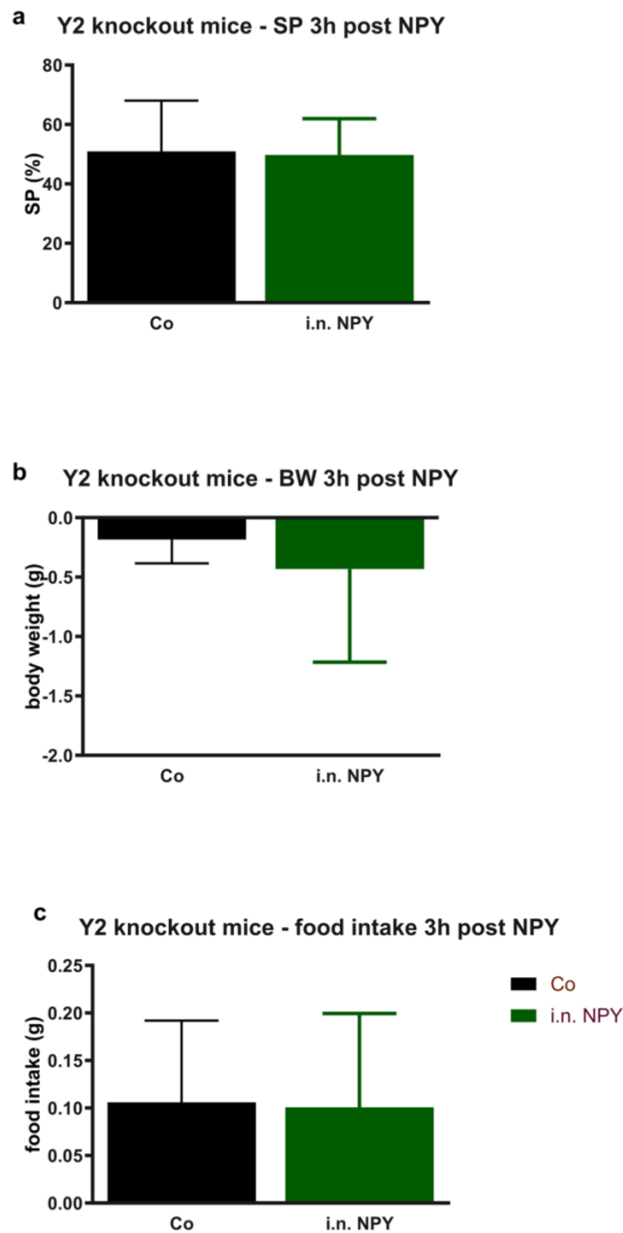

Figure 10: Intranasal NPY has no effect on SP (a), body weight (b) and food intake (c) of HFD fed Y2 knock-out mice 3h after treatment. *Mice were treated with either i.n. NPY or sterile distilled water. 3h later SP, body weight and food intake was measured. Values represent means ± SEM,* n=12 *vs. control (water)/control diet* ***(two-way ANOVA, Tukey's multiple comparisons test)***

4 Discussion

4.1 Sucrose preference, food intake and weight loss after intranasal NPY

The data collected show that i.n. NPY induces a decrease in sucrose intake in HFD-fed mice alongside with lower food intake and body weight. These effects are accompanied by elevated corticosterone levels in plasma. By contrast, in Y2 KO mice fed an HFD, NPY failed to produce any significant change in behavior, food intake, or body weight. In addition, HFD increased the expression of Il-1b and decreased the expression of NPY in the hypothalamus, and i.n. application of NPY decreased that of Bdnf in the control group.

Anhedonia is a major symptom of depression that can be observed in humans as well as in mice. In the mouse model, anhedonia can be measured by a decrease in SP in order to objectify depression-like behavior (48). Various experiments have already shown that HFD can induce depressive-like behavior. Feeding HFD with 60% of calories from fat for 8 to 12 weeks reduces SP in mice (38, 43, 75). In another study, there was no reduction in SP after 8 weeks of HFD with 60% fat, but anhedonia was observed after 15-17 weeks (76). In the current experiment, HFD does not induce anhedonia after 8 weeks. This might be due to the lower fat content of the HFD (48% of kJ from fat), which is closer to the composition of the Western diet of humans (77, 78), and therefore may give a more realistic representation of the effect of a HFD in humans. The SP test was used in this case because strong effects were observed in previous experiments (43). However, it would be useful to repeat the experiment with other behavioral tests like the forced swimming test or elevated plus maze test to see if there is a change in other aspects of depressive-like behavior.

Although the reduction in SP after HFD was not statistically significant, absolute sucrose intake was statistically lower after 8 weeks of HFD compared to control diet. In other experiments with rats in a model of chronic unpredictable mild stress, a commonly used model of depression in rodents, SP was not constantly reduced as well (79). In addition, there is a study with rats that sees equally stronger effects in absolute sucrose intake than in sucrose preference with the same sucrose concentration as used in this experiment (1% sucrose) (80). One possible explanation brought by the authors is that water intake is also influenced by other physiological processes besides the reward circuit and therefore the results of SP differ from those

of absolute sucrose intake (80). Moreover, the increased absolute sucrose intake in this experiment was associated with higher dopamine-dependent activation of dopamine receptors in the nucleus accumbens (80). Since dopamine is currently thought to be involved in mediating the rewarding effect of sucrose, it can be hypothesized that the change in absolute sucrose intake is also indicative of changes in the reward circuit even though sucrose preference is statistically unchanged. Therefore, reduced sucrose intake in our experiment may still be a sign of a change in the reward circuit and may be interpreted as a sign of anhedonia.

NPY can modify mood and behavior because it has anxiolytic effects and reduces depression-like behavior (50). This is evidenced not only by the antidepressant effect of i.c.v. administered NPY (81, 82) and anxiolytic effect of i.c.v. administration of the Y1, Y2 and Y5 receptor agonist NPY(2-36) (65) but also by the fact that lower expression of NPY was found in people with mild depressive disorder (83). Furthermore, feeding mice a HFD decreases NPY mRNA in the arcuate nucleus (ARC) of the hypothalamus (58) and depression-like behavior induced by HFD is associated with lower expression of NPY in the hypothalamus and hippocampus (43). Therefore, we hypothesized that i.n. NPY may improve HFD-induced anhedonia. Contrary to expectations, i.n. NPY lowered SP in the HFD group. This finding was accompanied by lower food intake and weight loss in the HFD group after i.n. NPY.

NPY is anxiolytic at lower doses but sedative at higher doses, as measured by the home cage and open field activity of the rats studied (84). In addition, NPY administered i.c.v. is capable of enhancing the sedative effects of anesthetics, for example of the GABA agonist pentobarbital (85). Because isoflurane, a GABA-agonist, was used in this experiment to sedate the mice for i.n. application, the sedative effect of NPY, but also an enhancement of the sedation of the isoflurane used, could explain the reduced SP and food intake. The dosage of NPY was derived from a previous experiment with i.n. NPY, in which food intake was also lower after NPY but not statistically significant (72). One consideration to make is that NPY could have led to local irritation of the nasal cavity, resulting in reduced SP. However, this is contradicted by the fact that there are already experiments with i.n. NPY in rats in which i.n. NPY was able to reverse behavioral changes such as signs of anxiety and depressive-like behavior, showing the potential of i.n. NPY (63).

According to current knowledge, the appetite-stimulating effect of NPY is mediated via the PVN and the anxiolytic effect via the amygdala. However, intra-amygdalar

administration of NPY in satiated rats was able to produce a reduced preference for HFD without changing total food intake. This effect appeared independent of anxiety. In this experiment, the authors hypothesized that the reduced preference for HFD was due to the NPY-induced change in the emotional well-being of the rats (86). In our experiment, the mice showed reduced SP after NPY treatment, which is indicative of anhedonia and therefore makes it difficult to distinguish whether the reduced food intake after i.n. NPY was due to the changes in the behavior of the mice or to previously unknown mechanisms linking the amygdala and PVN.

4.2 Involvement of the Y2 receptor

Another possible explanation for the decreased food intake in the HFD group after i.n. NPY is that HFD might desensitize the effect of NPY. There is evidence that HFD-induced obesity comes along with a reduced sensitivity of downstream circuits regulating food intake following the stimulation of Agouti-related protein (AgRP)/NPY neurons in ARC. Therefore, a stronger activation of AgRP/NPY neurons, and thus more NPY release, is required to initiate food intake (87). On the other hand, neurons in the ARC presynaptically express Y2 receptors that cause inhibition of further NPY release after NPY binding (53, 88). It is possible that i.n. NPY did not sufficiently activate the downstream circuit after application but resulted in negative feedback via the Y2 receptors and thus reduced food intake in comparison to the HFD only group. This presumption also fits with the discovery that NPY specifically from AgPR/NPY neurons is relevant to the regulation of food intake (89). Using NPY knockout mice with specific knockout of NPY in AgRP/NPY neurons demonstrated that NPY from AgRP/NPY neurons is primarily required for initiation of food intake but also for its duration (89). To further test the hypothesis of an involvement of the Y2 receptor in our observations, we performed the same experiment with Y2 KO mice. The fact that there was no difference in food intake and weight loss between i.n.NPY and i.n. water in HFD Y2 KO mice is a strong indication that the observed effect in the first experiment is mediated via Y2 receptors. Although the exact mechanisms are not yet known, it is suggested that the Y2 receptor plays a role because, as a presynaptic receptor, it inhibits the further release of NPY from neurons and therefore tends to mediate an anorexigenic effect (90). This is also reflected by the fact that mice consumed less food and lost significant weight after ICV administration of a Y2 agonist (91).

4.3 NPY expression under HFD

Since NPY is anxiolytic (92) and it has been observed that HFD with 60% of kcal from fat diminishes NPY expression in the hypothalamus (43, 58, 93), we wanted to analyze whether this could also be reproduced by a HFD with a lower fat content. Indeed, HFD reduced NPY expression in hypothalamus as shown by both mRNA and peptide level measurements. Hypothalamic NPY neurons express leptin receptors (94), through which leptin controls NPY expression (58). Hypothalamic content of NPY peptide under HFD feeding is negatively correlated with plasma leptin levels (95), which suggests that the diminished NPY after HFD feeding, that was observed in many experiments, might be due to a negative feedback by leptin which intends to inhibit the orexigenic effect of NPY and prevent further food intake and weight gain by reducing the release of NPY in the hypothalamus. The fact that there was no significant change in NPY expression after i.n. NPY application is possibly due to the fact that adaptation of this circuit takes longer. When feeding rats a HFD with 30 % of kcal from fat the decrease in NPY expression was only visible after 9 weeks of feeding (95).

4.4 Hypothalamic inflammation in response to HFD

In addition to diminished NPY expression in the hypothalamus, we observed a trend of increased IL-1b by HFD ($p=0.062$). There may also be a relationship between increased IL-1b and lower NPY expression in the hypothalamus. Because expression of NPY was increased in the ARC of the hypothalamus in an IL-1 receptor-deficient mouse model, it can be hypothesized that increased IL-1b after HFD feeding inhibits expression of NPY in the hypothalamus(96). Our finding of a tendency to increased Il-1b after HFD is consistent with previous research showing that HFD can increase levels of proinflammatory cytokines in the hypothalamus of rodents (97-99). In more detail, saturated fatty acids initiate the Toll-like-receptor 4 (TLR-4) pathway (100), which leads to the release of proinflammatory cytokines such as IL-1b via the nuclear factor kB (NF-kB) pathway (30, 101) and to endoplasmic reticulum stress (102), which drives further inflammation (103). In addition, some regions of the hypothalamus have an imcomplete BBB (104), the permeability of which is also increased by HFD (105). This allows circulating proinflammatory cytokines such as IL-1b from the periphery to overcome the BBB, cross over into the hypothalamus, and exert their proinflammatory

effects (30). This explains the strong tendency of increased IL-1b expression in the hypothalamus in response to HFD.

4.5 Intranasal NPY and Bdnf

A strong trend for reduced hypothalamic Bdnf was observed in response to i.n. NPY within the control diet group (p=0.0613). Bdnf is a neurotrophin that is primarily relevant for neuronal differentiation and survival during CNS development, but is also important in adulthood for neuronal processes such as neuroplasticity (106, 107). In the hypothalamus, Bdnf influences the HPA axis, among others, by stimulating the expression of Crh (108). In experiments with intraperitoneal NPY, it was observed that Bdnf expression in the hypothalamus decreased after NPY administration (109, 110) which was accompanied by a significant reduction in corticosterone plasma levels in the NPY treated group (110). As this is indicative of a lower HPA axis activity, and on the other hand NPY is known to reduce HPA axis activity (111), Bdnf may be involved in mediating the effect of NPY on the HPA axis. In fact, we also observed a reduction of Crh in the control group after treatment with i.n. NPY. Since NPY neurons in the ARC and Crh neurons in the PVN can communicate via synaptic interactions (112), this is thought to be a mechanism through which NPY increases resilience against stress (50).

4.6 Corticosterone levels and intranasal NPY in HFD fed mice

Current knowledge on the influence of NPY on the HPA axis is not consistent. One of the main actions of NPY is the counteraction of the reactivity of the HPA axis to a stress stimulus (50) but there is also evidence for increased corticosterone levels in response to NPY. For example, Wahlestedt et. al injected NPY into the PVN and detected increased circulating ACTH and corticosterone levels compared to controls (113). Albers et. al also injected NPY into the PVN of rats and also observed higher plasma corticosterone levels than in saline injected rats (114). A recent study showed that AgRP neurons in the hypothalamus of mice activate the HPA axis and lead to higher corticosterone levels in the blood plasma (115). Their results strongly suggest that HPA axis activation by AgRP neurons happens through their projections to the PVN. Since the exact mechanism of how AgRP neurons activate the Crh producing neurons in the PVN are not known yet, Douglass et al. tested if AgRP neuron

stimulation is able to increase corticosterone levels in the absence of NPY and GABA (NPY KO mice, mice lacking the vesicular GABA transporter and double-KO mice). AgRP neuron stimulation was able to induce a corticosterone response in the absence of NPY or GABA but not in the double KO mice (115). This implies a possible involvement of NPY and GABA in the activation of the HPA axis mediated by AgRP neurons. In the current experiment i.n. NPY also increased corticosterone levels in the HFD group. This might be explained by the mechanisms mentioned above. Since reduced SP is indicative of anhedonia and this in turn is in context of depressive-like behavior associated with HPA axis activation (116), higher plasma corticosterone after i.n. NPY could mediate reduced sucrose preference. This is in line with a study by Sturm et. al that demonstrated that chronic corticosterone administration reduces SP of male mice (117).

4.7 Limitations of this project

As in any experiment, it is possible that external circumstances or biases may have affected the results of the current study. One limitation of this study is that only male mice were used. Since there is a possibility that the hormonal cycle of female mice changes the behavior independently of the experiment, this could also influence the behavior in the SPT and thus bias the results. Therefore, only male mice were used, but the results cannot be generalized to both sexes and the experiment should be repeated again with female mice, if necessary to make a general statement. A further limitation can be the use of only one behavioral test, the SPT. Since this test examines only one symptom of depression-like behavior, it is possible that some results would be stronger or weaker in other behavioral test. Although other experiments have seen strong results in SPT it is still possible that in this experiment other tests would have shown results as well. In addition to this, the SP can be affected when the food intake changes because the solution contains sucrose in the SPT. Therefore, a decrease in SPT could also be due to a decrease in (palatable) food intake, independent of depression-like behavior. Last, the duration of the experiment may also have been a limitation. Since we chose a HFD with lower fat content but still chose the same time period to feed (8 weeks), it may be that we would have had to feed longer to see signs of anhedonia in the SPT before NPY administration.

5 Conclusion

Since obesity and depression are a growing problem for our health care system, it is of societal interest to keep developing and improving therapies. To do this, it would be advantageous to not only be able to proceed by remedying the causes of obesity, but also to be able to alleviate the depressive symptoms. Since the current state of research strongly suggests that NPY is involved in the development of obesity-induced depression, it is certainly worthwhile to further explore the possibilities of therapeutic administration of NPY. Although we did not observe the expected antidepressant effect of NPY in this experiment, we did observe interesting evidence of Y2 receptor involvement. Since the Y2 receptor is not yet as well studied in this context as the Y1 receptor, it would be interesting to perform further experiments in the future that analyze the expression of the Y2 receptor. Nevertheless, this experiment demonstrated the potential of i.n. drug delivery. Since there are few publications to date on experiments with i.n. NPY in disturbances of mood and behavior, it would be interesting to try different dosages of i.n. NPY and Y1 specific agonist in future experiments.

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