Thesis

Relevance of hormones and peptides to food intake: Effects of JNJ-31020028

submitted by

Ulrich Severin Dischinger

Mat.Nr.: 0534262

for the degree of

Dr. med. univ.

at the

Medical University of Graz

Institute of Experimental and Clinical Pharmacology

Supervisor

Univ.-Prof. Mag.rer.nat. Dr.phil. Peter Holzer

Graz, July 12, 2011

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am

Unterschrift

Acknowledgments

I would like to thank Univ.-Prof. Mag.rer.nat. Dr.phil. Peter Holzer for supervising my work and Mag. Dr.rer.nat. Evelin Painsipp and Ing. Margit Eichholzer for helping hands in every difficult situation. The gift of JNJ-31020028 by Dr. Pascal Bonaventure (Johnson&Johnson, San Diego, CA, USA) is greatly appreciated.

Zusammenfassung

Die Anorexia nervosa gehört zu den prognostisch ungünstigsten Erkrankungen aus dem Fachbereich der Psychiatrie. Dies ist auch durch beschränkte Möglichkeiten in der medikamentösen Therapie dieser Erkrankung bedingt. Um so wichtiger ist es, die pathophysiologischen Grundlagen von Anorexia nervosa weiter zu erforschen sowie geeignete Tiermodelle zu entwickeln, um so auf mögliche medikamentöse Angriffspunkte zu stoßen und die Auswirkungen potentieller Therapeutika präklinisch zu erfassen.

Diese Arbeit gibt dementsprechend im ersten Teil einen Überblick über ausgewählte Peptide und Hormone, die im Rahmen der Nahrungsaufnahme eine Rolle spielen, und ihre Bedeutung bei Anorexia nervosa. Behandelt werden: Das Neuropeptid Y, das Agouti-related Peptid, das Melanocortin-System, der Corticotropin-releasing Faktor, das Peptid YY, die Hormone Leptin und Ghrelin, Stickstoffmonoxid und das Endocannabinoid-System. Entsprechend ihrer Auswirkung auf die Nahrungsaufnahme lassen sich diese Hormone/Peptide prinzipiell als orexigen, d.h. die Nahrungsaufnahme fördernd, oder anorexigen klassifizieren. Allerdings erweist sich diese Einteilung nicht grundsätzlich als sinnvoll, wie am Beispiel des Neuropeptid Y genauer erläutert werden soll. Ferner werden zu Beginn der Arbeit verschiedene Tiermodelle vorgestellt, die für die präklinische Anorexia nervosa-Forschung zur Verfügung stehen. Besonderes Augenmerk wird dabei auf das Aktivitäts-Stress Modell nach Routtenberg und Kuznesof gerichtet, welches bei den Versuchstieren u.a. eine Hyperaktivität beinhaltet, wie sie auch bei Anorexia nervosa PatientInnen häufig zu beobachten ist.

Im zweiten Teil dieser Arbeit wird der Effekt des spezifischen Y2-Antagonisten JNJ-31020028 auf Nahrungsaufnahme, Flüssigkeitsaufnahme sowie Aktivität von Mäusen genauer untersucht. Zu diesem Zweck wurde diese Substanz weiblichen Tieren in drei Versuchsansätzen intraperitoneal injiziert. Im ersten und zweiten Ansatz, die sich lediglich in der Dosierung des injizierten Antagonisten unterscheiden (10 mg/kg, bzw. 20 mg/kg), erhielten die Tiere Nahrung ad libitum, während im dritten Ansatz (10 mg/kg) die Nahrungszufuhr auf drei Stunden pro Tag beschränkt wurde, um so eine Hyperaktivität der Mäuse zu induzieren. Die Versuchstiere rekrutierten sich dabei aus dem Stamm DBA/2, der im Fall von Nahrungsrestriktion ein hyperaktives Verhalten aufweist. Gemessen wurden das Gewicht der Tiere an festgelegten Punkten der Versuchsreihen und mit Hilfe des LabMaster-Systems Nahrungs- und Flüssigkeitsaufnahme sowie Aktivität während des gesamten Versuchszeitraumes. Dabei zeigte sich im ersten Versuchsansatz (10 mg/kg) ein positiver Effekt auf die Nahrungsaufnahme der Tiere, der im zweiten Ansatz mit verdoppelter Dosis der Substanz (20 mg/kg) jedoch nicht bestätigt werden konnte. Eine deutliche Auswirkung auf das Aktivitätsniveau konnte in keinem Ansatz gezeigt werden. Eine mögliche Bedeutung von Neuropeptid Y/Y2-Rezeptoren für die Anorexie bleibt somit offen.

Abstract

Anroexia nervosa is one of the most severe diseases in psychiatry. A lack of pharmacotherapeutical possibilities is one reason for this fact. Therefore it is of great concern to gain further information about the pathophysiological background of anorexia nervosa and to find appropriate animal models, with the aim to determine new pharmacological possibilities and to test their effects in a preclinical environment.

In the first part of this paper, a review of several peptides and hormones in the regulation of ingestion and their impact in anorexia nervosa is given. Discussed are neuropeptide Y, agouti-related peptide, melanocortin system, corticotropin-releasing factor, peptide YY, the hormones leptin and ghrelin, nitric oxide and the endocannabinoid system. Regarding food intake, these hormones/peptides can be classified as orexigenic, meaning food intake stimulating, or anorexigenic. However, this classification is not generally suitable, as is shown here for the case of neuropeptide Y. At the beginning of the first part, several animal models which might be appropriate for preclinical anorexia nervosa research are presented. Attention is focused especially on the activity-stress model by Routtenberg and Kuznesof. This model imitates among other things a hyperactive behaviour which is often observed in anorexia nervosa patients.

The second part of this paper reports on the effects of the Y2 receptor antagonist JNJ-31020028 in mice regarding food and water intake as well as activity. This substance was injected intraperitoneally to female mice in three experimental approaches. The first and second approach only differed in substance dose (10 mg/kg, resp. 20 mg/kg) and the mice received food ad libitum, while in the third approach (10 mg/kg) the access to food was restricted to three hours a day. Mice of the strain DBA/2 were used because this strain was reported to become hyperactive in case of diet-restriction. The weight of the mice was measured at defined points in each trial, while the LabMaster system was used to measure feeding, drinking and activity over the whole time. An effect of JNJ-31020028 to increase food intake was shown in the first approach (10 mg/kg), an effect that was not present in the second approach with a twofold dose of the substance (20 mg/kg). A significant effect of JNJ-31020028 on the activity level of the experimental animals was not seen. Thus, any role of neuropeptide Y acting via Y2 receptors in anorexia nervosa remains questionable.

Contents

1 Introduction

Anorexia nervosa (AN) is one of the most dangerous psychiatric disorders. Only 25% of the patients achieve long-time recovery of this frustrating sickness, with a death rate of nearly 1% per therapy-year (Herold, 2008 : p. 656 f.). Mortality from all causes in AN patients is six-fold higher than in the general population (Rask-Andersen et al., 2010). Therapeutic options are still limited: By evidence-based criteria only cognitive behavioural therapy seems to have some therapeutic value. Furthermore the pathogenesis of AN is not completely clear, but the following points seem to be of interest: In sociocultural matters the dominance of a slimness and juvenileness oriented ideal of beauty seems to play the main role. Otherwise overestimation of this fact is probably unrealistic: Even in former times and also in culture areas with other ideals of beauty AN was/is existent. For the first time described more precisely by W.W. Gull in 1868 and E.C. Lasègue in 1873 (Vandereycken et al., 1989), there are even earlier descriptions. To take a single example, Mary Stuart (1542-1587), queen of Scots, seems to have suffered from AN (McSherry, 1985) in a time when the ideal of beauty was definitely not slimness, as we see in portraits of Rubens or Vermeer. Moreover, affected people do not stop to loose weight, even if their body proportion has reached a level that brings social disadvantage. This might indicate that AN is a sort of addiction. Furthermore certain personality traits (perfectionism, compulsivity) and developmental-psychological factors (experience of loss, abuse) may promote the development of AN. Also genetic analyses indicate a multilayer pathogenesis of AN, showing familial accumulation and a concordance-rate of 66% within monozygotic gemini. The frequency of AN in women in western civilization is at 3.5%, and 90% of AN patients are female (Rothenhäusler et al., 2007 : p. 406 ff.).

There are two types of AN, which refer to eating-related behaviour. Patients who suffer from restricting-type AN lose weight mainly by disciplined dieting, while binge-eating anorexics sometimes engage in bingeing and/or purging, as seen in bulimia nervosa. The DSM-IV criteria for AN diagnosis are: Weight loss to under 85% of individually expected body weight, distinct fear to gain weight, disturbance of self-perception and amenorrhoea in postmenopausal women (Kaye et al., 2009).

In many cases of neuroendocrinological changes in AN patients, it is hard to say, if they are cause or consequence of AN. In the following, the main endocrinological axes in terms of their changes in AN are briefly mentioned. The hypothalamic-pituitary-gonadal axis displays an hypogonadotropic hypogonadism, whose manifestation (i.e. amenorrhoea) correlates with available fat mass. In the growth hormone (GH) - insulin-like growth factor 1 (IGF-1) axis, IGF-1 levels are low, while GH levels are high. The hypothalamic-pituitary-adrenal (HPA) axis displays high cerebrospinal fluid levels of corticotropin-releasing factor (CRF), normal adrenocor-

ticotropic hormone (ACTH) levels and high cortisol levels (similar to stress induced response). Regarding the hypothalamic-pituitary-thyroid axis, thyrotropin-releasing hormone (TRH) levels are slightly decreased as well as thyroid-stimulating hormone (TSH) levels. Triiodothyronine (T3) levels are low and free thyroxine (T4) levels are normal or low (Misra et al., 2010; Casper, 2006).

Several brain areas are involved in feeding. The hindbrain contains motor neurons responsible for actions like chewing and swallowing. Viscerosensory information, such as blood glucose, gastrointestinal distension and hepatic function, is processed in the hindbrain (Watts et al., 2007). The telencephalon (cerebral cortex, striatum and pallidum) integrates cognitive components of feeding, for example foraging. The hypothalamus takes centre stage in the process of spontaneous feeding. In this area of the brain, many hormones and neuropeptides interact. Humerosensory information, such as insulin and leptin levels, is processed here (Watts et al., 2007). Section 3.1 gives more insight into this area.

Because of currently limited therapeutic options, it is absolutely necessary to elucidate the pathogenesis of AN. Correspondingly, the first ambition of this paper is to review the neuroendocrinological background of AN. Therefore, the general relevance of several neuropeptides and hormones for food intake and their AN-specific changes are presented, their hypothalamic interaction being of special interest. Regarding food intake, it makes sense to classify these hormones and neuropeptides either as orexigenic - food intake stimulating - or anorexigenic. However, with some of these messengers, their effect seems to be situation-dependent. It has to be suggested, that feeding is the consequence of preponderance of orexigenic signalling, while anorexigenic signalling prevails in case of anorexia.

In the second part of this paper, an experiment is presented, which was realized within the framework of this final year project. Therein the effect of an injection of the Y2 receptor antagonist JNJ-31020028 on weight, food intake, activity and drinking of DBA/2-mice was tested. Additionally, the substance was tested in mice under diet-restriction (activity-stress model), especially for its effect on the activity-level. As background for further explanations several animal models, that are more or less appropriate for the examination of AN, are presented in the following section.

Part I

Animal models and (neuro-)peptides

2 Animal models for anorexia nervosa

In general, animal models are used for research on human diseases, particularly if a disease-like condition cannot be induced in humans for ethical reasons. Especially in case of psychiatric diseases, such as AN, the animals in use should display a behaviour resembling that found in the patients (face validity). Of course, right at this point animal models for AN are very limited. A spontaneous development of AN in wild animals is very unrealistic, because of the very human aspects of this disease. Causes or exact physiological mechanisms of AN are hardly known, although some progress in their investigation has been made. So, following the classification of animal models by Smith (1989), the available animal models for AN are isomorphic. This means, that the main phenotypic points of AN, weight loss and reduced food intake, can be generated by various approaches, while the human aspects - like those mentioned above - can hardly be simulated. Basically, anorexia and/or cachexia in an experimental animal can be caused by a change in the environment, injection of anorexia-inducing substances, changes on an organic level, or genetically.

In this context, the terms cachexia and anorexia should be clarified. While cachexia means an abnormal emaciation with a body-mass-index (BMI) under 18 in humans, anorexia, as a clinical symptom, describes a decreased sensation of appetite, that can of course lead to cachexia. Hence, cachexia is objectifiable in humans as well as in animals, whereas anorexia can hardly be objectified, especially in animals. Because of this, it makes sense to speak of anorexia in cases, when experimental animals eat less food than available, although they have not reached their daily requirements. AN, however, is the name of a psychiatric disorder, of which anorexia is one symptom.

Still, if anorexia is present in an animal model, analysis of neuroendocrinological changes can shed light on the pathophysiological backgrounds of AN. Furthermore, the administration of substances that are assumed to play a role in the regulation of food intake, can be tested in such animals. In this way, animal models for AN may help to find new therapeutic strategies for AN.

2.1 Anorexia inducing environmental conditions

The best way to simulate AN seems to be the activity-stress model (activity-based anorexia (ABA)) by Routtenberg and Kuznesof (1967). Rats under diet-restriction (food access 60-120 min/d) and with access to a running wheel show the following behaviour: Increased running wheel activity (RWA) with primarily compensatory increased food intake. However, food intake stagnates at some point while running wheel activity increases further and leads to weight-loss, amenorrhoea and finally to death of starvation (Casper et al., 2008). Female mice display more RWA than male mice and younger mice more than older mice (van Leeuwen et al. 1997). The following (neuro-)endocrinological changes were detected in ABA: Increased plasma corticosterone and ACTH levels (van Leeuwen et al. 1997), elevated serotonin levels in the hypothalamus (Siegfried et al., 2003), increased neuropeptide Y (NPY) and agouti-related peptide (AGRP) and decreased pro-opiomelanocortin (POMC) in the hypothalamus, hyperghrelinaemia and hypoleptinaemia. The development can be counteracted through the administration of L-tryptophan, serotonin agonists, alpha-2-adrenoceptor agonists, naloxone (opioid antagonist), cis-flupenthixol (dopamine antagonist) and benzodiazepines. The mentioned substances lead to modest improvement, but do not abolish ABA (Pirke et al., 1993; Lewis et al., 2010). Administration of tyrosine also significantly improves food consumption, cognitive function and delays the onset of fatigue within experimental animals in this model (Siegfried et al., 2003; Avraham et al., 2001). Administration of AGRP increases the survival rate of rats in this model, probably by inhibiting the anorectic melanocortin system (see section 3) (Adan et al., 2003). For the development of ABA, the presence of a running wheel seems to be inevitable. After a period of re-feeding, RWA of ABA animals returns to normal (Dixon et al., 2003). Taking everything into consideration, this animal model provides many aspects of AN: Hypophagia, hyperactivity, increased HPA axis, weight-loss and amenorrhoea.

In contrast to rats, ABA develops only in few mice strains (Rikke et al., 2003), maybe because of less nutritional reserves. Instead, mice under ABA conditions more often show episodes of torpor (Lewis et al., 2010). Gelegen et al. (2010) could match ABA to the chromosomes 4, 12 and 13 of the mouse-strain A/J, while the strain C57BL/6J showed reduced activity under the same conditions. However, Lewis et al. (2010) used an optimised ABA paradigm to induce ABA in C57BL/6 mice, too (see section 10).

In nature, the increased activity shown by ABA animals could even be useful in terms of foraging, when not enough food is available. The behaviour shown by ABA animals could be present at least in some AN patients, too. So, the described increase of casual activity in many AN patients could at least partly be caused by this archaic way of behaving. The prevalence of hyperactivity in AN patients varies between 31% and 80% (Hillebrand et al., 2008).

Additionally, it has to be mentioned that behavioural thermoregulation is one mechanism to

maintain a stable body temperature and might be present in ABA. Decrease of body temperature is at least partly due to hypoleptinaemia during food restriction, because leptin is known to increase thermogenesis in the brown adipose tissue. Gelegen et al. (2007) could show that A/J as well as DBA/2J mice increase their locomotor activity and display a decrease of body temperature under ABA conditions (which is more distinct in DBA/2J mice), while C57BL/6J mice display a larger decrease of body temperature than A/J mice and no significant change of locomotor activity. Interestingly, hypoleptinaemia was not nearly as distinctive in A/J as in DBA/2J and C57BL/6J mice. Still A/J and DBA/2J mice reached nearly the same level of locomotor activity. Furthermore, although DBA/2J mice increased their locomotor activity, the decrease of body temperature is nearly as large as in C57BL/6J mice. This might be due to the fact that Y1 receptors are upregulated only in DBA/2J mice under ABA conditions and NPY might mediate an antithermogenic effect through Y1 receptors. Different expression patterns of the melanocortin 3 receptor in the three groups (downregulation in A/J and C57BL/6J but not in DBA/2J) might be of further relevance. Taking everything into consideration, these observations indicate that there are different adaptation strategies to food restriction, which depend on different genetic situations (Gelegen et al., 2007). Hypoleptinaemia might be a cause of increased locomotor activity (see section 7), depending on the genetic background.

It is important to consider that exercise can show similarities to addiction. Using the activity-stress model (wheel access and 1h-feeding), it could be shown that rats display significantly more symptoms of withdrawal following injections of 1.0 mg/kg naloxone, than 24h-fed rats with wheel access and inactive rats. This indicates that endogenous opioids take part in the behaviour precipitated by the activity-stress model. (Kanarek et al., 2009) This means, that locomotor activity is satisfying and addictive. In AN patients this is very unwanted and a therapeutic hurdle.

Of note, rats that underwent the activity-stress model in adolescence, show as adults increased anxiety-like behaviour on an elevated plus maze and in an open field (Kinzig et al., 2010).

For detailed information on the relevance of NPY, the melanocortin system, leptin and ghrelin in this animal model, see the corresponding sections.

Certain pure stress models, for example the swim test or tail pinching, are less appropriate, because of their very artificial approach. The main point of criticism might be that in these models the applied stress is acute. This is probably not the case in human AN patients, who are most probably suffering from chronic stress.

Most suitable in context group seems to be the separation model (van Leeuwen et al. 1997), which combines separation stress and diet restriction and is very easy to perform. Mice were

housed in single cages of plexiglass while seeing and smelling each other. For a certain period of time (ideal 1h per day), mice of each group were fed altogether in one cage. In comparison to mice housed in groups of ten in one cage (C-1H), to mice under diet restriction (60% of their daily requirements, 60%-group) and slightly even to mice under activity-stress-conditions (ASC) (all with 1h-feeding per day), separated mice lost more weight over a period of 18 days. Interestingly, while the C1-H group and the ASC group reached 80% of their daily requirement, the separated mice reached an intake of only 65% of their daily requirement and lost even more weight than the 60%-group. The fact that the groups C1-H and ASC displayed nearly the same intake, stands in contrast to the aforementioned activity-stress model by Routtenberg and Kuznesof (van Leeuwen et al. 1997). An impaired cognitive function in the T maze (restorable through tyrosine) can also be found in experimental animals in the separation-model, as well as elevated serotonin and catecholamine levels in the hypothalamus (Siegfried et al., 2003).

Animal models with pure diet-restriction are always subject to the criticism that the state is not chosen by the experimental animal and so by definition is not anorexia, but a state forced by the scientist, being a contradiction to AN. To examine neuroendocrine changes, these models still can be useful. Using diet-restriction, a positive impact of weight-loss on the opioid system could be detected (Siegfried et al., 2003). This might be one reason for the development and maintenance of AN in humans. Moreover, Siegfried et al. (2003) could show an improved maze performance (improved cognitve function) in mice under a 60% diet restriction and an impaired maze performance under a 40% restriction, that can be brought to the level of the 60% group by tyrosine administration.

When fed hypertonic (2.5%) saline instead of normal drinking water (and no food restriction), rats develop anorexia, starting in the second night of saline administration. This so called dehydration-associated (-induced) anorexia (DIA) is reversed within minutes after rehydration (Watts, 1999). As expected, NPY mRNA is increased in the arcuate nucleus of rats with DIA, while POMC mRNA is decreased. Circulating glucocorticoid levels are elevated, leptin and insulin levels instead, are reduced. CRF mRNA expression in the paraventricular nucleus (PVN) is decreased, while CRF and neurotensin mRNAs in the lateral hypothalamic area (LHA) are increased. As CRF mRNA (and neurotensin) expression in the LHA strongly correlates with the intensity of DIA and anticipates the onset of anorexia, it was suggested that LHA CRF efferents mediate this kind of anorexia, possibly at the PVN (see section 5) (Watts et al., 1999). Injections of high doses of NPY (1µg) into the LHA (but not the PVN) can reverse DIA. As the ability of 1µg NPY to reverse anorexia correlates negatively with the intensity of DIA, it was suggested that the sensitivity of downstream elements to NPY neurons is reduced during DIA (Watts et al., 2007). Expression of the anorexigenic TRH in the PVN is upregulated in animals

with DIA compared to animals under food restriction (FR). Since factors affecting PVN TRH synthesis, such as leptin or NPY, are not different in DIA and FR animals, it was suggested that orexin expressing neurons from the LHA to the PVN, changed leptin receptor properties on TRH neurons or changed Y1 receptor activity (NPY reduces TRH mRNA expression) might be responsible for elevated TRH expression (García-Luna et al., 2010). Additionally, DIA is significantly attenuated in oxytocin knockout mice (Rinaman et al., 2005).

A newer approach to anorexia is to administer a diet that lacks essential components, for example essential amino acids. It could be shown that mice fed a valine-deficient diet display significant reductions of food intake and body weight, starting on the first day of administration of this diet. Both parameters can be restored by valine supplementation in a dose dependent manner. This approach takes advantage of the fact, that animals have developed the ability to recognize and reject a deficient diet, possibly through a chemosensor in the anterior piriform cortex of the brain. Further, hypothalamic somatostatin mRNA expression is increased in experimental animals and an intraventricular administration of somatostatin leads to reduced food intake, too (Goto et al., 2010; Nakahara et al., 2011).

2.2 Anorexia inducing substances

Injection of lipopolysaccharide (LPS) or inflammatory cytokines such as tumor necrosis factor (TNF) alpha or interleukin (IL) 1-beta leads to a decrease of food intake. This model induces fever, anorexia and reduced activity (Konsman et al., 2002). A single peripheral injection leads to a decrease of food intake by 40% to 50% over a time period of 12 hours. After 24 hours the baseline value of intake is reached again. Repeated injections result in a tolerance to the stimulus. Therefore, it can be criticised that a very acute state is induced, what makes this model less useful for the investigation of therapeutics for chronic diseases (DeBoer, 2009). Some substances can attenuate the effect of these inflammatory substances: AGRP can prevent anorexia caused by intraventricular administration of IL-1beta (DeBoer et al., 2009). For the role of CRF in this model, see section 5, for the relevance of nitric oxide see section 9.

Administration of O,O,S-trimethyl-phosphorothioate induces anorexia in mice. Elevated CRF levels in this case might be responsible for the phenotype (Huang et al., 2007).

Induction of anorexia by other neuropeptides and hormones is discussed in the corresponding sections.

2.3 Modifications of organ function

Several organic modulations can lead to cachexia. Subcutaneous implantation of tumour cells, preferably cells of non-metastasing tumours like the "Lewis Lung Carcinoma", colorectal

tumours or syngenic sarcomas, lead to cancer cachexia and anorexia, probably through the release of inflammatory cytokines or prostaglandines and subsequently CRF (see section 5). Other principles of animal models based on organic modulation would be myocardial infarction, aortic banding or a 5/6-nephrectomy. All these models are probably more suitable for the study of organic disease induced cachexia (DeBoer, 2009).

2.4 Genetics

The only spontaneous genetic mutation in animals that leads to anorexia is the autosomal recessive anx mutation in mice. The anx gene has not yet been completely identified, but it seems to be localized on chromosome 2. Affected animals (anx/anx) are characterized by reduced appetite, low body weight, hyperactivity during the late preweanling period, head weaving, body tremor and uncoordinated gait (Maltais et al., 1984). This phenotype becomes manifest at 5 to 8 days after birth, while the afflicted animals die at an age of 3 to 5 weeks (Siegfried et al., 2003). In anx/anx mice, compared to normal litter mates, serum leptin levels were significantly lower, probably due to the depletion of body fat (Johansen et al., 2000). By using immunochemistry, an accumulation of NPY and AGRP in the cell bodies of the arcuate nucleus and a decrease in the terminals could be shown. This indicates that a defect in NPY/AGRP-signalling could contribute to the anx/anx phenotype (Broberger et al., 1997; Broberger et al., 1998). Moreover, decreased levels of the anorexigenic pro-opiomelanocortin (POMC), the Y1-receptor and the Y5-receptor have been detected, as well as an increase in serotonergic fibers in the forebrain and arcuate nucleus of anx/anx mice (Siegfried et al., 2003). Based on the genetic expression profile Mercader et al. concluded that anx/anx mice simulate cancer caused cachexia/anorexia (Mercader et al., 2008).

Further models of anorexia are based on the possibilities of gene knockout, which means that a specific gene of an organism is made inoperable. NPY, PYY, leptin, ghrelin, CRF, the melanocortin system, NO and endocannabinoid deletion is are treated in detail in the corresponding sections following this section.

Dopamine deficiency, caused by knockout of the gene coding for tyrosine hydroxylase, leads to a hypophagic and hypoactive phenotype. Experimental animals die within three to four weeks after birth. Both decreased food intake and locomotor activity, can be elevated through administration of L-dopa (Szczypka et al., 1999).

Knockout of the gene for the M3 muscarinic receptor (M3R) leads to a significant decrease in food intake, reduced weight and low levels of insulin and leptin. While hypothalamic POMC mRNA expression is reduced in M3R -/- mice, hypothalamic mRNA levels of the orexigenic AGRP are increased. Paradoxically, the orexigenic melanin concentrating hormone (MCH) is reduced, too. Administration of AGRP does not increase food intake in M3R -/- mice, MCH

does. So both decreased MCH levels as well as reduced responsiveness to AGRP might be causes for the development of the phenotype of M3R -/- mice (Yamada et al., 2001; Siegfried et al., 2003).

Deletion of the MCH gene likewise leads to reduced body weight and hypophagia, while POMC and leptin levels are low in MCH -/- mice, as expected. This underlines the importance of MCH for the regulation of feeding (Shimada et al., 1998).

3 Neuropeptide Y and agouti-related peptide

3.1 Physiology of neuropeptide Y and agouti-related peptide

NPY is a neurotransmitter consisting of 36 amino acids and can be found in many brain regions, in the spinal cord and in the sympathetic nervous system. NPY plays a part in the regulation of energy balance, in the immune system, in memory and learning, in the regulation of mood and stress as well as fear and addiction, and in the regulation of heart activity. There even might be an involvement of NPY in sweet and umami taste sensation. NPY builds a family with the peptide YY (70% homology) and the pancreatic polypeptide (50% homology) (Brothers et al., 2010). Of special interest in this work is the relevance of NPY to food intake, which is increased by NPY. So, NPY is a potent orexigen, and food deprivation is a very important cause for elevated NPY levels (Chee et al., 2008; Kask et al., 2002; Heilig, 2004; Painsipp et al., 2008; Redrobe et al. 2004). Furthermore, NPY seems to be anxiolytic (Thorsell, 2010).

NPY binds to the $G_{i/o}$-coupled receptors Y1, Y2, Y4, Y5 and Y6 (Kask et al., 2002). Activation of these receptors by NPY results in decreased cyclic adenosine monophosphate (cAMP) production in the cell by inhibition of adenylate cyclase (Brothers et al., 2010). Regarding food intake, the receptors Y1, Y2, Y4 and Y5 seem to be relevant. While Y1 receptors are located only postsynaptically, Y2 receptors (Y2-R) are located post- and presynaptically (Chee et al., 2008). Y1, Y2 and Y5 receptors are expressed densely in the arcuate nucleus (ARC). NPY is expressed in neurons which project within the arcuate nucleus and from the arcuate nucleus to several other brain areas and nuclei, for example to the PVN, the LHA, the perifornical area (PFA), the ventromedial nucleus (VMN) and the dorsomedial nucleus (DMN). Further on, NPY is expressed in neurons of the dorsomedial nucleus (DMN). In case of excitation of NPY expressing neurons, NPY and - in case of co-localisation in the ARC - AGRP are set free (Chee et al., 2008).

So, the functionality of the AGRP is very closely linked to NPY, wherefore this section discusses both. Further to mention is the gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the CNS, which is produced by most of the neurons that express NPY and AGRP. So, some of the orexigenic effect of NPY and AGRP is in fact mediated by GABA (Wu et al., 2010).

As the hypothalamus and its nuclei discussed in the following are still not understood in every detail and research goes on, the probability exists that some of the facts presented will prove wrong, especially because many studies generate contradictory results.

An overview of selective ligands for the different Y receptors is given by Brothers et al. (2010).

3.1.1 The arcuate nucleus

In the ARC, NPY neurons densely innervate POMC neurons (figure 1). By binding to Y1 and Y2 receptors expressed on these neurons, NPY hyperpolarizes their membrane and prevents activity. In this way, orexigenic effects of NPY are already mediated, because POMC and its tissue specific cleavage products (especially alpha-melanocyte-stimulating hormone (-MSH)) are known to have anorexigenic effects (Chee et al., 2008). The anorexigenic effect of alpha-MSH is mediated by binding to melanocortin 4 receptors (MC4-R) and melanocortin 3 receptors (MC3-R), which are probably directly inhibited by AGRP (Wu et al., 2010). AGRP inhibits the action of alpha-MSH not only in the ARC, but in several other areas of the hypothalamus, for example in the dorsomedial nucleus. In this area, NPY neurons can be inhibited by alpha-MSH through MC4-R binding (Chee et al., 2008). In this sense, AGRP has to be called an orexigenic peptide, too. Interestingly, AGRP ablation (by diphteria toxin) can be compensated for if the neurons are ablated in newborn mice or ablated slowly in adult mice. If the ablation is done rapidly, severe anorexia develops, which cannot be attenuated by blockade of the melanocortin system. The importance of GABAergic signalling for food intake and in AGRP signalling can be shown in this case, because the reduction of food intake induced by rapid AGRP ablation can be restored by bretazenil, a $GABA_A$ receptor partial agonist. An increase in food intake could be shown for other $GABA_A$ binding benzodiazepines, too (Wu et al., 2010). For further explanation see figure 1.

The detailed function of MC3-R is still under question, while the function of MC4-R in regulating food intake is better understood. The MC3-R is expressed on both NPY and POMC expressing neurons. In the first case, inhibition of NPY neurons through POMC neurons can be mediated. In the second case, MC3-R acts as an autoreceptor. MC3-R knockout mice, however, show an increase in adiposity, but no hyperphagia or increased body weight (Renquist et al., 2011). Administration of AGRP to MC3-R as well as MC4-R knockout mice increases food intake (Irani et al., 2010). This means that synergistic as well as independent actions of AGRP on melanocortin receptors mediate its orexigenic effect. MC4-R antagonists increase food intake, contrary to MC4-R agonists (Wu et al., 2010). For further explanation of the melanocortin system, see section 4.

Activity of NPY neurons in the arcuate nucleus and thus release of NPY can be inhibited by several other hormones and neurotransmitters by binding to various receptors on NPY cells. In terms of a negative feedback, NPY itself binds to the Y2-R (just as 3-36-PYY, see section 5). Thus, by antagonism of this receptor, an increase of NPY levels in the hypothalamus and thereby an increase of food intake should be achievable (Brothers et al., 2010).

Nesfatin-1 is expressed in several nuclei of the hypothalamus. In vitro, an inhibitory effect on NPY neurons of the ARC could be detected. However, the receptor that mediates effects of

nesfatin-1 is still not identified. Anyhow, nesfatin-1 reduces food intake in a leptin independent way (Stengel et al., 2010).

Insulin, as a signal of satiety, inhibits neuronal firing of NPY neurons in the ARC by binding to its own receptor (InsR) and inhibits NPY mRNA expression, while it activates POMC neurons (Schwartz et al., 1992; Chee et al., 2008). By these mechanisms, insulin mediates its anorexigenic effect.

By binding to the Ob-Rb receptor, leptin, an indicator of available energy stores, inhibits the activity of NPY neurons of the ARC (Chee et al., 2008). Moreover, leptin increases the frequency of action potentials of POMC expressing neurons in the ARC (Cowley et al., 2001). Both facts explain the anorexigenic effect of leptin.

Ghrelin, which is produced in the stomach, is possibly essential for meal initiation, as ghrelin levels rise immediately before a meal and decrease after a meal. By binding to the growth hormone secretagogue receptor-1a (GHSR) on NPY/AGRP neurons (activating) and on POMC neurons (inhibiting), ghrelin mediates its orexigenic effect (Cowley et al., 2003).

Additionally, it has to be mentioned that POMC neurons in the ARC express serotonin receptors (5-HT_{2C}) and are activated by serotonin and serotonergic compounds. By binding to these receptors, D-fenfluramine, which was in former times used as an anti-obesity drug, mediates its anorexigenic effect (Xu et al., 2010).

Furthermore, POMC neurons of the ARC probably express receptors for interleukin 1 (IL-1-R). When IL-1-beta is injected into the lateral ventricles, an increased c-Fos activation of POMC neurons of the ARC can be detected. Consequently, food intake decreases in this experiment, which mimics inflammation-associated cachexia (DeBoer, 2010).

3.1.2 Further hypothalamic nuclei

Within the DMN NPY expressing neurons also exist. On the one hand, these neurons are inhibited by POMC/alpha-MSH projections from the ARC, with alpha-MSH binding to MC4 receptors. On the other hand, cholecystokinin (CCK) inhibits these neurons by binding to CCK1 receptors and CCK2 receptors (Chee et al., 2008).

In the VMN (or ventromedial hypothalamus), Y1 receptors are strongly expressed. Lesions of the VMN as well as NPY injection into the VMN lead to increased food intake. So, the VMN seems to have an anorexigenic impact. NPY projections from the ARC innervate this region and NPY mediates an inhibitory (and thus orexigenic) effect by binding to postynaptic Y1 receptors (Chee et al., 2008). Leptin receptors are expressed as well on cells of the the VMN. Thus, leptin might mediate its anorexigenic effect partly by activating VMN neurons. In case of satiety, when ARC NPY neurons are inactive and leptin levels are high, activity of glutamatergic VMN efferent neurons to anorexigenic ARC POMC cells is increased (Chee et

al., 2010).

Injection of NPY as well as injection of an Y1 antagonist into the perifornical area (PFA) not only increases food intake, but also hoarding and foraging in Siberian hamsters (Dailey et al., 2009). This means, that at least Y1 receptors should be present in this area, too.

The LHA contains neurons expressing orexins. Orexins (orexin-A and orexin-B) stimulate food intake, but to a lesser extent than NPY (Edwards et al., 1999). After a period of fasting, the expression of orexin as well as the expression of orexin receptors are increased and orexin neurons of the LHA are activated. These neurons innervate and activate NPY/AGRP neurons of the ARC, which express orexin receptors (OX-A receptor and OX-B receptor). The NPY neurons, by contrast, inhibit the orexin neurons of the LHA, namely by NPY binding to Y4 receptors expressed on orexin neurons. Taking everything into consideration, orexins induce NPY activity and hence induce food intake after a period of fasting, while a short-loop regulation by inhibitory NPY neurons exists (Chee et al., 2008).

The PVN receives both NPY and POMC projections. PVN NPY injections increase food intake and food hoarding, but decrease foraging (Dailey et al., 2009, Stanley et al. 1984). However, NPY acts not directly on parvocellular PVN neurons, but increases their activity by inhibiting inhibitory GABAergic interneurons by binding to the presynaptic receptors Y1, Y2 and Y5. Alpha-MSH, in contrast, increases the GABA signal by binding to the presynaptic MC4-receptor (Chee et al., 2008). Newer research shows that there is a stimulating postsynaptic effect of alpha-MSH on MC4-R expressing neurons of the PVN, too. Secondly, a direct inhibition of MC4-R expressing PVN neurons through leptin was assumed, which stands in contrast to the assumption, that leptin acts mainly by increasing POMC/alpha-MSH activity and inhibiting NPY/AGRP activity in the ARC (Ghamari-Langroudi et al., 2011). Besides magnocellular PVN neurons, there are two different kinds of parvocellular PVN neurons in this area, neurosecretory (NS) and preautonomic (PA) neurons. These neurons secrete TRH, oxytocin and CRF. In newborn animals, both cell types are sensitive to NPY and little sensitive to melanocortins. With aging, sensitivity of PA neurons to NPY decreases and sensitivity of NS neurons to melanocortins increases. This may be interpreted in a way that satiety is independent of melanocortin in early childhood (Chee et al., 2008). A stimulating effect of central administration of alpha-MSH on TRH and CRF expression was shown. TRH and CRF are known to be anorexigens (Kamisoyama et al., 2009). Finally, Nesfatin-1 stimulates oxytocin (anorexigen) release in the PVN (Maejima et al., 2009). For further explanation see figure 1.

The lack of melanocortin action in early childhood might be one reason for the interesting fact that NPY gene knockout does not lead to hypophagia or a lean phenotype. In contrast, when NPY/AGRP neurons are ablated rapidly in adult animals, starvation takes place. Instead, POMC ablation and POMC gene knockout lead to hyperphagia (Gropp et al., 2005; Chee et

al., 2008). In addition, it could be shown that ablation of NPY/AGRP neurons in newborn mice leads to only a slight reduction in body weight, which might indicate correspondingly that the melanocortin system is not fully developed in newborns. The fact that such mice show nearly normal feeding behaviour and growth as adults, too, indicates that the development of an alternative network regarding food intake takes place (Luquet et al., 2005).

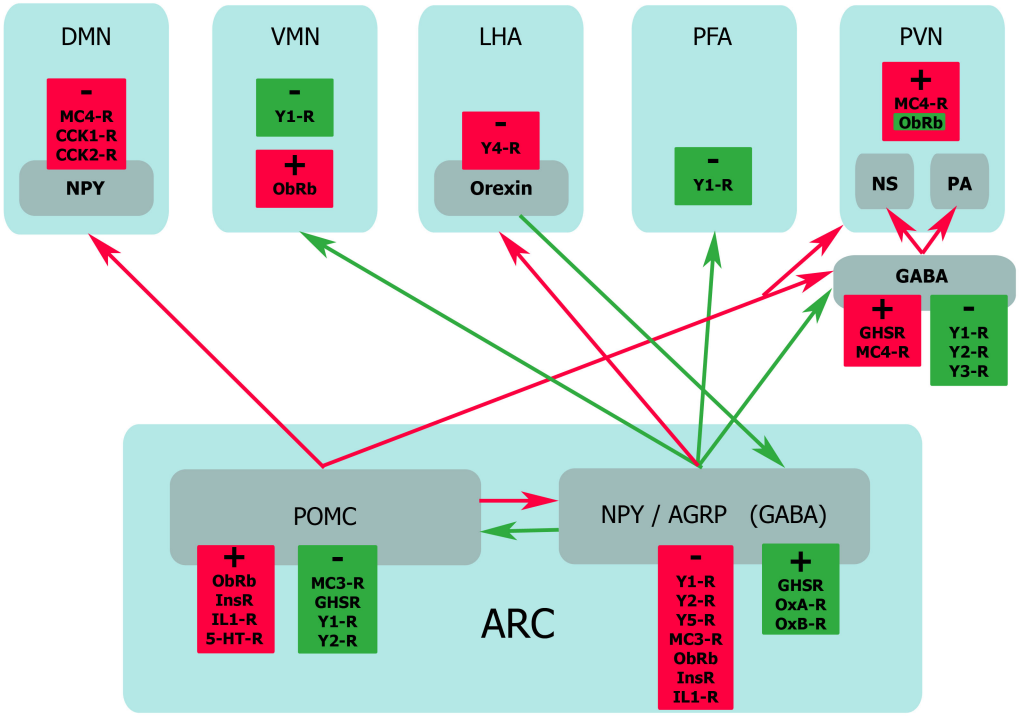

Figure 1: Physiology of NPY: The colour "red" indicates an inhibitory effect on food intake, while the colour "green" indicates a stimulating effect. The green and red boxes contain the receptors which are expressed by the corresponding neurons. "Minus" in these boxes indicates an inhibitory effect of agonists on these receptors, "plus" indicates an excitatory effect. ARC = arcuate nucleus, DMN = dorsomedial nucleus, VMN = ventromedial nucleus, LHA = lateral hypothalamic area, PFA = perifornical area, PVN = paraventricular nucleus. For the sake of clearness, not all connections between the displayed nuclei are shown. For further explanation, see paragraph 3. Modified after Chee et al., 2008, Fig. 1.

3.1.3 Is neuropeptide Y a pure orexigen?

It has often been suggested that NPY is more important for the actions which stand before food intake, than for food intake itself. The above mentioned activity-stress model (2.1) may illustrate this suggestion, because NPY levels are elevated in experimental animals in this model. Since hyperactivity is also present in many AN patients and goes along with elevated plasma

and liquor levels of NPY, there might be parallels. Nergårdh et al. (2006) could show that rats under diet-restriction and with access to a running wheel, increase running and decrease food intake more when treated with NPY, than rats under the same conditions treated with vehicle. This suggests that at least in special situations NPY has no orexigenic effect and that AN patients might equally elevate their activity level not voluntarily, but "driven" by elevated NPY levels (Nergårdh et al., 2006). It has to be mentioned, though, that other groups found no increased activity after NPY administration to rats in the activity-stress model (Hillebrand et al., 2008).

Furthermore it could be shown that rats visit an empty bottle more frequently and drink less from another source after administration of NPY (10µg) and that male animals withdraw attention from female animals in favour of offered food (Södersten et al., 2008). These observations might underline the implication of NPY in foreaging.

According to these discoveries, the classification of NPY as a pure orexigen has to be reconsidered, especially in case of AN. Furthermore, it is most likely that AGRP is a more potent and a more direct acting orexigenic peptide compared to NPY.

3.2 Genetic studies of the neuropeptide Y / agouti-related peptide system

In female NPY knockout mice, a significant decrease in food and water intake during the light phase and a significant decrease in locomotor and exploratory behaviour during the light and dark phase can be detected (Edelsbrunner et al., 2009). Leptin deficient ob/ob mice with NPY knockout show reduced body-weight compared to ob/ob mice (Herzog, 2003).

Administration of Y1 agonists increases body weight without inducing hyperphagia (Henry et al., 2005), while administration of a Y1 antagonist suppresses food intake and reduces body-weight in high-fat-diet fed C57BL/6 mice, an action that is enhanced when combined with a Y5 antagonist (Mashiko et al., 2009). Therefore, it has to be suggested, that Y1 knockout should reduce body weight. Instead the Y1-/- mice by Kushi et al. showed moderate obesity without hyperphagia, more white adipose tissue and elevated plasma insulin levels in later life, particularly in female mice (Kushi et al., 1998). This phenotype was confirmed in later studies, when additionally no changes in NPY and AGRP expression in the ARC, but decreased POMC levels were detected (Herzog, 2003). Further on, the Y1 receptor might play an important role in mediating the inhibition of the gonadotrope axis under poor metabolic conditions, as Y1-deficient food-restricted mice go through puberty with only a slight delay in sexual maturation, while wild-type food-restricted controls show a distinct delay (Gonzales et al., 2004). When leptin deficient ob/ob mice additionally lack Y1 receptors, hyperphagia is less distinct than in

ob/ob / Y1+/+ mice (Pralong et al., 2002). Y1, Y1/Y2 and Y1/Y4 knockout mice display increased body weight gain compared to wild types when fed a chow diet or a high fat diet, while the triple knockout Y1/Y2/Y4 leads to decreased body weight gain when fed a high fat diet (Sainsbury et al., 2006).

Since Y2 agonists (for example 3-36-PYY) reduce food intake (Moriya et al., 2009; Brothers et al., 2010), one would expect that Y2 knockout increases food intake. Instead, the pertinent studies are contradictory. Using a conditional Y2 knockout mouse model, Sainsbury et al. could show that hypothalamic Y2 destruction leads to a significant decrease in body weight and an increase in food intake. In such mice, hypothalamic NPY, AGRP and POMC levels are increased (Sainsbury et al., 2002). In contrast, inactivation of the Y2 receptor by disrupting the encoding gene, leads to increased body weight, food intake and fat deposition in mice (Naveilhan et al., 1999). A significant change of body weight compared to ob/ob mice is not present in ob/ob / Y2-/- mice, but ob/ob mice with Y2 and Y4 knockout display reduced body weight (Lee et al., 2008). Y2 and Y4 knockout seems to protect against diet induced obesity, too. Compared to wild type mice, Y2/Y4 knockout mice seem to have a higher intake, but spill most of the food and so display reduced body weight (Sainsbury et al., 2006). Additionally, Y4 receptor knockout mice show increased energy expenditure and increased physical activity, which is not present in Y2 knockout mice, but is present in Y2/Y4 knockout mice in an even greater extent, which suggests synergism of Y2 and Y4 receptors in these matters (Zhang et al., 2010). Edelsbrunner et al. found that diurnal locomotion, exploration, drinking and feeding are reduced, while nocturnal locomotion is enhanced in Y2 -/- mice, and to the contrary that Y4 -/- mice show increased activity and decreased drinking and feeding during the photophase, while feeding is increased and activity decreased during the scotophase (Edelsbrunner et al, 2009).

Knockout of the Y4 receptor alone leads to a slightly reduced body-weight gain and food intake. However, ob/ob mice with Y4 knockout show no reduced food intake compared to ob/ob mice (Herzog, 2003).

Y5 knockout mice display hyperphagia and obesity in later life, compared to wild-type mice (Higuchi et al., 2008).

3.3 Neuropeptide Y and agouti-related peptide in anorexia nervosa

Plasma levels of NPY are elevated in AN patients (Beranova et al., 2009; Baranowska et al., 2001; Baranowska et al., 2003). The same applies to liquor concentrations (Kaye et al., 1990). During therapeutical refeeding over a period of six weeks, plasma NPY levels do not

significantly change (Beranova et al., 2009). Plasma AGRP levels are increased in AN patients (Moriya et al., 2006). A polymorphism (G760A) of the gene encoding AGRP was found to be associated with AN (Rask-Andersen et al., 2010).

3.4 Other implications of the neuropeptide Y system

As mentioned above, NPY plays a role in many physiological functions. Because this paper focuses on its role in food intake, only some newer insights in its implication in other functions will be presented in the following.

Y2-Rs are expressed in visceral fat. Stimulation of these receptors by NPY leads to fat angiogenesis, macrophage infiltration and the proliferation and differentiation of new adipocytes and thereby obesity (Kuo et al., 2007).

Furthermore, in contrast to solitary chronic stress, which decreases weight in mice, the combination of chronic stress and a high fat/sugar diet (HFS) leads to abdominal obesity. Administration of chronic stress to HFS fed mice stimulates NPY release and Y2-R expression in visceral fat. This suggests that Y2-R antagonists might be of therapeutic value in obesity and metabolic syndrome (Kuo et al., 2008). Moreover, NPY seems to mediate accelerated atherosclerosis and restenosis (Abe et al., 2010). So, the NPY system might be substantial in the development of metabolic syndrome associated with psychiatric disorders and new therapeutic approaches might be found when this connection is further elucidated (Rasmusson et al., 2010).

Several polymorphisms of the Y2-R encoding gene are connected with development and maintenance of addiction. In a Japanese population, a higher prevalence of the allele rs4425326 was found in smokers than in ex-smokers. Besides, a higher score in the Fagerström test[1] was reached by test persons who exhibit this allele (Sato et al., 2010).

The NPY system might present therapeutic targets for stress related disorders, as Y1 agonism and Y2 antagonism has anti-stress effects (Heilig, 2004).

Finally, NPY seems to play a role in the attention-deficit/hyperactivity disorder (ADHD) (Lesch et al., 2010).

[1] Test for determination of physical addiction of smokers

4 The melanocortin system

This system consists of alpha-, beta- and gamma-MSH and ACTH, cleavage products of POMC, which bind to the melanocortin receptors 1 to 5 (MC1-R - MC5-R, G-protein coupled), while AGRP is an endogenous antagonist at these receptors. Only the MC3 receptor and the MC4 receptor seem to be essential for food intake and energy homeostasis, whereas their detailed function is still not elucidated. Most of the insight into the melanocortin system, especially the MC3 receptor, was acquired by studying knockout models, because of the lack of synthetic agonists. Basically, POMC mRNA levels are decreased by fasting (Renquist et al., 2011). Central administration of alpha-MSH and beta-MSH reduces food intake in rats, mice and chickens (Kamisoyama et al., 2009).

The MC3 receptor is expressed on both POMC and NPY/AGRP neurons. In case of POMC neurons the MC3 receptor has an auto-inhibitory character. In this sense, peripheral administration of an MC3-R agonist (D-trp^8-gamma-MSH) increases food intake in wild-type, but not in MC3 receptor knockout mice, and decreases POMC mRNA expression after a while when administered i.c.v.. This effect follows a U-shape dose-response relationship, as higher doses reduce food intake, probably through MC4-R activity (Renquist et al., 2011). MC3-R knockout mice show a very interesting phenotype: No significant overweight, increased food intake or energy expenditure, but increased adipose mass. Additionally, male MC3-R -/- mice exhibit reduced locomotor activity on the running wheel (Butler et al., 2000). Furthermore, the MC3-R -/- mice seem to be protected from the development of metabolic syndrome, maybe due to defects in the inflammatory response to obesity (Ellacott et al., 2007). The MC3-R -/- phenotype might at least partly be caused by changes in circadian rhythm (Begriche et al., 2009). Another cause of this phenotype might be the lack of autoinhibition of the anorexigenic POMC neurons in the ARC when MC3 receptors are missing (as mentioned above), although a decline of weight would be expected. Administration of a mixed MC3-R partial agonist/antagonist (JRH322-18) and a MC4-R agonist reduces food intake in wild-type mice, as well as in MC4-R -/- mice (Irani et al., 2010).

MC4 receptors are expressed widely in the CNS. MC4 receptor knockout mice show increased food intake and body weight (Ellacott et al., 2007). Mutation of the MC4-R might be the most common monogenic cause of obesity in humans and a certain polymorphism of the encoding gene seems to bring some resistance to cancer cachexia (DeBoer, 2010). Administration of MC4-R antagonists increases food intake. This could also be shown for the orally active antagonists SNT207707 and SNT209858, which reduce cancer-induced cachexia in mice (Weyermann et al., 2009).

Recent studies show, that the thyroid transcription factor-1 (TTF-1) stimulates AGRP and

inhibits POMC transcription by binding to the promoters of these genes. Thereby, TTF-1 positively affects food intake (Kim et al., 2011).

To the contrary, the neuropeptide W (NPW) increases POMC and decreases AGRP mRNA expression (no influence on NPY). Moreover, NPW decreases firing activity of NPY/AGRP neurons, while inhibitory synaptic input to POMC neurons is expectedly decreased, too. Consequently, i.c.v. injection of NPW (40 µg/kg) leads to decreased food intake in rats. Therefore, NPW has to be called an anorexigenic peptide (Date et al., 2010).

Interestingly and paradoxically, the above mentioned ABA (see paragraph 2.1) goes along with increased melanocortin receptors in the VMH, while AGRP is increased and POMC decreased as expected. Administration of AGRP (via central infusion), an antagonist of the melanocortin system, attenuates self-starvation, physical hyperactivity, and deregulated body temperature. Therefore, the survival rate of AGRP treated rats in this model is elevated compared to vehicle treated rats (Kas et al., 2003). The meaning of the melanocortin system in ABA is further affirmed by the fact that administration of alpha-MSH increases running-wheel activity in the light phase, decreases food intake and body-weight and increases activation of the HPA axis in 2h-fed rats (Hillebrand et al., 2005). Heat application (32°C) increases weight gain and food intake and decreases activity in rats in the activity-stress model (compared to ABA rats at 21°C), which goes along with reduced MC4 receptor, AgRP and POMC expression (no changes in controls). This positive effect of heat application in ABA might be due to the fact that hyperactivity is a way of behavioural thermoregulation (see 2.1) (Gutiérrez et al., 2009).

Alpha-MSH induces release of the anorexigenic oxytocin from dendrites of magnocellular neurons in the paraventricular nucleus and the supraoptic nucleus of the hypothalamus (Douglas et al., 2007).

In rats, an age dependence of melanocortin sensitivity could be shown, with a decreased response in middle-aged rats and an increased response in old animals (Pétervári et al., 2010).

Taking everything into consideration, the melanocortin system seems to be the most important anorexigenic system. It might be interesting as a therapeutic working point in AN-associated hyperactivity.

5 The corticotropin-releasing factor

CRF is a polypeptide consisting of 41 amino-acids. It is expressed in the hypothalamus, mainly in the PVN, and heads the HPA axis. CRF regulates the release of ACTH and POMC from the pituitary. Expression and release of CRF in the hypothalamus is stimulated by many cytokines such as IL-1, IL-2, IL-6, TNF-alpha and IFN-gamma. Thus, CRF plays a role in tumor cachexia (Inui, 1999). The actions of CRF, like those of similar peptides (e.g. urocortin), are mediated by the CRF receptors 1 and 2 (CRF1-R and CRF2-R, both G-protein coupled), which are both found throughout the central nervous system and periphery. The CRF2-R is highly expressed in the the VMN and the PVN, while CRF1-R is expressed in the ARC of the hypothalamus. The action of CRF (and urocortin) in these areas might be mainly responsible for its effects on food intake: CRF is known to be an anorexigen. Stress induced anorexia is reversed by administration of a CRF receptor antagonist, while food intake is decreased by manipulations that increase CRF levels (stress, tumour) and by administration of CRF agonists. CRF also influences diet selection, sexual activity and anxiety. Urocortin seems to be even more anorexigenic than CRF (Krahn et al., 1990; Heinrichs et al., 1999).

The anorexigenic effects of CRF seem to be mediated by CRF2-R (late effect) and by CRF1-R (acute effect) receptor agonism. CRF2-R knockout mice display higher nocturnal food intake under basal conditions. CRF-induced anorexia can be blocked by administration of the CRF2-R antagonist antisauvagine-30, but not by the CRF1-R antagonist NBI27914 (Tabarin et al., 2007). In dehydration-induced anorexia (see section 2.1), injection of antisauvagine-30 into the PVN increases food intake. Additionally, PVN TRH mRNA and serum TSH levels are decreased. This strengthens the hypothesis that CRF activates TRHergic neurons of the PVN through CRF2-R binding (de Gortari et al., 2009).

As mentioned above (see 3.1.2), CRF is a downstream mediator of the anorexigenic effect of alpha-MSH, as alpha-MSH increases CRF mRNA expression in the PVN, as does beta-MSH in chicks (Kamisoyama et al., 2009). The acute (0–4 h) anorexigenic effect of alpha-MSH (NDP-MSH) is not present in CRF knockout mice - in contrast to the late effect (4-12 h) - and after administration of a CRF receptor antagonist (astressin) (Kawashima et al., 2008). On the other hand, injection of urocortin 3, a CRF-related peptide with high affinity for the CRF2-R, reduces food intake and increases POMC mRNA expression in the ARC. Hence, the melanocortin system might be a downstream target of CRF2-R expressing neurons of the VMN (Chen et al., 2010). An inhibition of the NPY system by CRF and an attenuation of the orexigenic NPYergic effect by CRF was shown (Inui, 1999), which might be the consequence of the stimulating effect of CRF on POMC neurons, that inhibit NPY/AGRP neurons (see figure 1).

Because of their positve effect on food intake, CRF receptor antagonists seem to be promising potential therapeutics in AN.

6 Peptide YY and glucagone like peptide 1

The peptide YY (PYY) is a hormone consisting of 36 amino acids. It belongs to a family with the pancreatic polypeptide and the neuropeptide Y. Along with the glucagone like peptide 1 (GLP-1) PYY is secreted by enteroendocrine L-cells of the distal small intestine, mainly of the distal jejunum and the terminal ileum and of the colon and rectum. As shown in figure 2, PYY circulates in two forms: 1-36-PYY (60%) and 3-36-PYY (40%). The enzyme dipeptidyl peptidase IV (DPP-IV) generates 3-36-PYY from 1-36-PYY and thereby changes receptor binding abilities. While 1-36-PYY binds to all Y receptors, 3-36-PYY interferes mainly with Y2 and moderately with Y1 and Y5 receptors. Both forms are able to cross the blood-brain barrier. Plasma PYY levels are higher after intake of a high-protein meal than after the intake of high-fat or high-carbohydrate meals (Ueno et al., 2008). In case of fat intake, the amount of released PYY depends on the chain length of fatty acids, as dodecanoic acid increases PYY, while decanoic acid does not (Feltrin et al., 2006). Vasoactive intestinal polypeptide (VIP) as well as CCK stimulate PYY release, while gastrin inhibits PYY release. The half-life period of PYY is at nine minutes (Ballantyne, 2006; Huda et al., 2006). For further explanation, see figure 2.

PYY plays an important role in the concept of the ileal brake, which is a feedback mechanism that slows gastric emptying and intestinal transit when nutrients are available in the small intestine (Read et al., 1984; Grudell et al., 2007). PYY is known to slow down gastric emptying and mouth to caecum transit time. Moreover, PYY regulates GI secretion, as it inhibits gastric acid secretion, pancreatic exocrine and insulin secretion and chloride secretion in the small bowel and the colon (Ballantyne, 2006). It seems, that 1-36-PYY inhibits glucose-stimulated insulin secretion more than 3-36-PYY, while 3-36-PYY increases insulin sensitivity (Boey et al., 2007). For the sake of completeness, further functions of PYY shall be mentioned: In the kidneys, PYY decreases the glomerular filtration rate and inhibits renin release. Furthermore, PYY promotes vasoconstriction and increases systolic and diastolic blood pressure (Ballantyne, 2006).

GLP-1, which is co-localized with PYY in the L-cells, is also released in proportion to energy content and suppresses gastric emptying. It stimulates insulin release and suppresses glucagon release. Moreover, GLP-1 might play a role in an autoregulatory inhibitory feedback mechanism

of the PYY/GLP-1 system (Huda et al., 2006).

It was shown that ghrelin attenuates anorexic responses to intravenous infusions of PYY and GLP-1 (Chelikani et al., 2006).

6.1 Peptide YY and food intake

As 1-36-PYY is a Y1 and Y5 agonist, central administration increases food intake, weight gain and exploratory activity in rodents. Interestingly, this orexigenic effect of 1-36-PYY persists in rats with Y1 and Y5 receptor deletions (Boggiano et al., 2005). In contrast, peripheral infusion of 1-36-PYY decreases high-fat intake, probably due to conversion of 1-36-PYY to 3-36-PYY through DPP-IV (Ballantyne, 2006).

As mentioned above, Y2-R agonism reduces food intake probably by inhibition of orexigenic NPY/AGRP expressing neurons of the ARC, which hereafter are less inhibitory on anorexigenic POMC neurons (see section 3). 3-36-PYY is known to be a potent Y2 receptor agonist. Accordingly, in several studies an anorexigenic effect of peripheral 3-36-PYY administration in humans and animals could be shown. The fact that this effect of 3-36-PYY is Y2-R mediated, could be affirmed by the observation that Y2-R blockade and Y2-R knockout reduce 3-36-PYY caused inhibition of feeding (Ballantyne, 2006). Peripheral administration of PYY leads to decreased NPY mRNA expression in the ARC, while POMC mRNA and c-fos expression are increased and MCH mRNA levels are not changed (Batterham et al., 2002; Challis et al., 2003). Thus, the anorexigenic effect of 3-36-PYY is probably mainly mediated by its binding to Y2 receptors at vagal afferent terminals of the nodose ganglion projecting to the nucleus tractus solitarii (NTS), as abdominal vagotomy annihilates the anorectic effect of 3-36-PYY in rats (Koda et al., 2005; Ueno et al., 2008). PYY deficiency might contribute to the pathogenesis of obesity, as fasting PYY levels correlate negatively with the body-mass index and food intake is reduced after PYY infusion to obese subjects nearly to the same dimension as in lean subjects (normal PYY sensitivity in obese people) (Batterham et al., 2003). Despite several studies showing an anorexigenic effect of 3-36-PYY, this "fact" is not generally accepted. Boggiano et al. (2005) reviewed 41 studies, of which 33 showed no anorectic effect of 3-36-PYY. Still, more recent studies confirmed the anorexigenic effect of 3-36-PYY. Moriya et al. (2009) found, that i.p. injection of 3-36-PYY dose-dependently reduces food intake in lean and obese mice, especially when combined with a Y5-R antagonist. For further explanation, see figure 2.

PYY knockout also leads to inconsistent results. While one study showed hyperphagia and increased body weight, other groups found this effect only in female PYY-/- mice and two groups found no effect of PYY knockout (Kirchner et al., 2010). Batterham et al. (2006) found that PYY knockout leads to hyperphagia, obesity and hypersensitivity to PYY. Different strategies to cause PYY knockout could be responsible for different results in the different

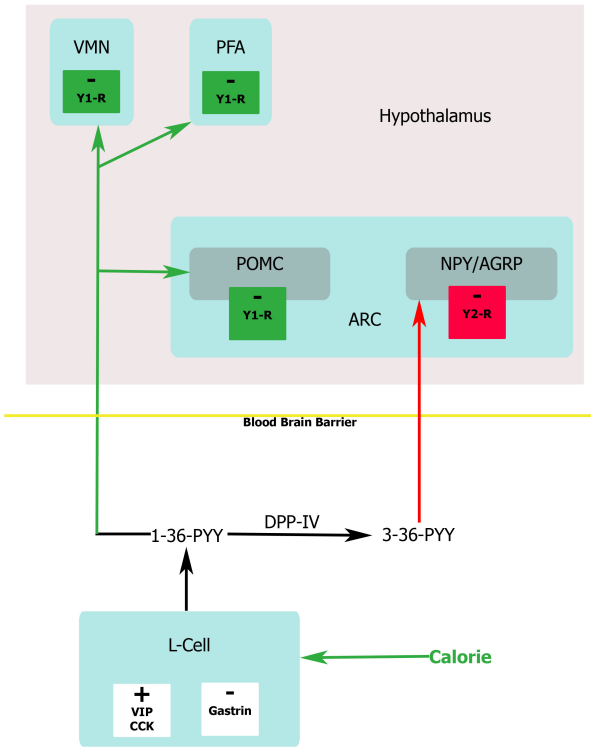

Figure 2: Physiology of PYY: Enteroendocrine L-cells release 1-36-PYY, which is converted by DPP-IV to 3-36-PYY, depending on caloric intake. While 1-36-PYY mediates an orexigenic effect, probably by binding to Y1 receptors of the arcuate nucleus (ARC), the ventromedial nucleus (VMN) and the perifornical area, 3-36-PYY is an anorexigen, as it inhibits orexigenic NPY/AGRP neurons in the ARC. The colour "red" indicates an inhibitory effect on food intake, while the colour "green" indicates a stimulating effect. The green and red boxes contain the receptors, which are expressed on corresponding neurons. "Minus" in these boxes indicates an inhibitory effect of agonists at these receptors on neurons, "plus" indicates an excitatory effect. For further explanations, see figure 1 and section 3.1.

knockout studies (Batterham et al., 2006).

PYY reaches its nadir in humans during mid-puberty. This might promote pubertal progression and growth (Lloyd et al., 2010). Higher levels of PYY after gastric bypass might at least partly mediate the anorexigenic effect (and subsequently weight-loss) of this surgery (Ashby et al., 2007).

6.2 PYY/GLP-1 and anorexia nervosa

Paradoxically, since serum PYY seems to be anorexigenic, many groups found elevated serum PYY levels in AN patients (Misra et al., 2006; Pfluger et al., 2007; Prince et al., 2009; Lawson et al., 2011). This might be a reason for decreased food intake in AN. However, other studies found normal or low PYY levels in AN patients, which would be more expectable and might be due to differences in assay sensitivity (Germain et al., 2007). Additionally, it is to mention that in subjects with constitutional thinness (CT) PYY levels are significantly higher than in AN patients, while ghrelin is lowest in CT subjects (Germain et al., 2007). Moreover, an elevated PYY level is associated with reduced bone density, which suggests a role of PYY in bone metabolism (Utz et al., 2008). GLP-1 levels are lower in AN patients compared to healthy controls (Huda et al., 2006).

7 Leptin

Leptin, a product of the ob gene, is a hormone consisting of 167 amino acids and weighs 16 kDa. It is mainly released from adipocytes, which are the dominant cells of adipose tissue. Therefore, the leptin plasma level is positively correlated with body fat mass. In case of fasting, plasma leptin levels decrease, while overeating leads to an increase. The main function of leptin is the transmission of available energy stores to the CNS and a decrease of food intake. Hence, leptin can be called an anorexigen. Other implications of leptin are the regulation of motor activity, sleep, of the hypothalamic-pituitary-gonadal axis and the hypothalamic-pituitary-thyroid axis (Ehrlich et al., 2009; Blüher et al., 2004).

7.1 Leptin and the regulation of food intake and activity

The rate at which leptin is transported across the blood brain barrier is inverse to the triglyceride level. According to that, high triglyceride levels attenuate leptin transport into the CNS. In case of fasting, serum triglyceride levels are high due to fat mobilisation from adipose tissue (Banks, 2006). By this mechanism, the level of leptin in the CNS and accordingly its anorexigenic effect are decreased in case of starvation. However, this mechanism is disadvantageous in case of obesity, when serum triglyceride levels are also elevated and the anorexigenic effect of leptin would be desirable. On the other hand, drastically reduced food intake in this case could be disadvantageous, too, as vitamin-deficiency might be the consequence (Banks, 2006).

Leptin administration decreases food intake (Huang et al., 2006). In the hypothalamus, leptin binds to receptors (ObRb) expressed on POMC neurons and NPY/AGRP neurons in the ARC and the VMN. While it activates anorexigenic POMC neurons directly by depolarising their membrane and increasing mRNA expression, NPY neurons, which inhibit POMC neurons, are inhibited by leptin (Cowley et al., 2001; Thornton et al., 1997). As mentioned above (3.1), leptin exhibits a postsynaptic inhibitory effect on MC4-R expressing cells of the PVN. This stands in contrast with the suggestion that leptin activates the melanocortin pathway, and requires further investigation (Ghamari-Langroudi et al., 2011). However, in the ventromedial nucleus, which exerts an anorexigenic tone, leptin has an activating effect (Chee et al., 2010). The orexigenic endocannabinoids anandamide and 2-arachidonoyl-glycerol are probably regulated by leptin, too, as defective leptin signalling (e.g. in ob/ob mice) is associated with elevated endocannabinoid levels and leptin treatment reduces these levels in case of defective leptin signalling (Di Marzo et al., 2001). Interestingly, the anorexigenic neuropeptide W is not upregulated by leptin and thus might play a role in energy homeostasis in case of leptin deficiency (Date et al., 2010). Serum levels of the brain-derived neurotrophic factor (BDNF) in AN patients depend on the degree of hypoleptinaemia (Ehrlich et al., 2009).

Hyperactivity in AN is linked to hypoleptinaemia (Exner et al., 2000; Baranowska et al., 2008). In ABA, leptin levels decrease over the course of time, probably due to loss of fat mass. Injection of leptin in the lateral ventricle and the ventral tegmental area (VTA) of experimental animals with ABA suppresses RWA, while food intake is not affected. In contrast, leptin injection into the VTA of ad libitum fed rats without hyperactivity does not affect the activity level, but reduces food intake. The VTA is known to be involved in reward and locomotion and leptin receptors have been identified in this area. As leptin alters reward aspects and activity is associated with reward, leptin might reduce activity by reducing its rewarding effect (Verhagen et al., 2011). On the other side, NPY is known to be elevated in ABA and might boost activity. Hence, the activity reducing effect of leptin in this model, might also be due to its ability to reduce NPYergic action (see 3.1.3). MCH does not seem to play a major role in the effect of leptin on RWA activity in ABA (Hillebrand et al., 2008). On the other hand, the effect of leptin on thermogenesis has to be considered. Leptin increases thermogenesis in brown adipose tissue and might subsequently decrease behavioural thermoregulation, as shown by decreased RWA activity. Moreover, continuous central leptin infusion has an inhibitory effect on the treadmill-running induced activation of CRFergic neurons of the PVN, while CRF-2-R expression in the VMN is increased (Huang et al., 2006). Accordingly, leptin deficient ob/ob mice show high circulating glucocorticoid levels, as do AN patients, who display low leptin levels (Blüher et al., 2004).

7.2 Leptin and Anorexia nervosa

Several studies found decreased plasma leptin levels in AN (Baranowska et al., 2001; Beranová et al., 2009; Janas-Kozik et al., 2011). Cerebrospinal fluid leptin levels are also decreased in AN (Mantzoros et al., 1997). Low body fat mass in AN is most probably responsible for these results. Moreover, leptin concentrations are significantly lower in AN subjects than in individuals with constitutional thinness (Germain et al., 2007). After six weeks of treatment of AN, plasma leptin levels significantly increase (Beranová et al., 2009). Thereby, plasma leptin reaches normal levels when underweight is still present. This might contribute to the problems in re-feeding of AN patients (Mantzoros et al., 1997).

As mentioned above, hyperactivity in AN is linked to hypoleptinaemia. When hypoleptinaemia is present, AN patients rank their motor restlessness higher than after reaching maximal leptin levels post-therapeutically (Exner et al., 2000). The negative correlation between leptin level and activity level, can also be objectified using accelerometers (Hillebrand et al., 2008). Additionally, low leptin levels in AN are related to a high "drive for thinness", an EDI-2[2] sub-

[2]Eating Disorder Inventory (first revision)

scale which refers to issues like preoccupation with weight and dieting, to the absence of sexual desire and intimate relationships and to depressive symptoms (Ehrlich et al., 2009). It is to mention that decreased TSH and free T3 levels in AN might contribute to depression and that hypoleptinaemia might be responsible for the dysfunction of the hypothalamic-pituitary-thyroid axis (Reinehr et al., 2008). Several links between leptin and this axis could be found, but the exact connections are still not clear (Blüher et al., 2004).

Finally, many implications of leptin in the hypothalamic-pituitary-gonadal axis are documented. Administration of leptin to starving rats restores regular ovulation. In case of infertile leptin-deficient ob/ob mice, leptin administration restores fertility and sexual behaviour. Leptin deficient children undergo puberty, when treated with leptin. The increase of leptin levels in weight-gaining AN patients goes along with an increase of LH and FSH levels. All these observations suggest that leptin activates the hypothalamic-pituitary-gonadal axis, which might be suppressed in case of and because of energy shortage as present in AN (Kiess et al., 2000; Blüher et al., 2004).

The implication of leptin for several elements of AN makes it a valuable diagnostic criterion. Following Föckner et al., a cutoff value of leptin in the range of 2 µg/L (mean 0.87 µg/L) has high specificity and sensitivity in predicting AN independently of BMI (Föckner et al., 2011).

Taking everything into consideration, leptin might be of future therapeutic value in AN - especially in AN related hyperactivity - and further studies should be performed in this direction (Støving et al., 2009).

8 Ghrelin

Ghrelin is a hormone consisting of 28 amino acids. It is mainly synthesized by X/A-like cells in the fundus of the stomach, and to a lesser extent also by neurons of the ARC, the DMN, the VMN, the PVN and the LHA. Ghrelin binds to the G-protein coupled growth hormone secretagogue receptor 1a (GHSR), which is expressed widely in the CNS (high expression in the ARC) and regulates the release of growth hormone (GH). As shown in several studies, ghrelin is a potent stimulator of food intake, even in satiated animals. In case of weight loss, ghrelin levels are elevated, while food intake decreases ghrelin levels (Olszewski et al., 2008; Kirchner et al., 2010). The latter seems to depend mainly on the chain length of the ingested fatty acids (Feltrin et al., 2006). Because ghrelin levels physiologically increase preceding a meal, it is hypothesised that ghrelin triggers meal initiation and food-anticipatory activity. Interestingly, ghrelin deficiency does not result in a hypophagic phenotype. Ghrelin knockout in mice only results in decreased body weight gain and fat mass compared to controls when fed a high-fat diet (HFD), but not in decreased food intake, not even in leptin deficient hyperphagic ob/ob mice. Knockout of the ghrelin receptor, however, leads to a reduction in food intake and food-anticipatory behaviour, but, similarly to ghrelin knockout, only when fed a HFD. Knockout of both ghrelin and GHSR leads to decreased body-weight under normal feeding conditions. Moreover, ghrelin influences the HPA axis, plays a role in glucose metabolism, in learning and memory and in food reward. Especially memory and reward might be essential factors in the feeding related properties of ghrelin (Olszewski et al., 2008; Kirchner et al., 2010). A relationship between hyperghrelinaemia and hypothalamic amenorrhoea in normal-weight women was suggested (Støving et al., 2009).

Ghrelin is probably capable of crossing the blood-brain barrier. Direct injection of ghrelin into the ARC stimulates food intake, as does injection into the PVN (Olszewski et al., 2008). In the ARC, ghrelin probably binds to GHSR receptors on NPY/AGRP expressing neurons and stimulates their activity. As mentioned in section 3.1, the NPY/AGRP system has an orexigenic effect. Moreover, ghrelin might activate orexin expressing neurons of the LHA and inactivate POMC neurons of the ARC, which would boost its orexigenic effect that might also be caused by its ability to increase gastric acid secretion and gastric motility (Huda et al., 2006). Ghrelin might in addition mediate its orexigenic effect by directly inhibiting PYY and GLP-1, which are known to delay gastric emptying (see section 6) (Chelikani et al., 2006).

As mentioned before, ghrelin might play an important role in food-anticipatory activity (FAA). Regarding this, it could be shown that FAA is significantly reduced in mice lacking ghrelin receptors. Moreover, ghrelin administration increases locomotor activity in the absence of food (LeSauter et al., 2009). Accordingly, plasma ghrelin levels are highly associated with

increased running wheel activity, which probably indicates food-anticipatory activity, in ABA (see 2.1). GHSR knockout mice do not anticipate food in this model and the FAA is suppressed by a GHSR antagonist (Verhagen et al., 2010). Moreover, in ABA chronic hyperghrelinaemia induces GHSR expression on visceral and subcutaneous fat which might indicate the prevention of lipid loss (Pardo et al., 2010). Still, ghrelin antagonism might be of potential therapeutic value in hyperactive AN patients.

In AN, plasma ghrelin levels are significantly increased compared to controls (Prince et al., 2009; Beranová et al., 2009). Ghrelin concentrations are significantly higher in AN subjects than in individuals with constitutional thinness (Germain et al., 2007). However, the elevation of ghrelin levels might depend on weight loss, as ghrelin levels of weight-stable and weight-gaining AN patients do not differ significantly from that of healthy controls (Støving et al., 2009). However, elevated ghrelin levels are expected only in restricting-type AN. In patients with AN-associated bingeing-purging behaviour, ghrelin levels are decreased (Germain et al., 2010). Elevated plasma ghrelin levels in AN might be due to an existing ghrelin resistance in this eating disorder. A negative correlation between plasma levels of ghrelin auto-antibodies and ghrelin itself might strengthen this hypothesis (Terashi et al., 2011). Finally, a genetic variation of the gene encoding the ghrelin activator enzyme "ghrelin O-acyltransferase" (GOAT) might be implicated in the etiology of AN (Müller et al., 2010).

9 Nitric oxide

Nitric oxide (NO) is a signalling molecule, produced in cells by the enzyme nitric oxide synthase (NOS) from L-arginine. NO activates the cytoplasmatic enzyme soluble guanylate cyclase (sGC) and thereby induces production of cyclic guanosine monophosphate (cGMP). NOS is found in different isoforms. In neurons, NO is produced by nNOS. sGC and nNOS are both found in the hypothalamic ARC (Morley et al., 2011; Riediger et al., 2006).

NO is known to be a central component in neuropeptide regulation of food intake. NO has been shown to be important in the action of NPY, leptin, ghrelin, orexin and CCK. Orexigenic peptides increase NO formation, while anorexigens decrease NO. The food intake inducing effects of NPY, ghrelin and orexin-A can be counteracted by administration of N-omega-nitro-L-arginine-methyl-ester (l-NAME), a NOS inhibitor (Morley et al., 2011). Leptin instead, which is known to be an anorexigen, decreases NO and nNOS in the hypothalamus, while the anorexigenic effect of leptin is greatly reduced in nNOS knockout mice. Furthermore, administration of NPY, ghrelin and orexin-A fail to enhance food intake in nNOS knockout mice, while CCK (an anorexigen) does not decrease food intake in these mice. It has further been shown that NO is essential in stressful situations, as food-deprived mice with NOS knockout fail to adapt to this situation (Morley et al., 2011). Administration of NOS inhibitors leads to decreased food intake and body weight in rodents. To the contrary, NOS gene expression in the PVN and NOS activity in the VMN and the ARC are reduced in case of food deprivation. NO production is lower in this case. This is surprising, as NPY and ghrelin are known to be elevated in case of food deprivation and to increase NO. Moreover, it has been shown that administration of the NO donor sodium nitroprusside (SNP) inhibits neurons in the ARC that are activated by ghrelin. As mentioned in section 8, ghrelin mediates its orexigenic effect partly by activating NPY/AGRP neurons in the ARC (Riediger et al., 2006).

As mentioned above (2.2), lipopolysaccaride (LPS) administration induces disease-related anorexia. Following this treatment, inducible NOS (iNOS) is expressed in the ARC. Blockade of iNOS by the specific inhibitor 1400W attenuates LPS-induced anorexia, as well as LPS-induced adipsia, hyperthermia and inactivity. So, iNOS dependent NO formation seems to be substantial in disease-related anorexia (Riediger et al., 2010).

In AN, plasma nitrite and cGMP levels are inversely correlated with BMI (Vannacci et al., 2006). Densitometric analysis of astrocytoma cells incubated with AN lipoproteins shows elevated iNOS and nNOS levels compared to cells incubated with control lipoproteins (Vignini et al., 2008).

10 Endocannabinoids

The endocannabinoids, e.g. anandamide (arachidonylethanolamide) and 2-arachidonoylglycerol (2-AG), which are derived from polyunsaturated fatty acids of the omega-6 series, are known to be involved in food intake and appetite. Endocannabinoids bind to the cannabinoid receptors CB1 and CB2, both localized in the brain and in peripheral organs (Støving et al., 2009). In addition, anandamide is now often referred to as "endovanilloid", as it binds to the vanilloid receptor TRPV1. Additionally, it was shown that the G-protein-coupled receptor 55 (GPR55) has also binding affinity for endocannabinoids (Ishiguro et al., 2010). The endocannabinoid system is essential in neonatal development, as its blockade prevents suckling in rat pups. Moreover, the CB1 receptor (CB1-R) antagonist rimonabant (SR141716A) was for some time approved as anti-obesity drug. Intake of the exocannabinoid delta-9 tetrahydrocannabinol (THC) has appetite stimulating effects in humans, which may be mediated through the rewarding properties of food. However, the effect of cannabinoids appears to be bi-phasic, as their food intake stimulating or inhibiting effects seem to be dose and preparation dependent, which was found in several animal experiments (Støving et al., 2009).

The positive effect of endocannabinoids on food intake seems to be CB1-R mediated, as CB1-R knockout mice eat less than controls, and the CB1-R antagonist SR141716A has no effect on feeding in these mice. Leptin administration reduces anandamide and 2-AG levels in the hypothalamus of leptin deficient ob/ob mice and normal rats (Di Marzo et al., 2001).

Lewis et al. (2010) investigated the effect of THC and (S)-N-oleoyl-(1′-hydroxybenzyl)-2′-ethanolamine (OMDM-2), an anandamide re-uptake inhibitor, in C57BL/6 mice under ABA conditions (see 2.1). Acute food restriction (3h/d) and access to a running wheel in C57BL/6 mice leads to the aforementioned characteristics of ABA which are: Increased RWA, decreased food intake and weight loss compared to controls without running-wheel access. But since hyperactivity is only present on the first day of food restriction and RWA then declines to levels beneath baseline RWA, this model offers only a very narrow window for pharmacological intervention. Therefore, the ABA model was optimized and, instead of acute induction, food restriction was progressively induced over 4 days (6, 5, 4 and finally 3 h/d) after habituation to a running wheel for 7 days (Lewis et al., 2010). Over the following 12 days, mice with running-wheel access showed significantly less food intake and body-weight than controls without running-wheel access. As the window for pharmacological interventions is much expanded, this optimized anorexia inducing model seems to be more appropriate for the investigation of potential pharmacological therapies in AN. RWA, however was significantly higher only in the period of progressive food restriction and shows an almost constant decline in the days following this period. Percentage activity in the light phase, though, increases in the period of

progressive food restriction and even more in the days after this period. The lack of persistent elevated RWA might be due to reduced nutritional reserves of mice (Lewis et al., 2010). In contrast to the above mentioned studies of Gelegen et al. (2007) (see 2.1), experimental animals were younger and wheel access was not permitted simultaneously with food presentation. Additionally, the effects of administration of THC or OMDM-2 were tested in this model. Wheel-running mice increased food intake after administration of THC (0.5 mg/kg) compared to vehicle treated mice. Body-weight, instead, showed no increase. An arguably higher mortality rate in THC treated wheel-running mice might be due to the hypothermic effect of THC. RWA was not affected by THC administration. OMDM-2 also increased food intake, but only in a dosage of 3 mg/kg and without ameliorating body-weight loss.

A recent study shows for the first time an effectiveness of THC in attenuating the weight loss in an ABA model (Verty et al., 2011).

Plasma levels of anandamide are significantly elevated in AN patients (Monteleone et al., 2005). This might account for a possible addictive component of AN. Furthermore, significantly higher plasma levels of CB1-R mRNA were found in AN patients compared to controls (Frieling et al., 2009). Impaired endocannabinoid signalling might be causative in AN. Moreover, two single nucleotide polymorphisms (SNPs) were shown to be associated with AN. The rs1049353 SNP of the gene encoding the CB1 receptor and the rs324420 SNP of the gene encoding fatty acid amide hydrolase (FAAH), an enzyme degrading endocannabinoids, are significantly more frequent in AN patients (Monteleone et al., 2009). The functional Gly195Val polymorphism in the GPR55 gene (mentioned above) was also shown to have a small effect for predisposing people to AN (Ishiguro et al., 2010).

Up to this day, no trial has been performed that clearly shows a beneficial effect of THC on food intake in AN patients. Earlier (small) trials exhibited ether no effect on food intake or are difficult to assess because of insufficient study design (Støving et al., 2009).

Part II

Effects of JNJ-31020028

This part describes an experiment, which was performed in the course of this final year thesis. The aim of this experiment was mainly to determine the effect of the selective Y2 receptor antagonist "JNJ-31020028" on food intake, locomotor activity and exploratory behaviour of mice under normal and activity-stress conditions. JNJ-31020028 (JNJ) was for the first time characterized by Shoblock et al. (2010) to be a selective brain penetrant small molecule antagonist of the Y2-R. As mentioned above (section 3), additionally to its postsynaptic expression, the Y2-R is expressed presynaptically on NPY/AGRP neurons of the ARC. NPY/AGRP neurons are known to exert an orexigenic effect, partly by inhibiting anorexigenic POMC neurons (Chee et al., 2008). However, agonists at Y2-Rs expressed on NPY/AGRP neurons exert inhibitory effects on NPY/AGRP neurons and probably anorexigenic effects. In terms of a negative feedback, NPY itself binds to Y2-Rs on NPY/AGRP neurons, just as 3-36-PYY, which is known to be an anorexigen (see section 6). Hence, it has been suggested that Y2-R antagonists might increase NPY levels in the CNS and therefore exhibit orexigenic effects (Brothers et al., 2010).

Accordingly, the first aim of this experiment was to demonstrate the effect of JNJ on feeding in mice. Moreover, the role of the NPY system in ABA was considered. As mentioned above, rats and certain mouse strains react to food-restriction with increasing running-wheel activity and decreasing food intake (activity-stress animal model for AN, see 2.1), a behaviour which might be present in some AN patients, too. NPY levels are elevated in this model. Additionally, Nergårdh et al. (2006) were able to show that in the activity-stress model rats increase running-wheel activity and decrease food intake when treated with NPY more than rats under the same conditions treated with vehicle. Furthermore, nocturnal locomotion is enhanced in Y2-R knockout mice (Edelsbrunner et al., 2009). Thus, Y2-R antagonism might as well lead to increased activity under normal conditions and/or in activity-based anorexia and subsequently to a higher energy expenditure. This would limit the use of Y2-R antagonists in AN, especially as hyperactivity is already a problem in many AN patients. In the present experiments DBA/2-mice were used as experimental animals, which have been shown to increase their activity under ABA conditions (Gelegen et al., 2007). As NPY is known to have anxiolytic effects (Thorsell, 2010), it has to be suggested that administration of a Y2 antagonist might have anxiolytic effects as well. Finally, Y2-R antagonism might increase drinking, since drinking has been shown to be slightly increased in Y2-/- mice (Edelsbrunner et al., 2009). As an aside, JNJ has already been tested for its effect on alcohol consumption, relapse-like behaviour and withdrawal-induced anxiety of rats (Cippitelli et al., 2010) as well as on nicotine abstinence-related social

anxiety-like behavior, NPY and CRF mRNA levels in the novelty-seeking phenotype (Aydin et al., 2011).

11 Methods

11.1 Experimental protocols

A total of 54 DBA/2-mice were used and allocated randomly to three groups. Allocation to subgroups took place randomly as well (figure 3).

The first group (group 1), consisting of 18 mice, was further subdivided into three subgroups, each consisting of six mice (groups 1.1, 1.2 and 1.3). One group at a time was examined in the LabMaster for one week, while the other two groups were housed in groups of three to five in cages of size II L (length x width x height = 26 cm x 20.5 cm x 14 cm). The six mice under examination were housed singly in LabMaster cages, after measuring their weight. For the first four days of the week the LabMaster measured activity, drinking and feeding of these mice. On the fifth day injection of JNJ to three mice and vehicle to the remaining three mice took place. After this, measurement of activity, drinking and feeding went on for another two days. On day seven, the weight of the six mice was measured again. Data of two mice of group 1 were not used for statistical evaluation, because their drinking bottles were leaking or blocked. One mouse of group 1 was excluded because of inexplicable high exploratory activity, with a peak of 3000 (counts) compared to peaks of 1000 in other mice.

The second group (group 2), consisting of 18 mice, was subdivided into groups 2.1, 2.2 and 2.3 and treated and housed just as group 1. The only difference to group 1 was that they were treated with a higher dose of JNJ (see 11.3).

The third group (group 3), consisting of 18 mice (groups 3.1, 3.2 and 3.3), was treated differently: To force an increase of activity, the mice received food only in the first three hours of the dark-phase (Gelegen et al. 2007). In this case, food consumption was determined by measuring the weight of the feeding bins before and after the three hour feeding period. Measurement of drinking and activity was performed by the LabMaster system. Additionally, the body weight was measured at the beginning, just before injection of JNJ or vehicle (on the fifth day) and at the end of each week. One mouse of group 3 was excluded because of inexplicable high exploratory activity. The data of six mice which died during the experiment were also excluded.

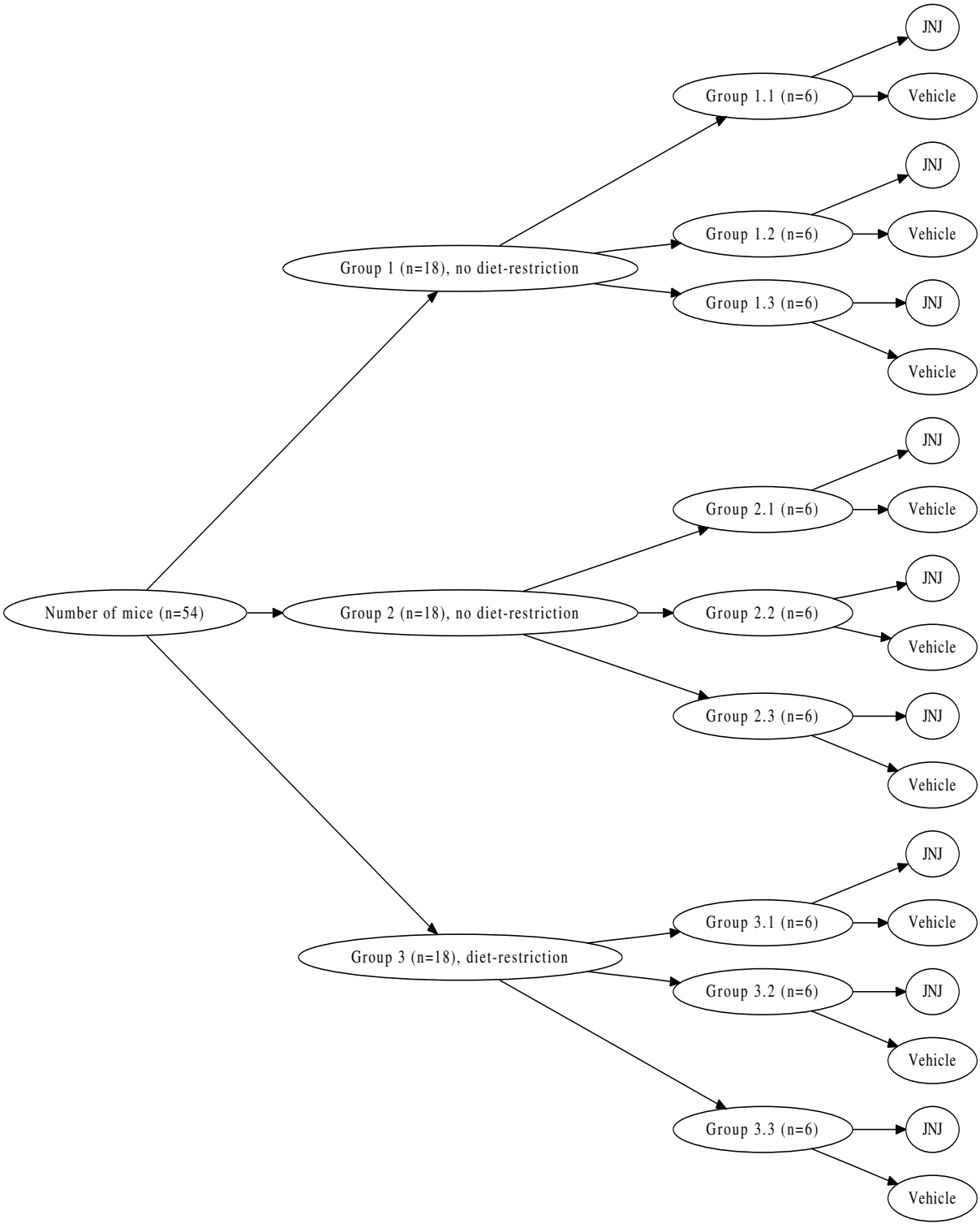

Figure 3: Study design. See section 11.1 for further explanation. All animals are shown in this figure, independently of whether or not the data were used for statistical evaluation.

11.2 Experimental animals

The study was carried out with female DBA/2-mice, which were housed under controlled temperature (set point 21° C), relative air humidity (set point 50%) and light conditions (lights on at 7:00 h, lights off at 19:00 h, maximal intensity 150 lux). The experimental procedures as well as the number of animals used in this trial were approved by an ethical committee at the Federal Ministry of Science and Research of the Republic of Austria and executed according to the "Directive of the European Communities Council" of 24 November 1986 (86/609/EEC). The experiments were designed and conducted with the aim to minimize the number of animals used as well as their suffering. Female mice were used because AN is highly prevalent in females. DBA/2-mice were used because of their reaction to diet-restriction. Under these circumstances DBA/2-mice show increased activity, a phenomenon that matches with the behaviour of many AN patients (Gelegen et al., 2007). The mice were bred at Charles River (Sulzfeld, Germany). The experiments were executed at the behavioural laboratory of the Experimental and Clinical Pharmacology Institute of Graz.

11.3 JNJ-31020028: Properties and administration

JNJ-31020028 (JNJ, Johnson & Johnson, San Diego, USA) is a brain-penetrant Y2-R antagonist. For this experiment, JNJ was dissolved in vehicle at a concentration of 5 mg/ml (mice 1-3 of groups 1.1, 1.2, 1.3, 3.1, 3.2, 3.3) and 10 mg/ml (mice 1-3 of groups 2.1, 2.2 and 2.3). As vehicle (VEH) a solution of 20% hydroxypropyl-beta-cyclodextrin (HPBCD) in pyrogen-free sterile saline (0.9% NaCl) was used (Shoblock et al., 2010). Both, JNJ and vehicle (mice 4-6 of all groups), were injected intraperitoneally (i.p.) at a volume of 2 µl/g. Accordingly, JNJ was injected in a dosage of 10 mg/kg (groups 1.1, 1.2, 1.3, 3.1, 3.2, 3.3) or 20 mg/kg (groups 2.1, 2.2 and 2.3). The injection took place on day 5 of each week between 6.15 p.m. and 6.45 p.m..

11.4 LabMaster system

The LabMaster system and basic recording protocol were as described by Edelsbrunner et al. (2009). The circadian pattern of locomotion, exploration, drinking and feeding was assessed with the LabMaster system (TSE Systems, Bad Homburg, Germany), which allowed continuous recording of these parameters for up to seven days while the animals remained mostly undisturbed by any investigator. The system consisted of six recording units, each unit comprising a test cage (type III, 42 cm x 26.5 cm x 15 cm, length x width x height), two external infrared frames and a cage lid fitted with two weight transducers. These devices were connected to a

personal computer which was used to collect and analyze the data with the LabMaster software (Edelsbrunner et al., 2009). The hardware sampling rate at the infrared frames was 100 Hz, while that at the drinking and feeding sensors was 1 Hz. In contrast, the minimal sampling interval of the LabMaster software was 1 min, which means that the recordings taken by the hardware over 1 min (6000 and 60, respectively) were summed up at 1 min intervals. In other terms, 720 values of each test data were collected over a 12 h interval. The two or three weight transducers were employed to quantify ingestive behaviour (Edelsbrunner et al., 2009). To this end, a feeding bin filled with standard rodent chow (altromin 1324 FORTI; Altromin, Lage, Germany) and a drinking bottle filled with tap water were each attached to a transducer on the cage lid. An additional drinking bottle filled with sucrose solution (2%) was provided in one experimental group. The drinking flasks were equipped with a special nipple that prevented the spontaneous leaking of water from the bottle. Water and food intake over time was measured in ml and g, respectively (Edelsbrunner et al., 2009). For recording locomotion and exploration, the two external infrared frames were positioned in a horizontal manner one above the other at a distance of 4.3 cm, with the lower frame being fixed 2.0 cm above the bedding floor. The bottom frame was used to record horizontal locomotion (ambulatory movements) of the mice, while the top frame served to record vertical movements (exploration). The measures of activity were derived from the light beam interruptions (counts) of the corresponding infrared frames. An ambulatory movement was defined as temporally subsequent interruption of any two different light beams in one axis, and the total locomotor activity was calculated by summing up the counts in the x- and the y-axes over selected time intervals. Moreover, a central area was defined in each cage, resulting in a rectangle of approximately 38 cm x 22.5 cm (length x width). Activity in this area was calculated by summing up the counts in the x- and the y-axes within this area to assess anxiety (Edelsbrunner et al., 2009).

11.5 Statistics

Statistical evaluations and plotting were performed with SigmaPlot 11.0 (Systat Software Inc., Chicago, Illinois, USA). The effects of JNJ were evaluated using Student's t-test after performing a normality test and an equal variance test. If the normality test failed, a Mann–Whitney U test was performed. Because of the exploratory nature of the study, probability values $P \leq 0.1$ were regarded as statistically significant (Winer, 1991; Kirk, 1995; Hays, 2007).

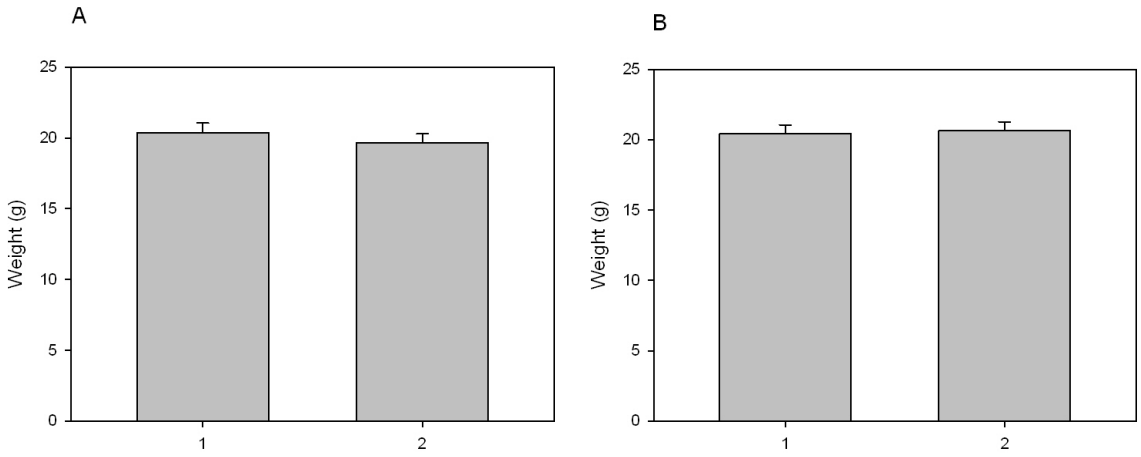

Figure 4: Mean body weight of JNJ-treated mice (10 mg/kg) (A) and of VEH-treated mice (B) at the beginning (1) and end (2) of the experiment (group 1).

12 Results

12.1 Groups 1.1, 1.2, 1.3

12.1.1 Body weight

The average body weight of the mice was 20.40 $\pm$ 0.45 g (mean $\pm$ SEM, n = 15). At the beginning of the experiment, there was no significant difference in weight between the group of mice which later received JNJ and the group of mice which later received VEH. There was also no significant difference between body weights at the beginning and end of each trial in both treatment groups of mice (figure 4).

12.1.2 Locomotion and exploration

The locomotor (ambulatory) and the exploratory behaviour showed characteristic circadian time courses over the seven days of observation. As is typical for nocturnal animals, the activity of the mice was considerably higher in the dark phase (7 p.m. to 7 a.m.) than in the light phase (7 a.m. to 7 p.m.). Regarding locomotion, there was no significant effect of JNJ (10 mg/kg) compared to VEH (figure 5 and 6). There was also no detectable effect of JNJ on exploratory behaviour (figure 7 and 8).

12.1.3 Ingestive behaviour

Drinking and feeding showed characteristic circadian time courses over the full seven days of observation with peaks during the dark phase. Feeding was higher in mice after injection of

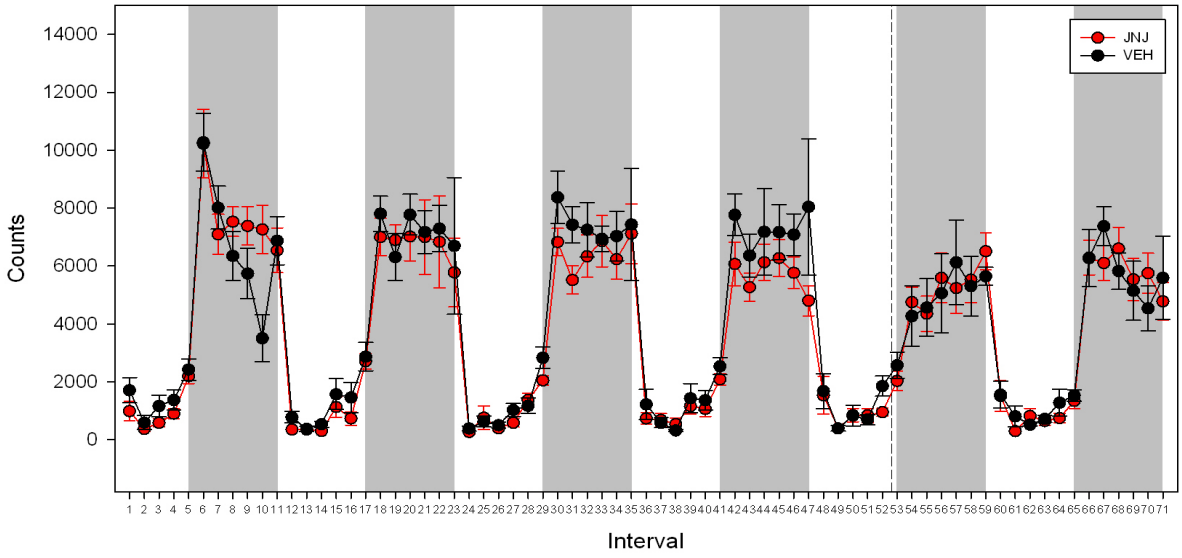

Figure 5: Locomotor activity of mice. Parameters for locomotion were summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of JNJ-injected mice, while the black curve represents locomotion of VEH-injected mice. The grey shading indicates the dark phase.

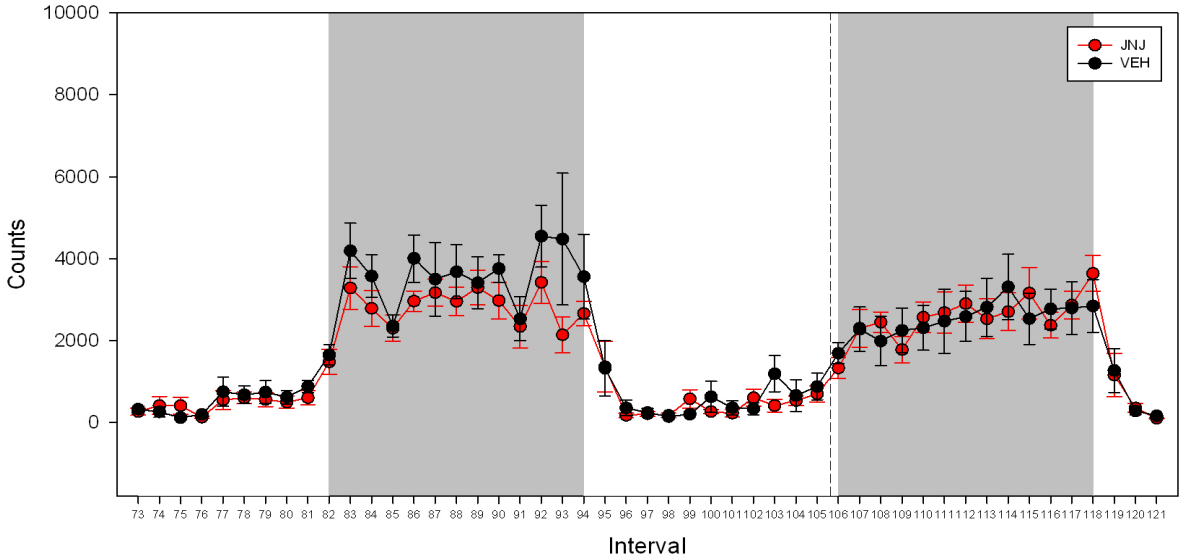

Figure 6: Locomotor activity of mice. Parameters for locomotion were summed up over 60 min, meaning that one interval represents one hour. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of JNJ-injected mice, while the black curve represents locomotion of VEH-injected mice. The grey shading indicates the dark phase.

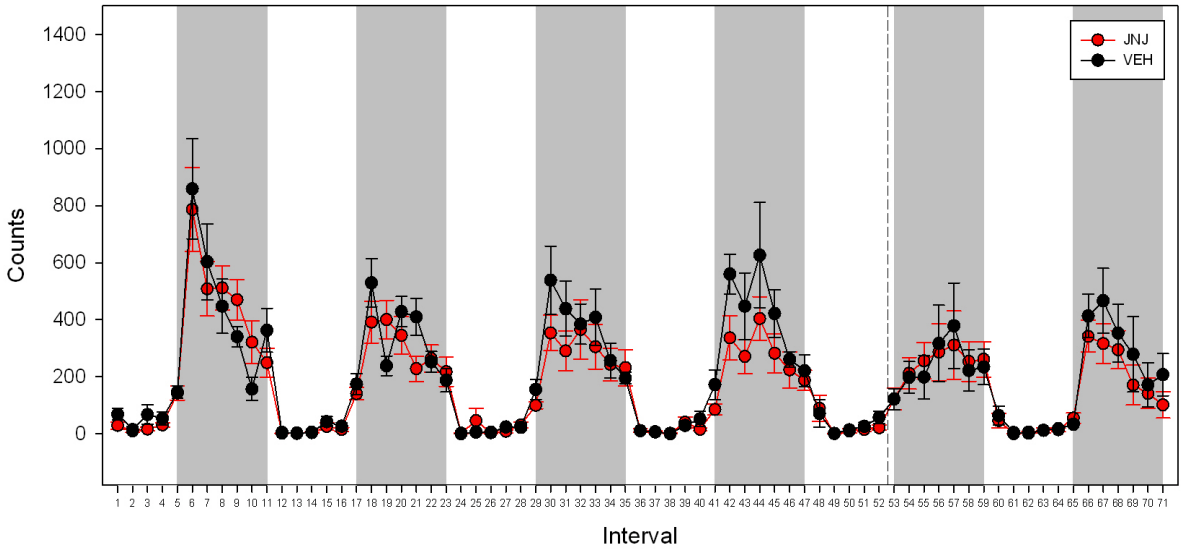

Figure 7: Exploratory behaviour of mice. The parameter for exploration was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents exploration of JNJ-injected mice, while the black curve represents exploration of VEH-injected mice. The grey shading indicates the dark phase.

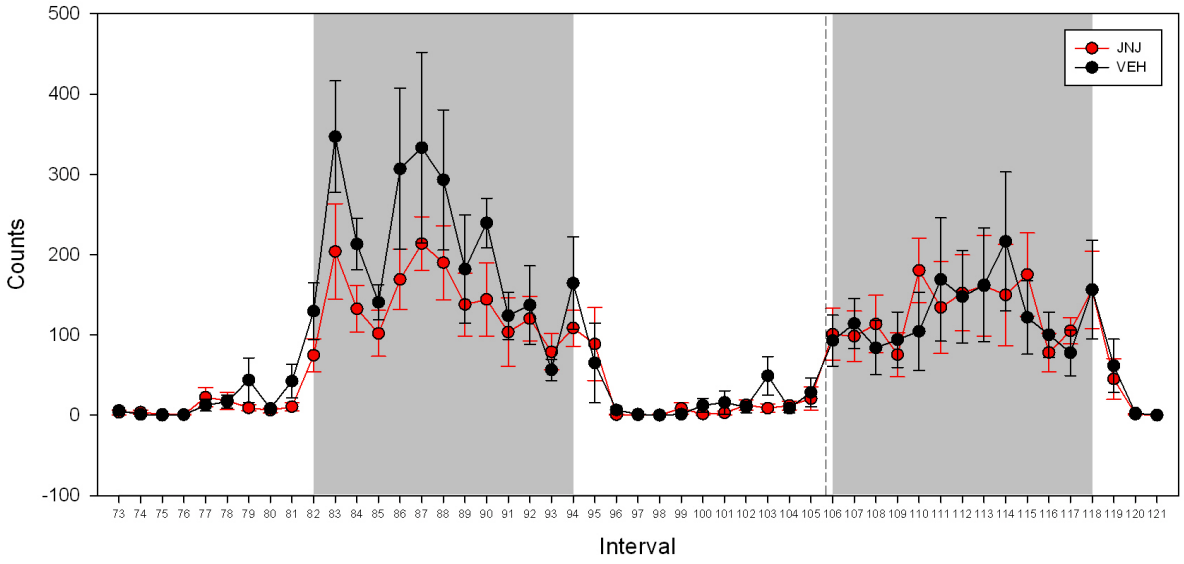

Figure 8: Exploratory behaviour of mice. The parameter for exploration was summed up over 60 min, meaning that one interval represents one hour. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents exploration of JNJ-injected mice, while the black curve represents exploration of VEH-injected mice. The grey shading indicates the dark phase.

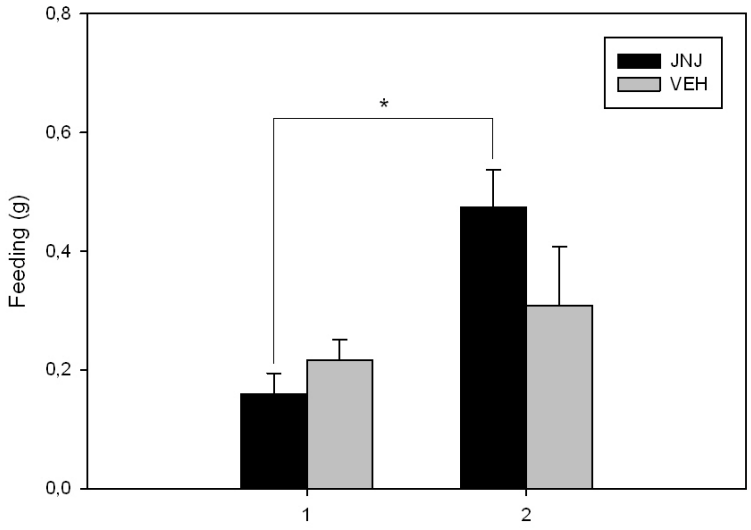

Figure 9: Food intake of mice between 5 p.m. and 7 p.m. (1) and between 7 p.m. and 9 p.m (2). The black bar represents JNJ-injected mice (10 mg/kg), the grey bar represents VEH-injected mice. * P = 0.005.

JNJ (10 mg/kg) than after injection of vehicle. However, this difference was not significant (P = 0.158). Still, it has to be mentioned that food intake between 5 p.m. and 7 p.m. and food intake between 7 p.m. and 9 p.m. differed significantly in JNJ-injected mice (P = 0.005), which was not the case in VEH-injected mice (P = 0.404) (figure 9, 53 and 54 in figure 10). Of course, it has to be taken into consideration that mice show a higher intake of food in the beginning of the dark phase than in the end of the light phase. Still, as VEH-treated mice do not follow this pattern here, probably due to injection stress, JNJ-treated mice, which most probably underwent equal stress, ate as mentioned significantly more food. In figure 11 and 12 the effect of JNJ on food intake is obvious. While there was no significant difference between the two treatment groups detectable in the first hour after injection (7 p.m. to 8 p.m., 107 in figure 12) (P = 0.648), the difference was highly significant in the second hour (8 p.m. to 9 p.m., 108 in figure 12) (P = 0.016). This positive effect of JNJ on food intake was further affirmed by the observation that food intake between 7 p.m. and 8 p.m. and food intake between 8 p.m. and 9 p.m. differed significantly in JNJ-injected mice (P = 0.003), which was not the case in VEH-treated mice (P = 0.666) (figure 11 and 12). Drinking was not affected by JNJ (figure 13 and 14).

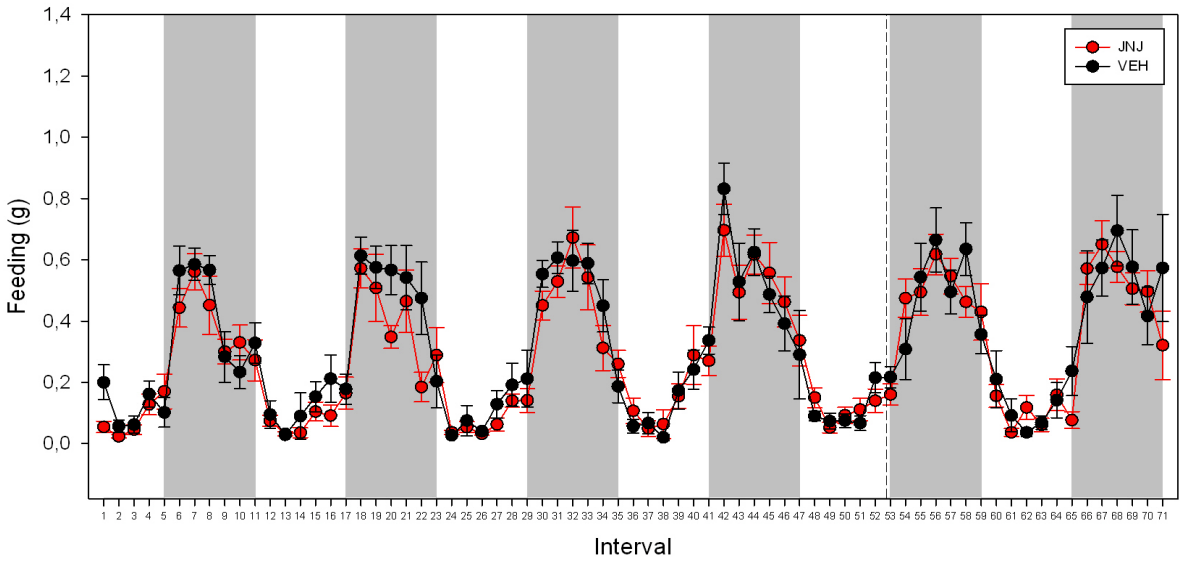

Figure 10: Food intake of mice. The parameter for feeding was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents food intake of JNJ-injected mice, while the black curve represents food intake of VEH-injected mice. The grey shading indicates the dark phase.

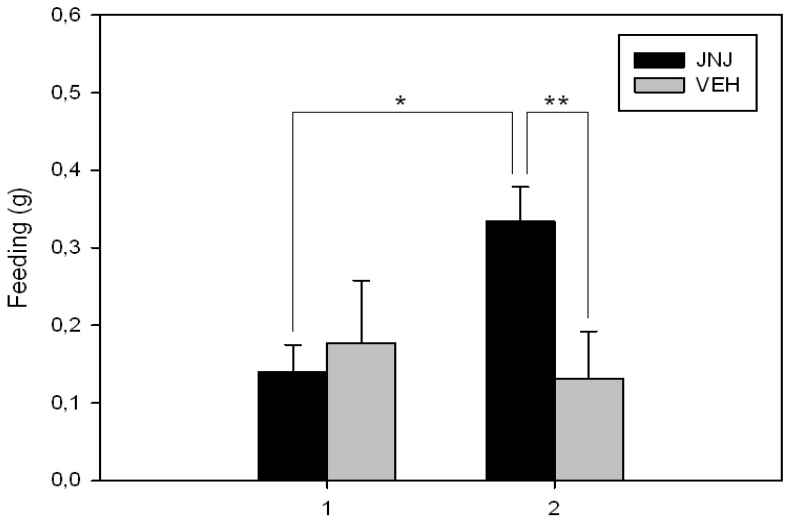

Figure 11: Food intake of mice between 7 p.m. and 8 p.m. (1) and between 8 p.m. and 9 p.m (2). The black bar represents JNJ-injected mice (10 mg/kg), the grey bar represents VEH-injected mice. * P = 0.003, ** P = 0.016.

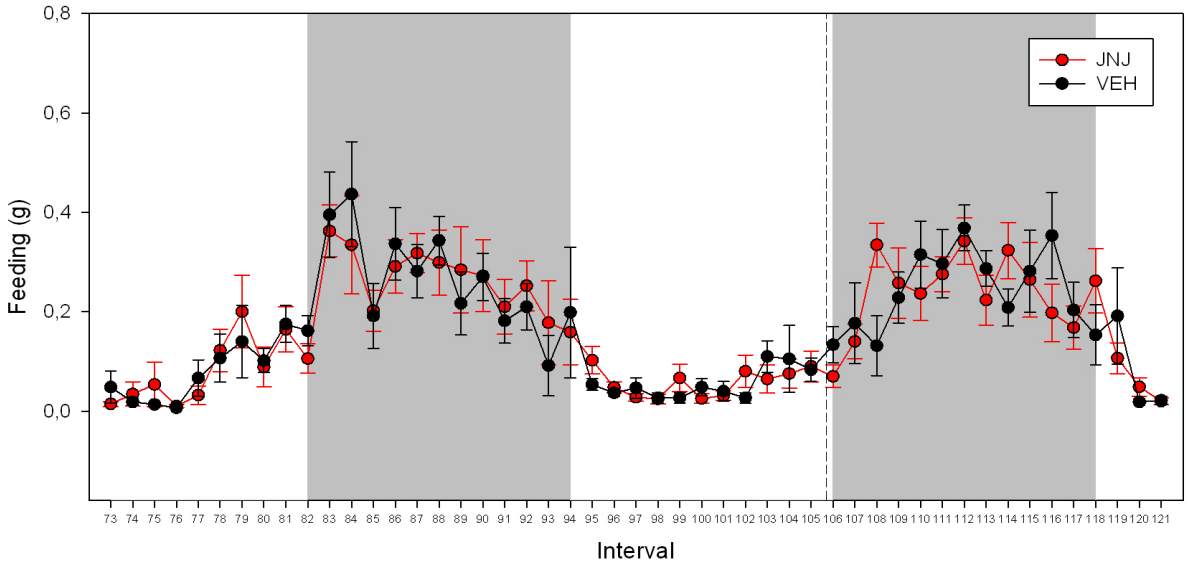

Figure 12: Food intake of mice. The parameter for feeding was summed up over 60 min, meaning that one interval represents one hour. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents food intake of JNJ-injected mice, while the black curve represents food intake of VEH-injected mice. The grey shading indicates the dark phase.

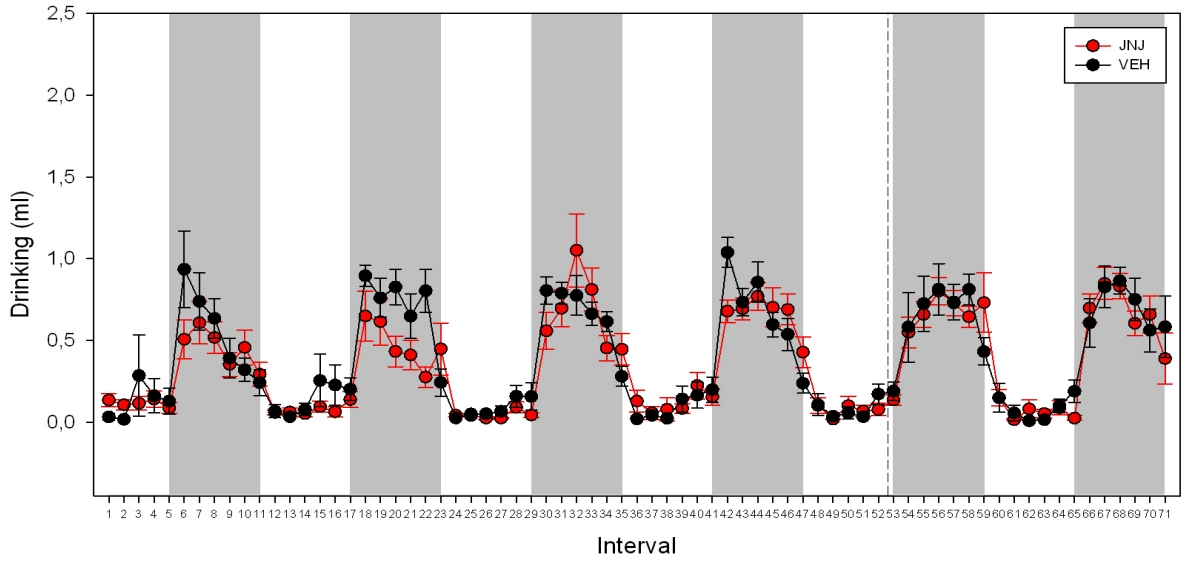

Figure 13: Drinking (water) of mice. The parameter for drinking was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

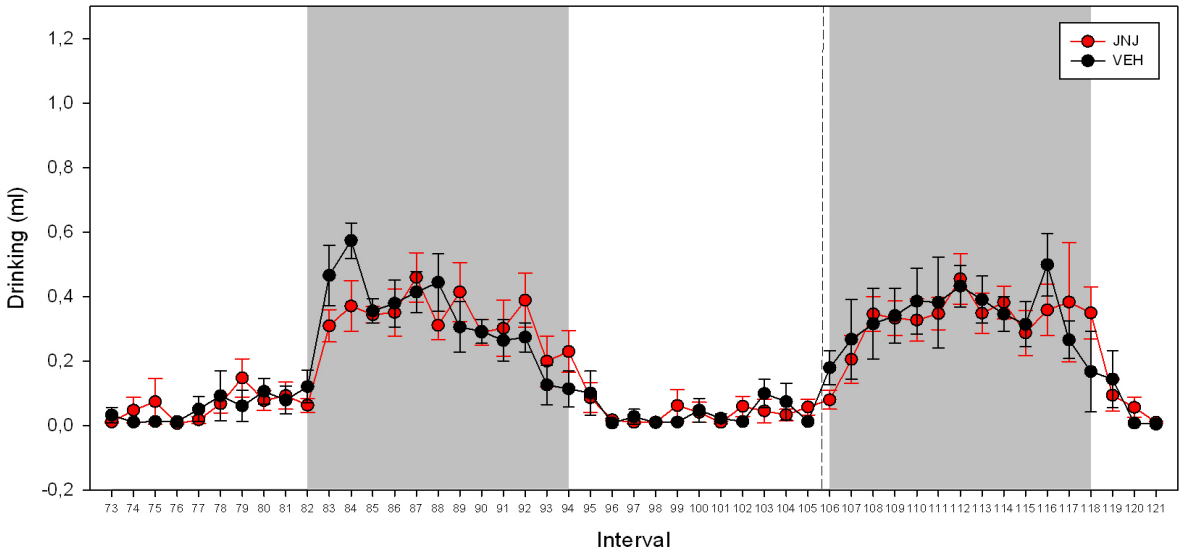

Figure 14: Drinking (water) of mice. The parameter for drinking was summed up over 60 min, meaning that one interval represents one hour. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

12.1.4 Anxiolysis

An anxiolytic effect of JNJ might be expressed by an elevated activity of a mouse within the central area of its cage. This area was defined as described in 11.4. Actually, as figure 15 shows, the activity in this central area appeared to be elevated in JNJ-treated mice (10 mg/kg) compared to VEH-treated mice. Yet, this difference was not significant.

12.2 Groups 2.1, 2.2, 2.3

12.2.1 Body weight

The average body weight of the mice, measured at the beginning of each experiment, was 20.22 $\pm$ 0.25 g (mean $\pm$ SEM, n = 18). Additionally, there was no significant difference in weight between the group of mice which later received JNJ and the group of mice which later received VEH. There was no significant difference between the body weights at the beginning and end of the trial in both treatment groups of mice (figure 16). There was also no significant difference between the average body weight of the mice of group 1 and the mice of group 2.

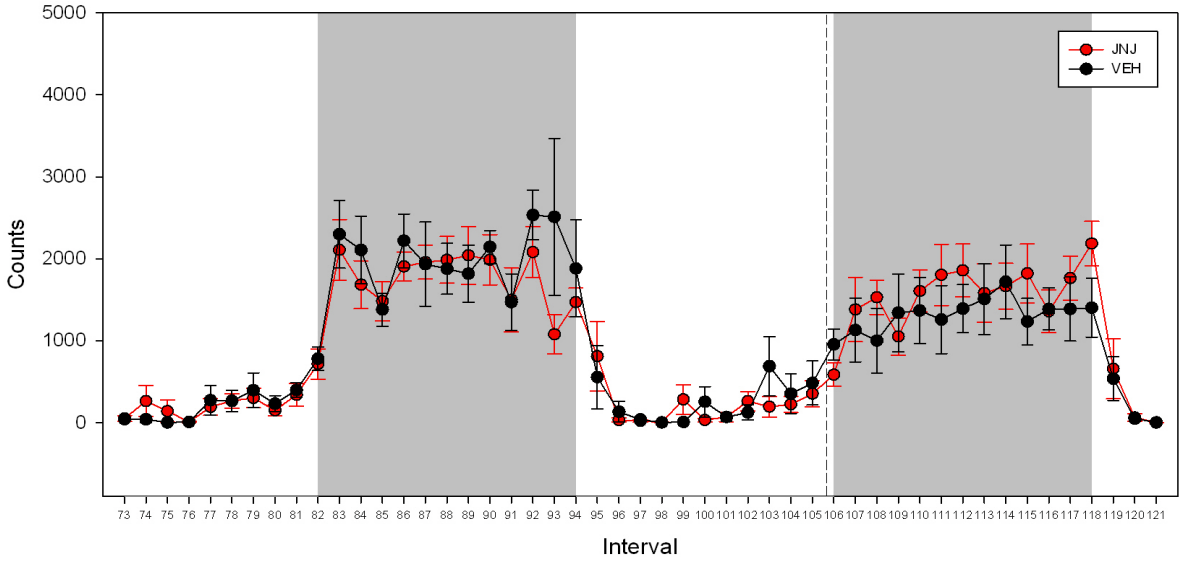

Figure 15: Activity of mice in the central area. The parameter for this activity was summed up over 60 min, meaning that one interval represents one hour. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents activity of JNJ-injected mice, while the black curve represents activity of VEH-injected mice. The grey shading indicates the dark phase.

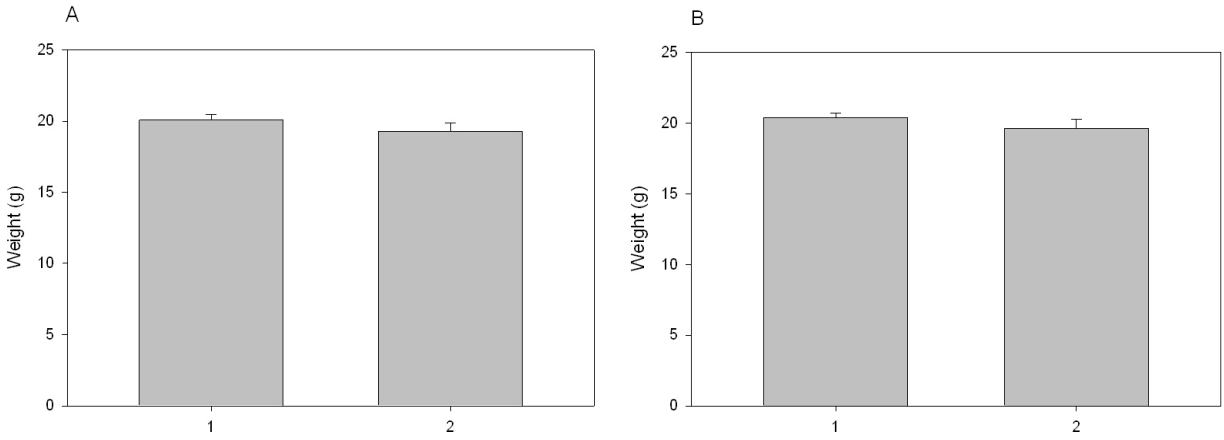

Figure 16: Mean body weight of JNJ-treated mice (20 mg/kg) (A) and of VEH-treated mice (B) at the beginning (1) and end (2) of the experiment (group 2).

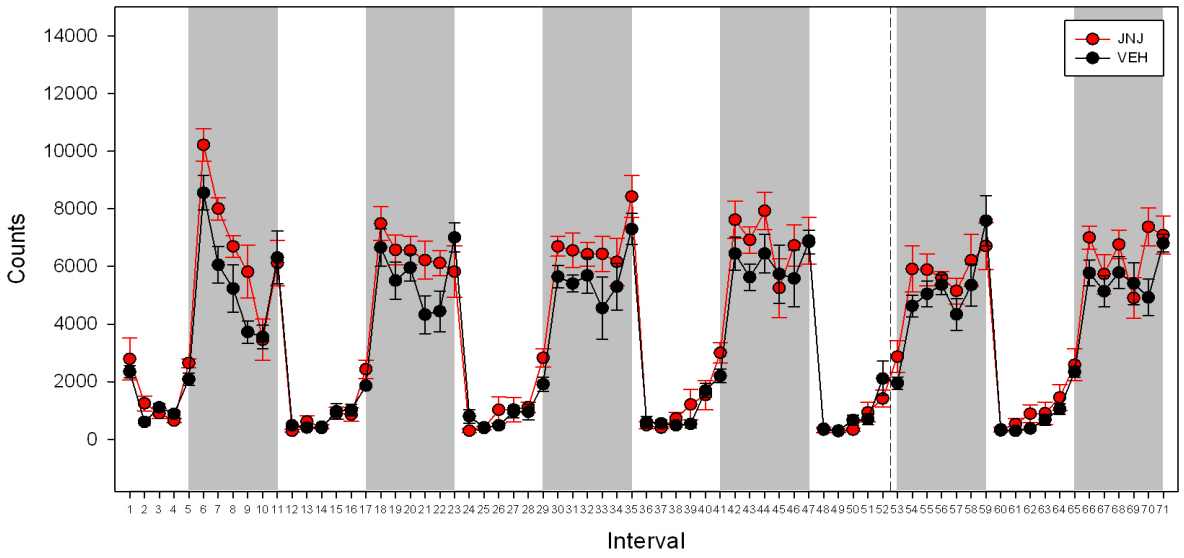

Figure 17: Locomotor activity of mice. Parameters for locomotion were summed up over 120 min, meaning that one interval represents two hours. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of JNJ-injected mice, while the black curve represents locomotion of VEH-injected mice. The grey shading indicates the dark phase.

12.2.2 Locomotion and exploration

The locomotor (ambulatory) and the exploratory behaviour showed characteristic circadian time courses over the seven days of observation. As is typical for nocturnal animals, the activity of the mice was considerably higher in the dark phase (7 p.m. to 7 a.m.) than in the light phase (7 a.m. to 7 p.m.). Regarding locomotion, there was no significant effect of JNJ (20 mg/kg) compared to VEH (figure 17 and 18). Although there was a non-significant visible difference between the two groups after injection, this was most probably so because of a generally higher locomotor level of JNJ-treated mice in this case. There was also no detectable effect of JNJ on exploratory behaviour (figure 19 and 20).

12.2.3 Ingestive behaviour

Drinking and feeding showed characteristic circadian time courses over the full seven days of observation, with peaks during the dark phase. As seen in figure 21 and 22, there was no noteworthy difference in feeding between JNJ-injected mice and VEH-injected mice. The positive effect of JNJ (10 mg/kg) on feeding which was detectable in group 1, was not confirmed in this group of mice receiving 20 mg/kg JNJ. Drinking (water) was also not affected by JNJ (figure 23). Likewise, there was no clear-cut effect of JNJ on sucrose drinking (figure 24, 25).

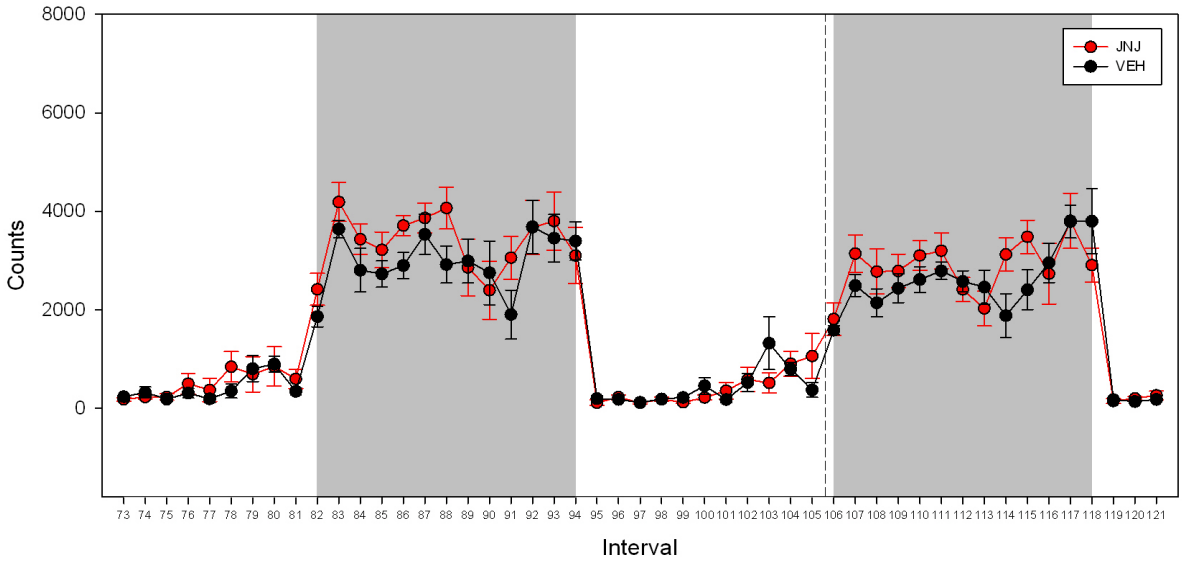

Figure 18: Locomotor activity of mice. Parameters for locomotion were summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of JNJ-injected mice, while the black curve represents locomotion of VEH-injected mice. The grey shading indicates the dark phase.

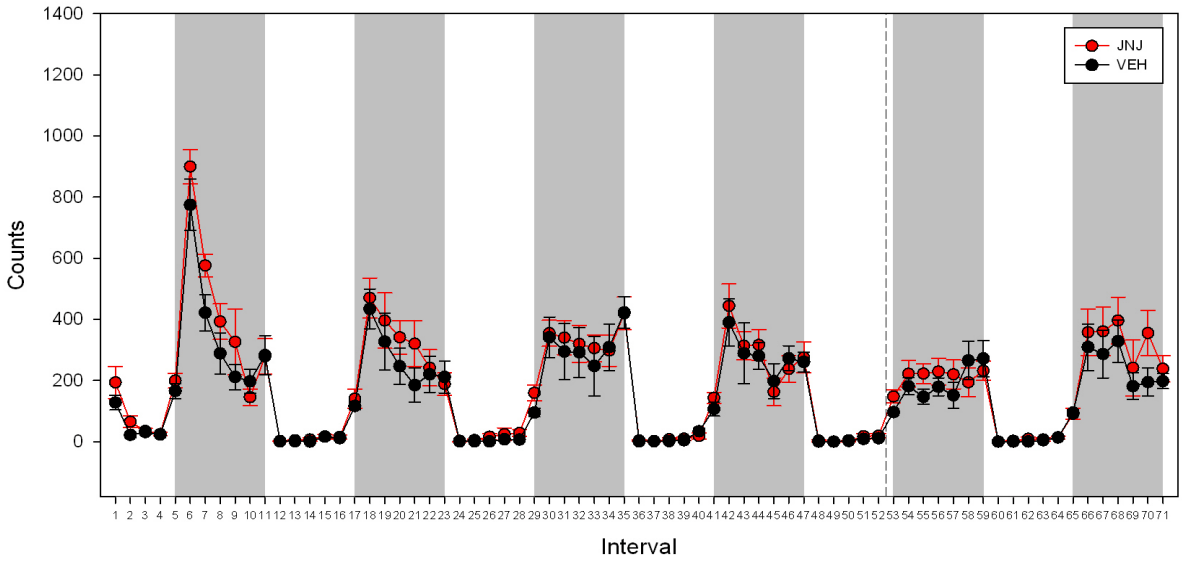

Figure 19: Exploratory behaviour of mice. The parameter for exploration was summed up over 120 min, meaning that one interval represents two hours. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents exploration of JNJ-injected mice, while the black curve represents exploration of VEH-injected mice. The grey shading indicates the dark phase.

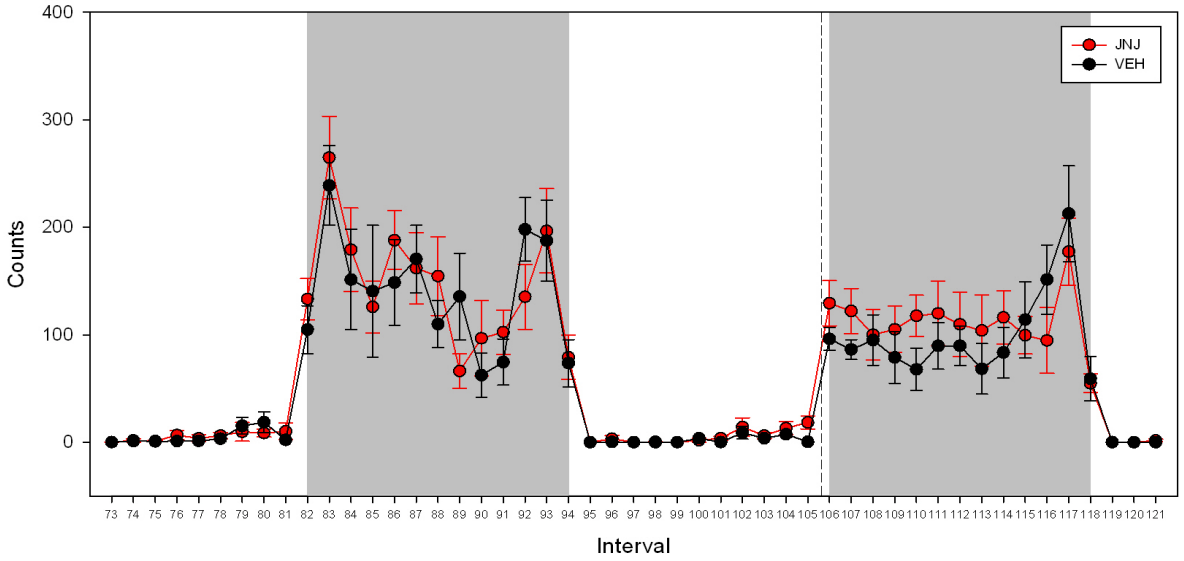

Figure 20: Exploratory behaviour of mice. The parameter for exploration was summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents exploration of JNJ-injected mice, while the black curve represents exploration of VEH-injected mice. The grey shading indicates the dark phase.

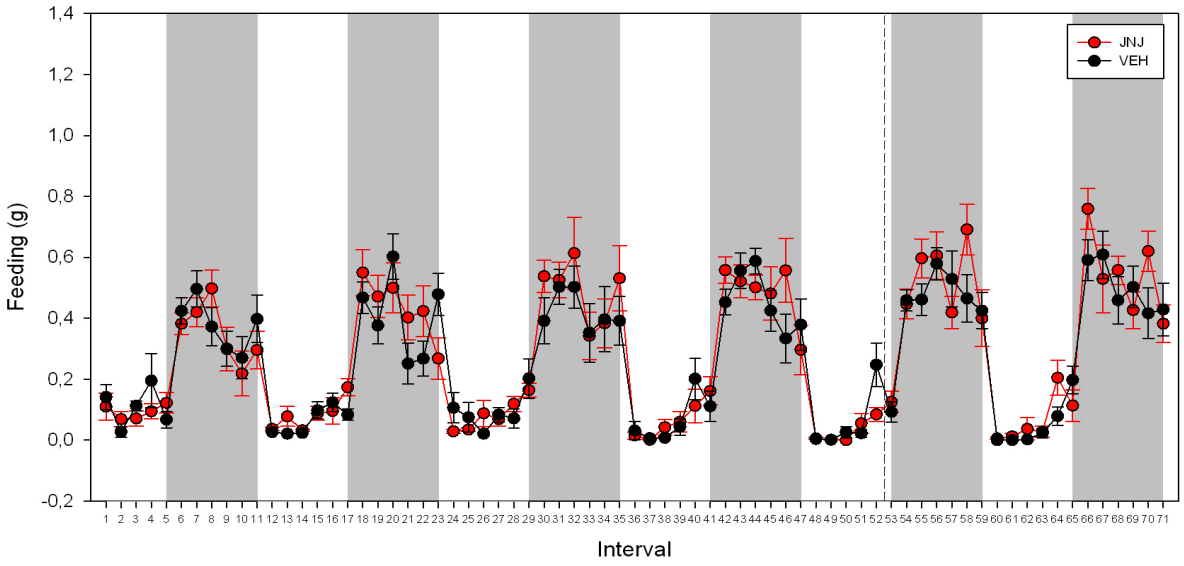

Figure 21: Food intake of mice. The parameter for feeding was summed up over 120 min, meaning that one interval represents two hours. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents food intake of JNJ-injected mice, while the black curve represents food intake of VEH-injected mice. The grey shading indicates the dark phase.

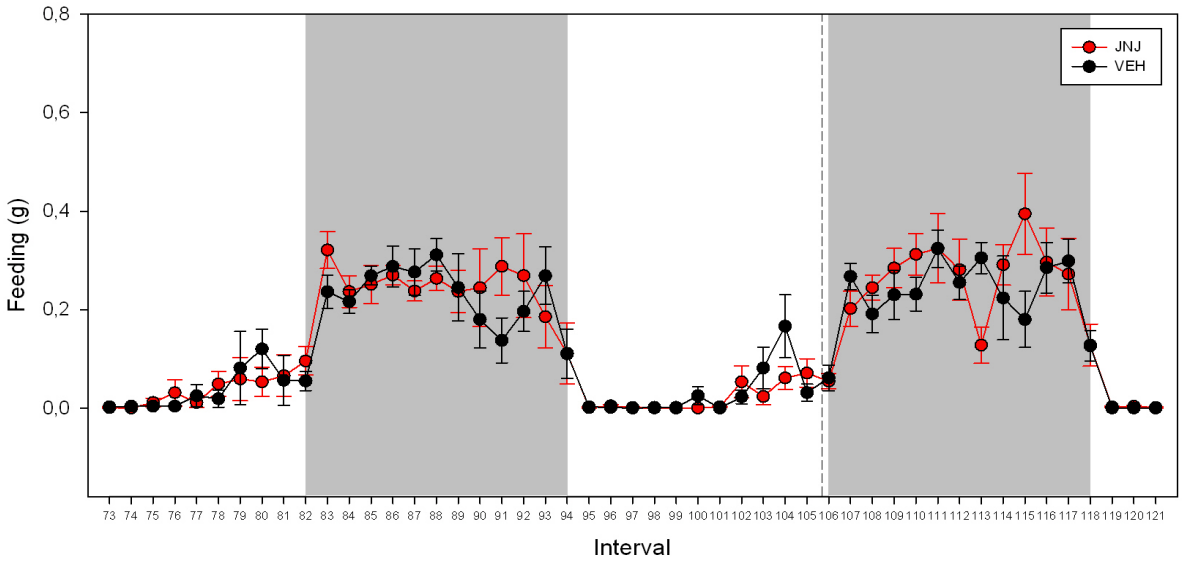

Figure 22: Food intake of mice. The parameter for feeding was summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents food intake of JNJ-injected mice, while the black curve represents food intake of VEH-injected mice. The grey shading indicates the dark phase.

12.2.4 Anxiolysis

An anxiolytic effect of JNJ might be expressed by an elevated activity of a mouse within the central area of its cage. This area was defined as described in 11.4. However, as figure 26 shows, the activity in this central area was not significantly altered by JNJ (20 mg/kg).

12.3 Groups 3.1, 3.2, 3.3

12.3.1 Body weight

The average body weight of the mice, measured at the beginning of each experiment, was 22.65 $\pm$ 0.47 g (mean $\pm$ SEM, n = 11). There was no significant difference in weight between the group of mice which later received JNJ and the group of mice which later received VEH. Figure 27 shows the weight loss caused by food-restriction over seven days. The weight loss was most pronounced during the days before injection of JNJ (10 mg/kg) or VEH. There was no significant difference between the two treatment groups on day 7.

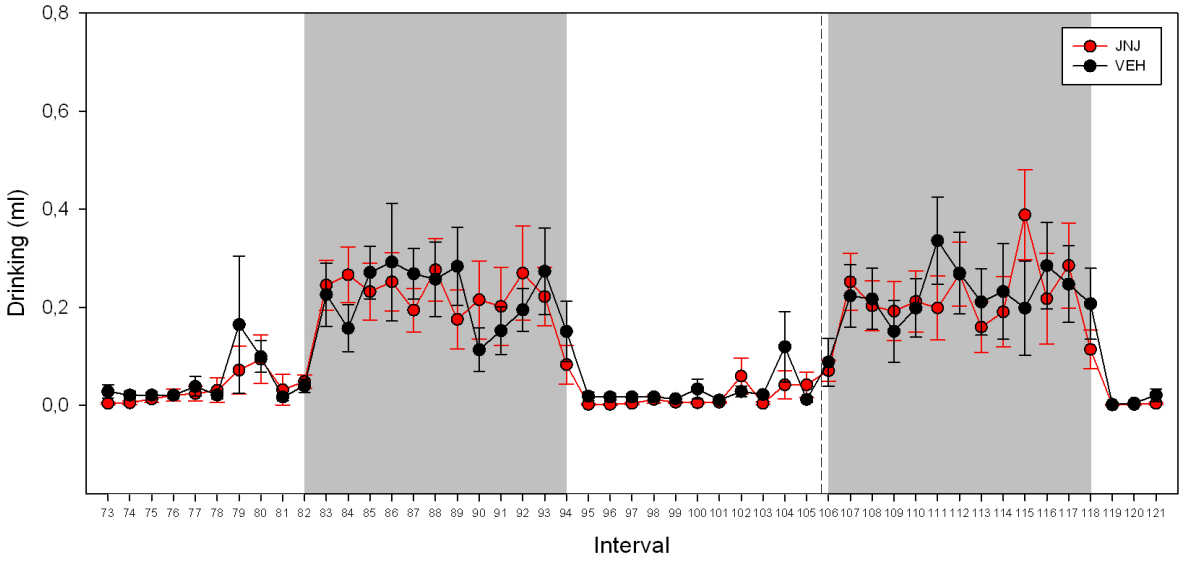

Figure 23: Drinking (water) of mice. The parameter for drinking was summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

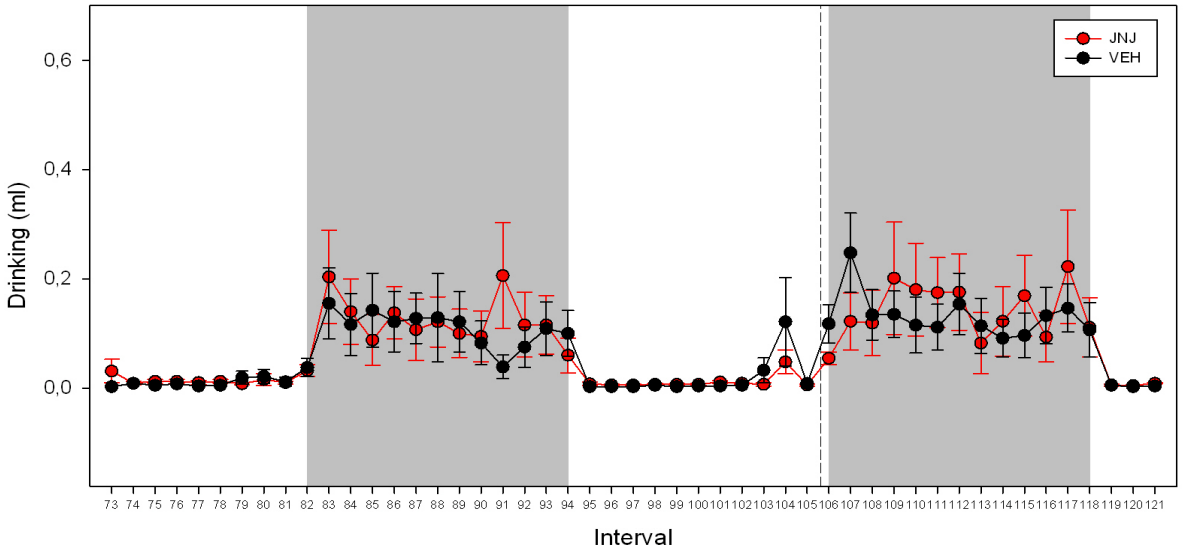

Figure 24: Drinking (sucrose) of mice. The parameter for drinking was summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

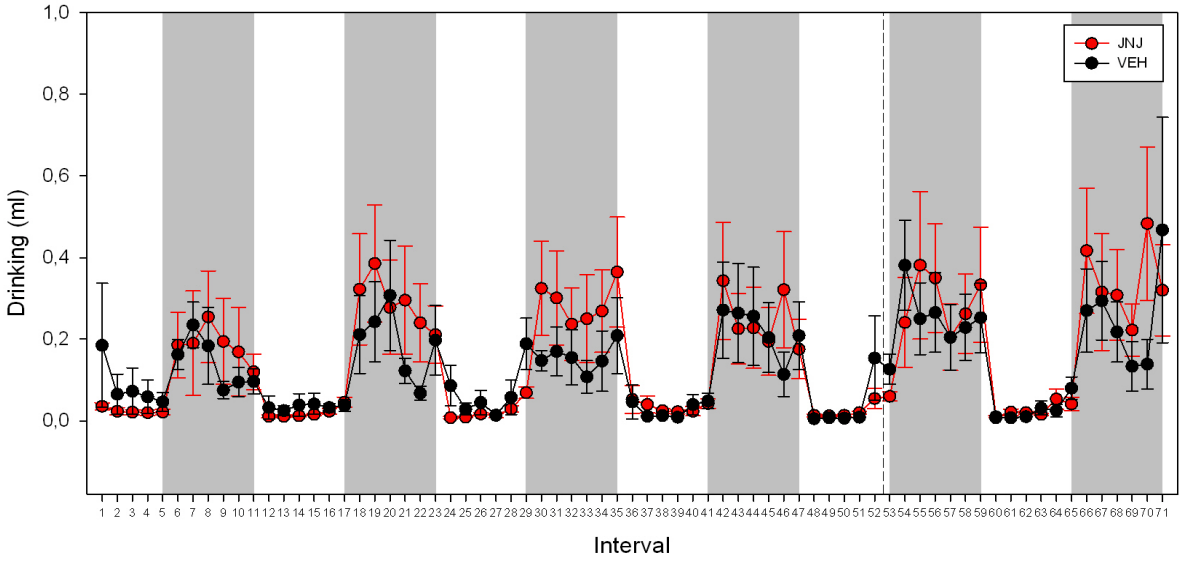

Figure 25: Drinking (sucrose) of mice. The parameter for drinking was summed up over 120 min, meaning that one interval represents two hours. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

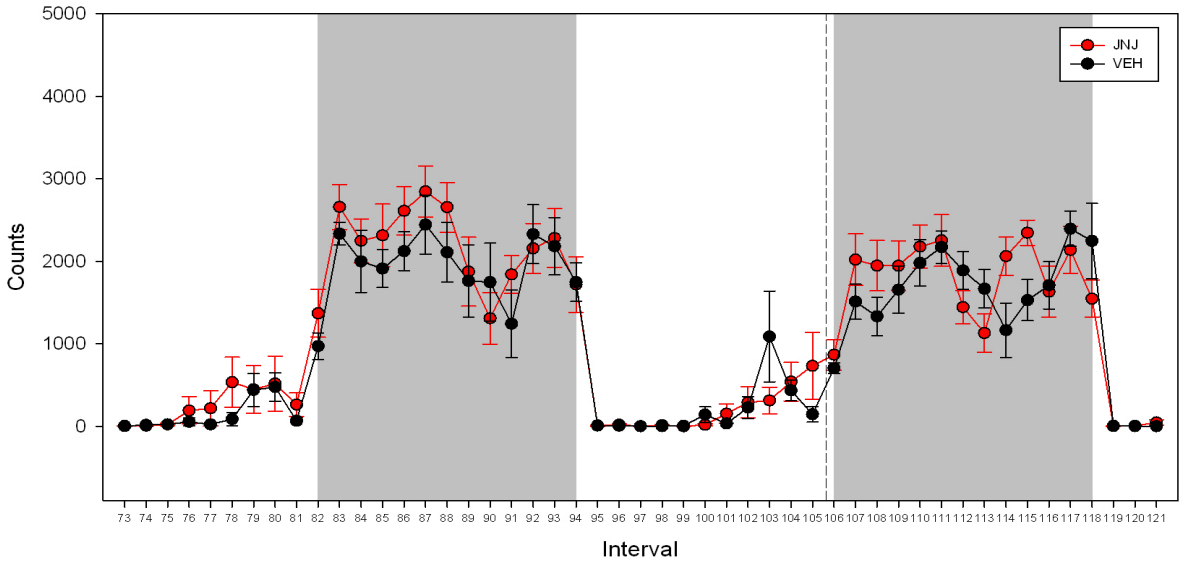

Figure 26: Activity of mice in the central area. The parameter for this activity was summed up over 60 min, meaning that one interval represents one hour. JNJ (20 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents activity of JNJ-injected mice, while the black curve represents activity of VEH-injected mice. The grey shading indicates the dark phase.

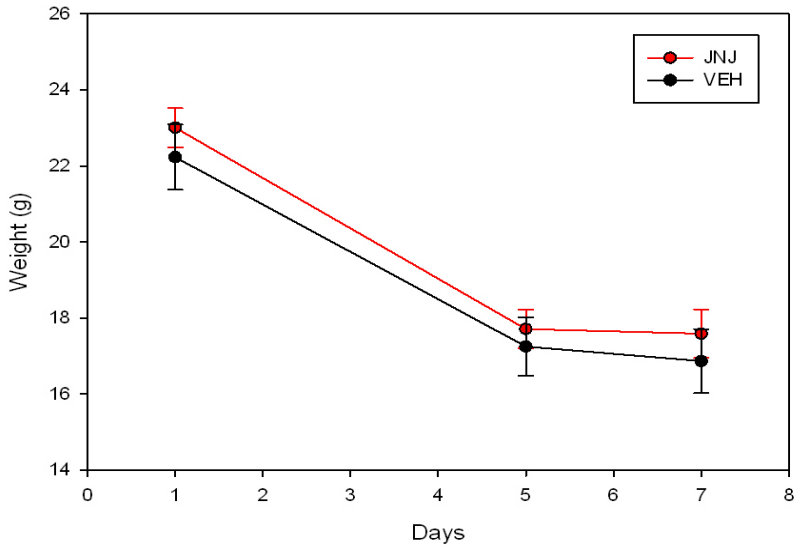

Figure 27: Mean body weight of mice on the days 1 to 7 of the diet-restriction experiment. JNJ-treated mice (10 mg/kg) are represented by a red curve while VEH-treated mice are represented by a black curve.

12.3.2 Locomotion and exploration

The locomotor (ambulatory) and the exploratory behaviour showed characteristic circadian time courses over the seven days of observation. As is typical for nocturnal animals, the activity of the mice was considerably higher in the dark phase (7 p.m. to 7 a.m.) than in the light phase (7 a.m. to 7 p.m.). However, compared to mice which received food ad libitum, diet-restricted mice showed a significant higher locomotor activity at the end of the light phase of days 4 to 6 (figure 28). This might be interpreted as food-anticipating activity and is also visible in figure 29, which shows the exploratory behaviour of diet-restricted mice and of mice fed ad libitum. There was no significant effect of JNJ (10 mg/kg) on locomotor activity (figure 30).

12.3.3 Ingestive behaviour

As seen in figure 31, there was no noteworthy difference in feeding between JNJ-injected mice (10 mg/kg) and VEH-injected mice following treatment on day 5. Interestingly, feeding of JNJ-injected mice is visibly but not significantly lower after injection on day 5 than on day 4 ($P = 0.195$), while there is no noteworthy difference in VEH-injected mice. Drinking (water) was not affected by JNJ-31020028 (figure 32).

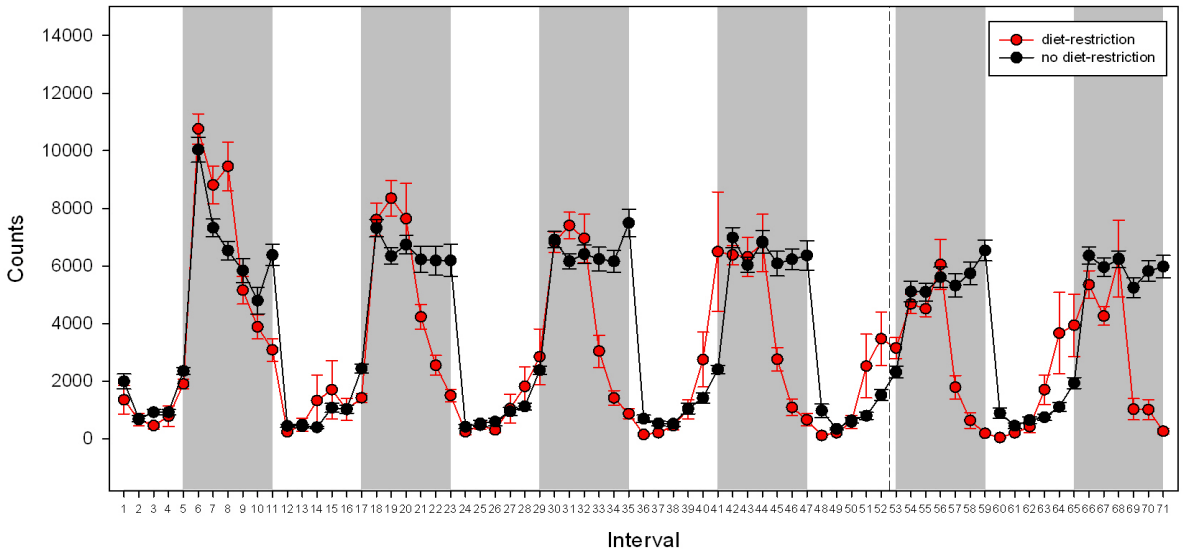

Figure 28: Locomotor activity of mice. Parameters for locomotion were summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of diet-restricted mice, while the black curve represents locomotion of mice fed ad libitum. The difference between the two groups at interval 52 is significant (P=0.084). The grey shading indicates the dark phase.

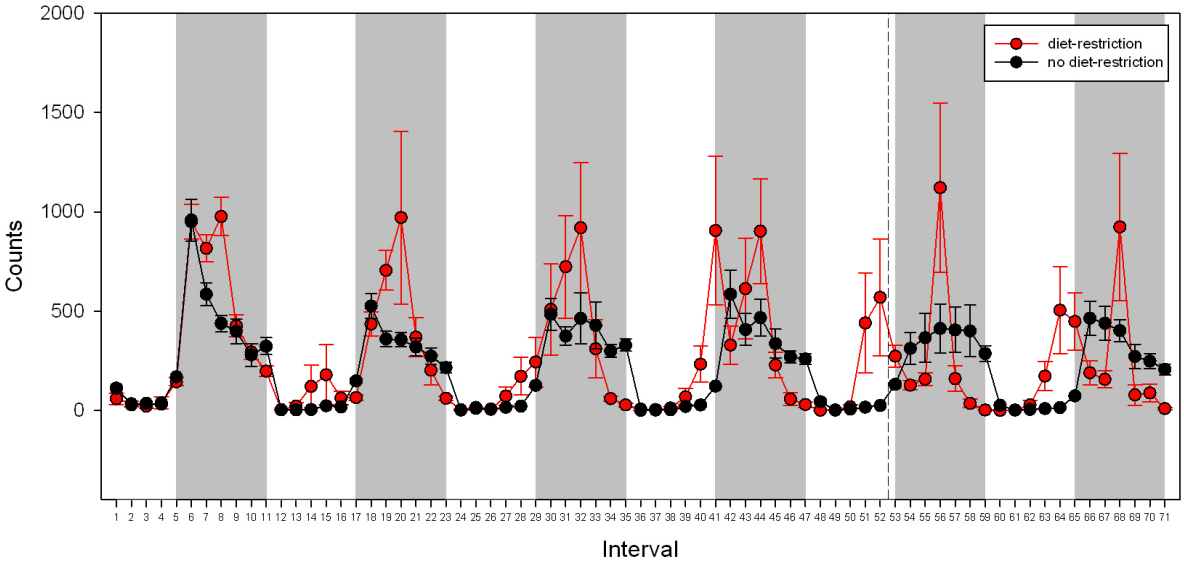

Figure 29: Exploratory behaviour of mice. The parameter for exploration was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of diet-restricted mice, while the black curve represents locomotion of mice fed ad libitum. The grey shading indicates the dark phase.

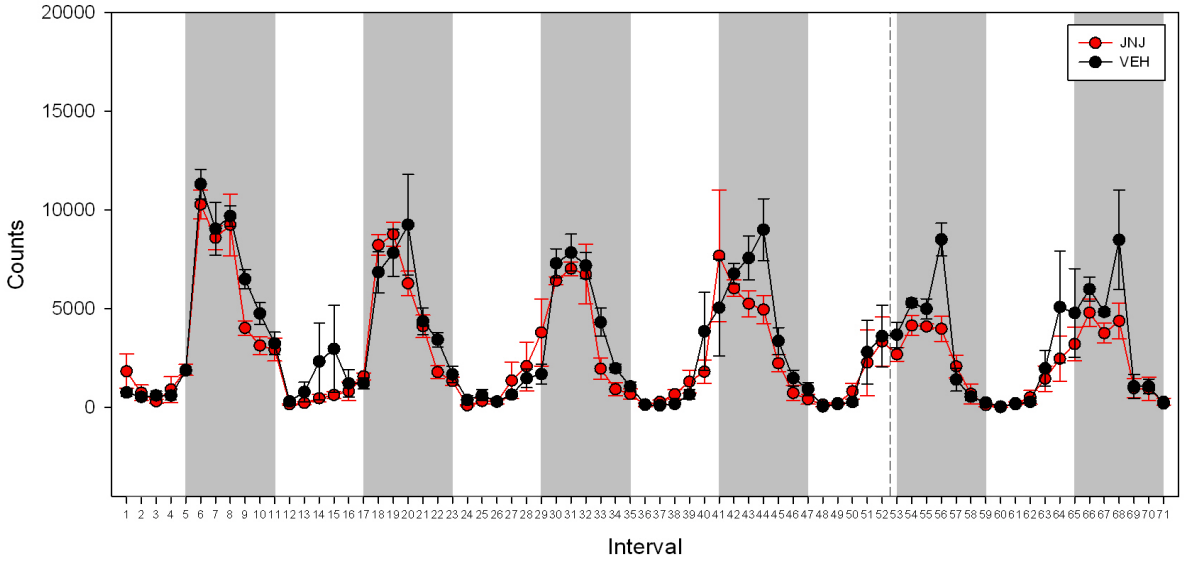

Figure 30: Locomotor activity of diet-restricted mice. Parameters for locomotion were summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents locomotion of JNJ injected mice, while the black curve represents locomotion of VEH injected mice. The grey shading indicates the dark phase.

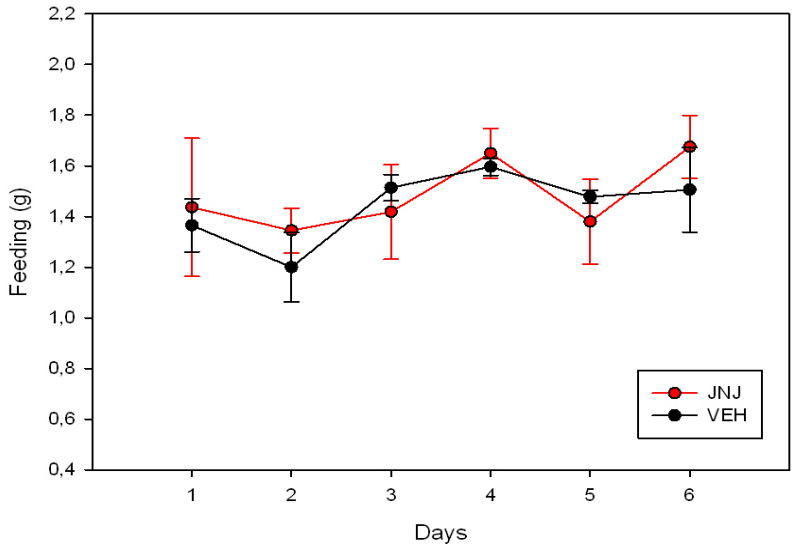

Figure 31: Food intake of diet-restricted mice. The parameter for feeding was summed up over the 180 min period of feeding in the dark phase. JNJ (10 mg/kg) or VEH was injected immediately before the feeding period on day 5. The red curve represents food intake of JNJ-injected mice, while the black curve represents food intake of VEH-injected mice.

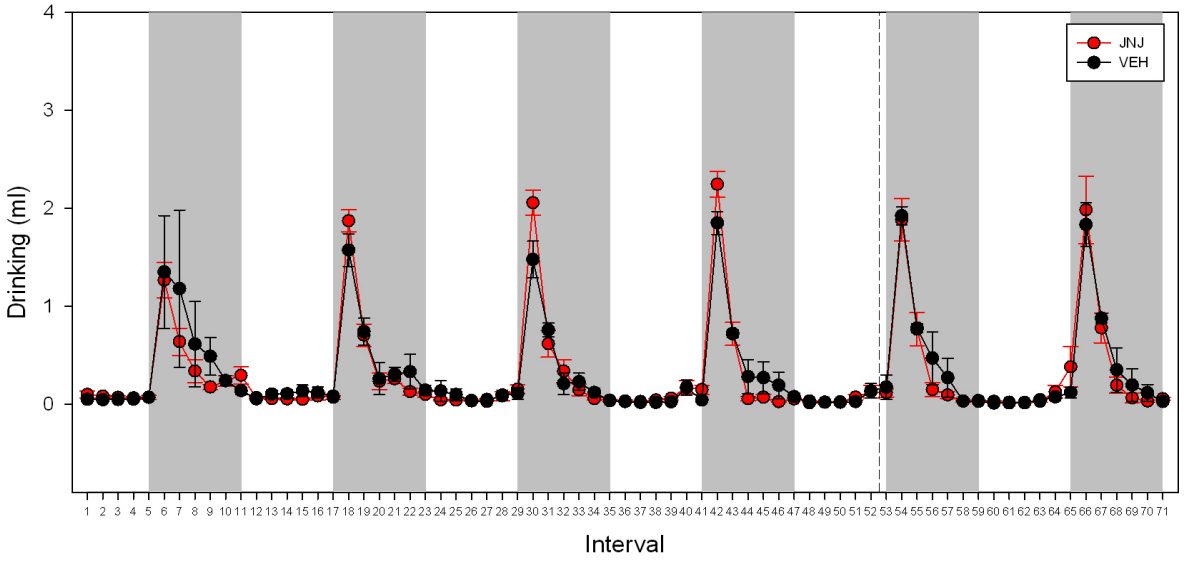

Figure 32: Drinking (water) of diet-restricted mice. The parameter for drinking was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents drinking of JNJ-injected mice, while the black curve represents drinking of VEH-injected mice. The grey shading indicates the dark phase.

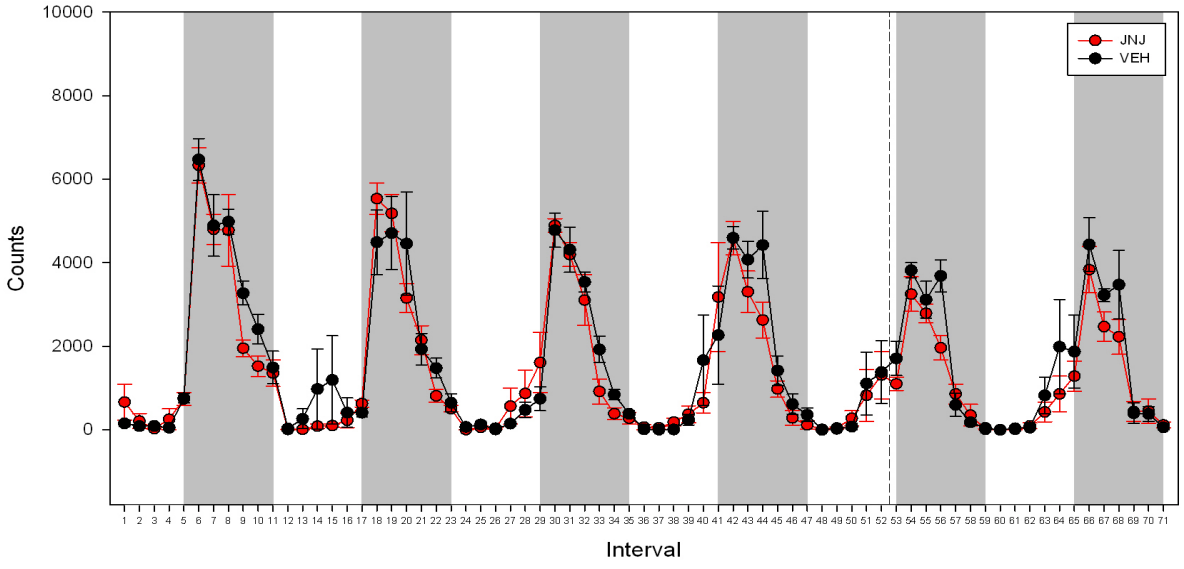

Figure 33: Activity of diet-restricted mice in central areas. The parameter for this activity was summed up over 120 min, meaning that one interval represents two hours. JNJ (10 mg/kg) or VEH was injected around the time indicated by a dashed line. The red curve represents activity of JNJ-injected mice, while the black curve represents activity of VEH-injected mice. The grey shading indicates the dark phase.

12.3.4 Anxiolysis

An anxiolytic effect of JNJ might be expressed by an increased activity of a mouse within the central area of its cage. This area was defined as described in 11.4. As figure 33 shows, there was no significant difference in the activity in this central area between JNJ-treated (10 mg/kg) and VEH-treated mice.

12.4 Discussion

The aim of this experiment was to determine the effect of the selective Y2-R antagonist "JNJ-31020028" on food intake, locomotor activity and exploratory behaviour of DBA/2 mice under normal and activity-stress conditions. As NPY is known to be an orexigenic peptide and Y2-R antagonists might increase NPY levels in the CNS (Brothers et al., 2010), it was hypothesized that JNJ increases food intake. Moreover, JNJ was expected to increase locomotor activity and/or exploratory behaviour, because it was shown that firstly nocturnal locomotion is enhanced in Y2-/- mice (Edelsbrunner et al., 2009) and that secondly in the activity-stress model rats increase running-wheel activity and decrease food intake when treated with NPY more than rats under the same conditions treated with vehicle (Nergårdh et al., 2006). Additionally, effects of JNJ on water and sucrose intake as well as anxiolytic properties of JNJ were examined. JNJ was expected to increase water intake, since nocturnal drinking has been shown to be slightly increased in Y2-/- mice (Edelsbrunner et al., 2009). Moreover, NPY plays a role in the regulation of mood (Brothers et al., 2010). To display effects of JNJ on mood, the mice of group 2 had access to a bottle filled with sucrose solution, given that sucrose preference is a test for depression-related behaviour. Finally, as NPY is known to have anxiolytic effects (Thorsell, 2010), it was suggested that administration of JNJ might affect anxiety-related behaviour as well.

JNJ was characterized by Shoblock et al. (2010) to be a selective brain penetrant small molecule antagonist of the Y2-R. In vitro, JNJ was shown to have high affinity for human and rodent Y2-Rs. When JNJ was administered subcutaneously, its bioavailability was 100% (Shoblock et al., 2010). Maximal Y2-R occupancy of JNJ (10 mg/kg) in rat hippocampus was observed 1 h following peripheral administration. Regarding dosage, highest Y2-R occupancy was seen at 10 and 30 mg/kg (Shoblock et al., 2010). In vivo, peripheral injection of JNJ increased hypothalamic norepinephrine (NE) release, which is consistent with an inhibition of NE release by Y2-R and indicates that JNJ acted in the brain. No anxiolytic effects of JNJ were detectable in the elevated plus maze test, Vogel test, light–dark test and stress-induced hyperthermia test in mice or rats (Shoblock et al., 2010). Additionally, JNJ (10 and 20 mg/kg) was shown to decrease corticosterone levels significantly under conditions of stress, but not in

nonstressed animals. JNJ-pretreated (20 mg/kg) stressed animals ate significantly more food than VEH-pretreated stressed animals (Shoblock et al., 2010). Moreover, JNJ was tested on nicotine abstinence-related social anxiety-like behavior as well as on NPY and CRF mRNA levels in the novelty-seeking phenotype. Thereby, JNJ (20 mg/kg, i.p.) was shown to reverse nicotine-induced social anxiety-like behavior during abstinence (Aydin et al., 2011). Finally, Cippitelli et al. showed that JNJ (15 mg/kg, s.c.) was able to reverse the anxiogenic effects of withdrawal. This might reflect anxiolytic-like properties of JNJ (Cippitelli et al., 2010).

In this experiment, JNJ was injected i.p. in dosages of 10 mg/kg or 20 mg/kg, because previous studies had shown that maximal Y2-R occupancy in the brain was achieved by this dose range (see previous paragraph). Intraperitoneal administration was chosen because of the lean phenotype of the experimental animals which made subcutaneous administration nearly impossible.

Regarding food intake, the effect of JNJ in this experiment was inconsistent. JNJ-treated mice of group 1 (10 mg/kg) ate significantly more food than before treatment, while there was no significant change in food intake of VEH-treated mice. Moreover, JNJ-injected mice ate significantly more than VEH-injected mice in the second hour after injection. However, this positive effect on food intake could not be confirmed in group 2 receiving a dosage of 20 mg/kg JNJ. It has to be mentioned that there was no significant difference in the mean body weight of the mice of group 1 and the mice of group 2 at the beginning and end of the experiment. A possible orexigenic effect of JNJ might therefore be dose dependent, possibly in form of a bell-shaped dose-response curve. Yet, it has to be taken into consideration that the mice of group 2 had access to a bottle filled with sucrose solution which was not the case in group 1. This limits the comparability of group 1 and group 2. However, Shoblock et al. found food intake to be significantly increased in stressed animals pretreated with JNJ at a dosage of 20 mg/kg but not at a dosage of 10 mg/kg (Shoblock et al., 2010). The dosage of JNJ necessary to increase food intake might therefore depend on corticosterone levels. The CRF-POMC/ACTH-cortisol axis is known to exert anorexigenic effects (see section 5). It might be that the method used by Shoblock et al. to induce stress led to higher corticosterone levels than were present in the mice of the current study and therefore JNJ at a dosage of 10 mg/kg was sufficient to achieve a significantly higher food intake. Although corticosterone or other indicators of stress were not measured in the present experiment, it has to be assumed that the animals were stressed, because they were kept separated (see section 2.1) and were briefly restrained for the i.p. treatment. Still, it is debatable why there was no positive effect of JNJ in group 2 (20 mg/kg) of the current experiment. In this context, it has to be taken into consideration that Y2-Rs are not only expressed presynaptically on NPY neurons of the ARC, but also postsynaptically on anorexigenic POMC neurons, and NPY inhibits POMC neurons by

binding to such Y2-Rs (and Y1-Rs) (Chee et al., 2008; see 3.1.1 and figure 1). By binding to Y2-Rs on POMC neurons, a Y2-R antagonist might cancel inhibitory effects of NPY on POMC neurons. As postsynaptic receptors may be less accessible for exogenous drugs and higher drug concentrations might increase the probability of postsynaptic effects, the positive effect of JNJ on food intake might be attenuated when the dose of JNJ is increased.

Although nocturnal drinking has been shown to be slightly increased in Y2-/- mice compared to WT mice (Edelsbrunner et al., 2009), it was not affected by JNJ in the current experiment. In this context, the question has to be raised if Y2-R knockout is comparable to Y2-R antagonism. It has to be taken into consideration that germ-line Y2-R knockout leads to compensatory changes to maintain function. Sucrose preference was also not affected by JNJ, which suggests that depression-related behaviour remained unaltered.

Although NPY is known to have anxiolytic properties (Thorsell, 2010), no such effects of JNJ were detectable. It has to be mentioned that the procedure to assess anxiolytic effects in the current experiment might be less sensitive than usual tests for anxiety. Moreover, anxiogenic peptides such as CRF were not measured in this experiment. It might be that the stress level of the experimental animals interfered with the evaluation of the effects under study and the JNJ dosage was inadequate. As mentioned by Shoblock et al., who also found no anxiolytic effects of JNJ, it has to be emphasized that there is no evidence that Y2-Rs are functional autoreceptors in anxiety-related brain areas (Shoblock et al., 2010).

There was no significant effect of JNJ on ambulatory or exploratory behaviour in mice fed at libitum (group 1 and 2). This is an interesting finding, since earlier studies showed that Y2-R knockout increased nocturnal locomotion compared to WT mice (Edelsbrunner et al., 2009). It has to be considered that the effect of JNJ to enhance activity would possibly attenuate or extinguish its possible positive effect on body weight, because increased activity goes along with increased energy expenditure.

As mentioned above, rats and certain mouse strains react to food-restriction with increasing running-wheel activity and decreasing food intake (activity-stress animal model of AN, see 2.1). DBA/2 mice have been reported to show this behaviour. In the activity-stress model rats increase running-wheel activity and decrease food intake when treated with NPY more than rats treated with vehicle (Nergårdh et al., 2006; see 3.1.3). As an Y2-R antagonist can elevate NPY levels in the hypothalamus, it has to be assumed that administration of JNJ to DBA/2 mice in the activity-stress model leads to increased activity and decreased food intake. This would limit the use of JNJ in AN, where hyperactivity is a common problem and might at least partly be due to high NPY levels. Therefore, it seemed reasonable to test the effect of JNJ in activity-based anorexia in DBA/2 mice. However, overall activity of the diet-restricted animals was not significantly higher than activity of mice fed ad libitum although body weight decreased. This

might be due to the lack of running wheels which might stimulate activity or because of the low mean weight of the mice, possibly leading to fatigue quickly because of low energy stores. Still, as described in 12.3.2, at the end of the light phase of days 4 to 6 locomotor activity was significantly higher in food-restricted mice than in mice fed ad libitum. This might be interpreted as food-anticipating activity. Administration of JNJ to food-restricted mice on day 5 did not cause significant changes in feeding, drinking, ambulatory or exploratory behaviour in the hours after injection compared to VEH-injected mice. If any, JNJ has a slight depressant effect on locomotor activity in this case. It might be that a higher dosage of JNJ will show clearer results in activity-based anorexia. As mentioned before, effects of JNJ might mainly depend on the stress level. In this case, the stress level of the mice was probably very high, while the dosage of JNJ might have been too low.

Taking everything into consideration, a significant positive but short-lasting effect of JNJ on food intake in a dosage of 10 mg/kg could be detected. The finding that any effects of JNJ are modulated by stress (Shoblock et al., 2010) needs to be taken into account in the further study of JNJ. In AN for example, the hypothalamic-pituitary-adrenal (HPA) axis is hyperactive displaying high cerebrospinal fluid levels of CRF, normal ACTH levels and high cortisol levels, which is similar under conditions of stress (Casper, 2006). To confirm or falsify an effect of JNJ on food intake, several doses of JNJ given over a prolonged period of time need to be investigated. To elucidate the connection between effects of JNJ and stress, it would be useful to measure parameters of stress, such as CRF or corticosterone. The effects of JNJ under activity-stress conditions should also be further elucidated. First of all, a range of JNJ doses in diet-restricted mice needs to be examined. Secondly, although diet-restricted mice actually displayed hyperactivity at times when no food was available, the lack of running wheels might have been a limiting factor. If JNJ would prove to reduce activity while increasing food intake, it could actually be a useful drug for AN in the future.

13 References

Abe K, Kuo L, Zukowska Z. Neuropeptide Y is a mediator of chronic vascular and metabolic maladaptations to stress and hypernutrition. Exp Biol Med (Maywood). 2010 Oct 1; 235(10): 1179-84.

Adan RA, Hillebrand JJ, De Rijke C, Nijenhuis W, Vink T, Garner KM, Kas MJ. Melanocortin system and eating disorders. Ann N Y Acad Sci. 2003 Jun;994:267-74.

Ashby D, Bloom SR. Recent progress in PYY research–an update report for 8th NPY meeting. Peptides. 2007 Feb;28(2):198-202.

Avraham Y, Hao S, Mendelson S, Berry EM. Tyrosine improves appetite, cognition, and exercise tolerance in activity anorexia. Med Sci Sports Exerc. 2001 Dec;33(12):2104-10.

Aydin C, Oztan O, Isgor C. Effects of a selective Y2R antagonist, JNJ-31020028, on nicotine abstinence-related social anxiety-like behavior, neuropeptide Y and corticotropin releasing factor mRNA levels in the novelty-seeking phenotype. Behav Brain Res. 2011 Sep 23;222(2):332-41.

Ballantyne GH. Peptide YY(1-36) and peptide YY(3-36): Part I. Distribution, release and actions. Obes Surg. 2006 May;16(5):651-8.

Banks WA, Farr SA, Morley JE. The effects of high fat diets on the blood-brain barrier transport of leptin: failure or adaptation? Physiol Behav. 2006 Jun 30;88(3):244-8.

Baranowska B, Baranowska-Bik A, Bik W, Martynska L. The role of leptin and orexins in the dysfunction of hypothalamo-pituitary-gonadal regulation and in the mechanism of hyperactivity in patients with anorexia nervosa. Neuro Endocrinol Lett. 2008 Feb;29(1):37-40.

Baranowska B, Wolinska-Witort E, Wasilewska-Dziubinska E, Roguski K, Chmielowska M. Plasma leptin, neuropeptide Y (NPY) and galanin concentrations in bulimia nervosa and in anorexia nervosa. Neuro Endocrinol Lett. 2001 Oct;22(5):356-8.

Baranowska B, Wolinska-Witort E, Wasilewska-Dziubinska E, Roguski K, Martynska L, Chmielowska M. The role of neuropeptides in the disturbed control of appetite and hormone secretion in eating disorders. Neuro Endocrinol Lett. 2003 Dec;24(6):431-4.

Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. N Engl J Med. 2003 Sep 4;349(10):941-8.

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. Nature. 2002 Aug 8;418(6898):650-4.

Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, Le Roux CW, Thomas EL, Bell JD, Withers DJ. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006 Sep;4(3):223-33.

Begriche K, Sutton GM, Fang J, Butler AA. The role of melanocortin neuronal pathways in circadian biology: a new homeostatic output involving melanocortin-3 receptors? Obes Rev. 2009 Nov;10 Suppl 2:14-24.

Beranová L, Sedlácková D, Kopecková J, Hainer V, Papezová H, Kvasnicková H, Nedvídková J.

Neuropeptide Y, ghrelin and leptin plasma levels in anorexia nervosa patients and their changes during six-week refeeding. Vnitr Lek. 2009 Oct;55(10):925-8.

Blüher S, Mantzoros CS. The role of leptin in regulating neuroendocrine function in humans. J Nutr. 2004 Sep;134(9):2469S-2474S.

Boey D, Sainsbury A, Herzog H. The role of peptide YY in regulating glucose homeostasis. Peptides. 2007 Feb;28(2):390-5.

Boggiano MM, Chandler PC, Oswald KD, Rodgers RJ, Blundell JE, Ishii Y, Beattie AH, Holch P, Allison DB, Schindler M, Arndt K, Rudolf K, Mark M, Schoelch C, Joost HG, Klaus S, Thöne-Reineke C, Benoit SC, Seeley RJ, Beck-Sickinger AG, Koglin N, Raun K, Madsen K, Wulff BS, Stidsen CE, Birringer M, Kreuzer OJ, Deng XY, Whitcomb DC, Halem H, Taylor J, Dong J, Datta R, Culler M, Ortmann S, Castañeda TR, Tschöp M. PYY3-36 as an anti-obesity drug target. Obes Rev. 2005 Nov;6(4):307-22. Review.

Broberger C, Johansen J, Johansson C, Schalling M, Hökfelt T. The neuropeptide Y/agouti gene-related protein (AGRP) brain circuitry in normal, anorectic, and monosodium glutamate-treated mice. Proc Natl Acad Sci U S A. 1998 Dec 8;95(25):15043-8.

Broberger C, Johansen J, Schalling M, Hökfelt T. Hypothalamic neurohistochemistry of the murine anorexia (anx/anx) mutation: altered processing of neuropeptide Y in the arcuate nucleus. J Comp Neurol. 1997 Oct 13;387(1):124-35.

Brothers SP, Wahlestedt C. Therapeutic potential of neuropeptide Y (NPY) receptor ligands. EMBO Mol Med. 2010 Nov;2(11):429-39.

Butler AA, Kesterson RA, Khong K, Cullen MJ, Pelleymounter MA, Dekoning J, Baetscher M, Cone RD. A unique metabolic syndrome causes obesity in the melanocortin-3 receptor-deficient mouse. Endocrinology. 2000 Sep;141(9):3518-21.

Casper RC, Sullivan EL, Tecott L. Relevance of animal models to human eating disorders and obesity. Psychopharmacology (Berl). 2008 Aug;199(3):313-29.

Casper RC. The 'drive for activity' and "restlessness" in anorexia nervosa: Potential pathways. J Affect Disord. 2006 May;92(1):99-107.

Challis BG, Pinnock SB, Coll AP, Carter RN, Dickson SL, O'Rahilly S. Biochem Biophys Res Commun. Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. 2003 Nov 28;311(4):915-9.

Chee MJ, Colmers WF. Y eat? Nutrition. 2008 Sep;24(9):869-77.

Chee MJ, Myers MG Jr, Price CJ, Colmers WF. Neuropeptide Y suppresses anorexigenic output from the ventromedial nucleus of the hypothalamus. J Neurosci. 2010 Mar 3;30(9):3380-90.

Chelikani PK, Haver AC, Reidelberger RD. Ghrelin attenuates the inhibitory effects of glucagon-like peptide-1 and peptide YY(3-36) on food intake and gastric emptying in rats. Diabetes. 2006 Nov;55(11):3038-46.

Chen P, Vaughan J, Donaldson C, Vale W, Li C. Injection of Urocortin 3 into the ventromedial hypothalamus modulates feeding, blood glucose levels, and hypothalamic POMC gene expression

but not the HPA axis. Am J Physiol Endocrinol Metab. 2010 Feb;298(2):E337-45.

Cippitelli A, Rezvani AH, Robinson JE, Eisenberg L, Levin ED, Bonaventure P, Motley ST, Lovenberg TW, Heilig M, Thorsell A. The novel, selective, brain-penetrant neuropeptide Y Y2 receptor antagonist, JNJ-31020028, tested in animal models of alcohol consumption, relapse, and anxiety. Alcohol. 2010 Dec 10.

Cowley MA, Smart JL, Rubinstein M, Cerdán MG, Diano S, Horvath TL, Cone RD, Low MJ. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature. 2001 May 24;411(6836):480-4.

Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. Neuron. 2003 Feb 20;37(4):649-61.

Dailey MJ, Bartness TJ. Appetitive and consummatory ingestive behaviors stimulated by PVH and perifornical area NPY injections. Am J Physiol Regul Integr Comp Physiol. 2009 Apr;296(4):R877-92.

Date Y, Mondal MS, Kageyama H, Ghamari-Langroudi M, Takenoya F, Yamaguchi H, Shimomura Y, Mori M, Murakami N, Shioda S, Cone RD, Nakazato M. Neuropeptide W: an anorectic peptide regulated by leptin and metabolic state. Endocrinology. 2010 May;151(5):2200-10.

DeBoer MD, Scarlett JM, Levasseur PR, Grant WF, Marks DL. Administration of IL-1beta to the 4th ventricle causes anorexia that is blocked by agouti-related peptide and that coincides with activation of tyrosine-hydroxylase neurons in the nucleus of the solitary tract. Peptides. 2009 Feb;30(2):210-8.

DeBoer MD. Animal models of anorexia and cachexia. Expert Opin Drug Discov. 2009 Nov 1;4(11):1145-1155.

DeBoer MD. Update on melanocortin interventions for cachexia: progress toward clinical application. Nutrition. 2010 Feb;26(2):146-51.

de Gortari P, Mancera K, Cote-Vélez A, Amaya MI, Martínez A, Jaimes-Hoy L, Joseph-Bravo P. Involvement of CRH-R2 receptor in eating behavior and in the response of the HPT axis in rats subjected to dehydration-induced anorexia. Psychoneuroendocrinology. 2009 Feb;34(2):259-72.

Di Marzo V, Goparaju SK, Wang L, Liu J, Bátkai S, Járai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos G. Leptin-regulated endocannabinoids are involved in maintaining food intake. Nature. 2001 Apr 12;410(6830):822-5.

Dixon DP, Ackert AM, Eckel LA. Development of, and recovery from, activity-based anorexia in female rats. Physiol Behav. 2003 Nov;80(2-3):273-9.

Douglas AJ, Johnstone LE, Leng G. Neuroendocrine mechanisms of change in food intake during pregnancy: a potential role for brain oxytocin. Physiol Behav. 2007 Jul 24;91(4):352-65.

Edelsbrunner ME, Herzog H, Holzer P. Evidence from knockout mice that peptide YY and neuropeptide

Y enforce murine locomotion, exploration and ingestive behaviour in a circadian cycle- and gender-dependent manner. Behav Brain Res. 2009 Oct 12;203(1):97-107.

Edelsbrunner ME, Painsipp E, Herzog H, Holzer P. Evidence from knockout mice for distinct implications of neuropeptide-Y Y2 and Y4 receptors in the circadian control of locomotion, exploration, water and food intake. Neuropeptides. 2009 Dec;43(6):491-7.

Edwards CM, Abusnana S, Sunter D, Murphy KG, Ghatei MA, Bloom SR. The effect of the orexins on food intake: comparison with neuropeptide Y, melanin-concentrating hormone and galanin. J Endocrinol. 1999 Mar;160(3):R7-12.

Ehrlich S, Burghardt R, Schneider N, Hein J, Weiss D, Pfeiffer E, Lehmkuhl U, Salbach-Andrae H. Leptin and its associations with measures of psychopathology in patients with anorexia nervosa. J Neural Transm. 2009 Jan;116(1):109-15.

Ehrlich S, Salbach-Andrae H, Eckart S, Merle JV, Burghardt R, Pfeiffer E, Franke L, Uebelhack R, Lehmkuhl U, Hellweg R. Serum brain-derived neurotrophic factor and peripheral indicators of the serotonin system in underweight and weight-recovered adolescent girls and women with anorexia nervosa. J Psychiatry Neurosci. 2009 Jul;34(4):323-9.

Ellacott KL, Murphy JG, Marks DL, Cone RD. Obesity-induced inflammation in white adipose tissue is attenuated by loss of melanocortin-3 receptor signaling. Endocrinology. 2007 Dec;148(12):6186-94.

Exner C, Hebebrand J, Remschmidt H, Wewetzer C, Ziegler A, Herpertz S, Schweiger U, Blum WF, Preibisch G, Heldmaier G, Klingenspor M. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. Mol Psychiatry. 2000 Sep;5(5):476-81.

Feltrin KL, Patterson M, Ghatei MA, Bloom SR, Meyer JH, Horowitz M, Feinle-Bisset C. Effect of fatty acid chain length on suppression of ghrelin and stimulation of PYY, GLP-2 and PP secretion in healthy men. Peptides. 2006 Jul;27(7):1638-43.

Frieling H, Albrecht H, Jedtberg S, Gozner A, Lenz B, Wilhelm J, Hillemacher T, de Zwaan M, Kornhuber J, Bleich S. Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudes in females with eating disorders. Psychoneuroendocrinology. 2009 May;34(4):620-4.

Föcker M, Timmesfeld N, Scherag S, Bühren K, Langkamp M, Dempfle A, Sheridan EM, de Zwaan M, Fleischhaker C, Herzog W, Egberts K, Zipfel S, Herpertz-Dahlmann B, Hebebrand J. Screening for anorexia nervosa via measurement of serum leptin levels. J Neural Transm. 2011 Jan 22.

García-Luna C, Amaya MI, Alvarez-Salas E, de Gortari P. Prepro-orexin and feeding-related peptide receptor expression in dehydration-induced anorexia. Regul Pept. 2010 Jan 8;159(1-3):54-60.

Gelegen C, Collier DA, Campbell IC, Oppelaar H, van den Heuvel J, Adan RA, Kas MJ. Difference in susceptibility to activity-based anorexia in two inbred strains of mice. Eur Neuropsychopharmacol. 2007 Feb;17(3):199-205.

Gelegen C, Pjetri E, Campbell IC, Collier DA, Oppelaar H, Kas MJ. Chromosomal mapping of excessive physical activity in mice in response to a restricted feeding schedule. Eur Neuropsychopharmacol. 2010 May;20(5):317-26.

Germain N, Galusca B, Grouselle D, Frere D, Billard S, Epelbaum J, Estour B. Ghrelin and obestatin circadian levels differentiate bingeing-purging from restrictive anorexia nervosa. J Clin Endocrinol Metab. 2010 Jun;95(6):3057-62.

Germain N, Galusca B, Le Roux CW, Bossu C, Ghatei MA, Lang F, Bloom SR, Estour B. Constitutional thinness and lean anorexia nervosa display opposite concentrations of peptide YY, glucagon-like peptide 1, ghrelin, and leptin. Am J Clin Nutr. 2007 Apr;85(4):967-71.

Ghamari-Langroudi M, Srisai D, Cone RD. Multinodal regulation of the arcuate/paraventricular nucleus circuit by leptin. Proc Natl Acad Sci U S A. 2011 Jan 4;108(1):355-60.

Gonzales C, Voirol MJ, Giacomini M, Gaillard RC, Pedrazzini T, Pralong FP. The neuropeptide Y Y1 receptor mediates NPY-induced inhibition of the gonadotrope axis under poor metabolic conditions. FASEB J. 2004 Jan;18(1):137-9.

Goto S, Nagao K, Bannai M, Takahashi M, Nakahara K, Kangawa K, Murakami N. Anorexia in rats caused by a valine-deficient diet is not ameliorated by systemic ghrelin treatment. Neuroscience. 2010 Mar 10;166(1):333-40.

Gropp E, Shanabrough M, Borok E, Xu AW, Janoschek R, Buch T, Plum L, Balthasar N, Hampel B, Waisman A, Barsh GS, Horvath TL, Brüning JC. Agouti-related peptide-expressing neurons are mandatory for feeding. Nat Neurosci. 2005 Oct;8(10):1289-91.

Grudell AB, Camilleri M. The role of peptide YY in integrative gut physiology and potential role in obesity. Curr Opin Endocrinol Diabetes Obes. 2007 Feb;14(1):52-7.

Gutiérrez E, Churruca I, Zárate J, Carrera O, Portillo MP, Cerrato M, Vázquez R, Echevarría E. High ambient temperature reverses hypothalamic MC4 receptor overexpression in an animal model of anorexia nervosa. Psychoneuroendocrinology. 2009 Apr;34(3):420-9.

Hays, W. L. (1994). Statistics (5th ed.). Fort Worth, TX: Harcourt Brace.

Heilig M. The NPY system in stress, anxiety and depression. Neuropeptides. 2004 Aug;38(4):213-24.

Heinrichs SC, Richard D. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides. 1999 Oct;33(5):350-9.

Henry M, Ghibaudi L, Gao J, Hwa JJ. Energy metabolic profile of mice after chronic activation of central NPY Y1, Y2, or Y5 receptors. Obes Res. 2005 Jan;13(1):36-47.

Herold G. Herold 2008. Köln 2008

Herzog H. Neuropeptide Y and energy homeostasis: insights from Y receptor knockout models. Eur J Pharmacol. 2003 Nov 7;480(1-3):21-9.

Higuchi H, Niki T, Shiiya T. Feeding behavior and gene expression of appetite-related neuropeptides in mice lacking for neuropeptide Y Y5 receptor subclass. World J Gastroenterol. 2008 Nov 7;14(41):6312-7.

Hillebrand JJ, Kas MJ, Adan RA. alpha-MSH enhances activity-based anorexia. Peptides. 2005 Oct;26(10):1690-6.

Hillebrand JJ, Kas MJ, van Elburg AA, Hoek HW, Adan RA. Leptin's effect on hyperactivity: potential downstream effector mechanisms. Physiol Behav. 2008 Aug 6;94(5):689-95.

Huang L, Toyoshima M, Asakawa A, Inoue K, Harada K, Kinoshita T, Koizumi A. Roles of neuropeptides in O,O,S-trimethylphosphorothioate (OOS-TMP)-induced anorexia in mice. Biochem Biophys Res Commun. 2007 Oct 12;362(1):177-82.

Huang Q, Timofeeva E, Richard D. Regulation of corticotropin-releasing factor and its types 1 and 2 receptors by leptin in rats subjected to treadmill running-induced stress. J Endocrinol. 2006 Oct;191(1):179-88.

Huda MS, Wilding JP, Pinkney JH. Gut peptides and the regulation of appetite. Obes Rev. 2006 May;7(2):163-82.

Inui A. Cancer Anorexia-Cachexia Syndrome: Are Neuropeptides the Key? Cancer Res. 1999 Sep 15;59(18):4493-501.

Irani BG, Xiang Z, Yarandi HN, Holder JR, Moore MC, Bauzo RM, Proneth B, Shaw AM, Millard WJ, Chambers JB, Benoit SC, Clegg DJ, Haskell-Luevano C. Implication of the melanocortin-3 receptor in the regulation of food intake. Eur J Pharmacol. 2011 Jan 1.

Ishiguro H, Onaivi ES, Horiuchi Y, Imai K, Komaki G, Ishikawa T, Suzuki M, Watanabe Y, Ando T, Higuchi S, Arinami T. Functional polymorphism in the GPR55 gene is associated with anorexia nervosa. Synapse. 2010 May 26.

Janas-Kozik M, Stachowicz M, Krupka-Matuszczyk I, Szymszal J, Krysta K, Janas A, Rybakowski JK. Plasma levels of leptin and orexin A in the restrictive type of anorexia nervosa. Regul Pept. 2011 Feb 19.

Johansen JE, Broberger C, Lavebratt C, Johansson C, Kuhar MJ, Hökfelt T, Schalling M. Hypothalamic CART and serum leptin levels are reduced in the anorectic (anx/anx) mouse. Brain Res Mol Brain Res. 2000 Dec 8;84(1-2):97-105.

Kamisoyama H, Honda K, Saneyasu T, Sugahara K, Hasegawa S. Corticotropin-releasing factor is a downstream mediator of the beta-melanocyte-stimulating hormone-induced anorexigenic pathway in chicks. Neurosci Lett. 2009 Jul 24;458(3):102-5.

Kanarek RB, D'Anci KE, Jurdak N, Mathes WF. Running and addiction: precipitated withdrawal in a rat model of activity-based anorexia. Behav Neurosci. 2009 Aug;123(4):905-12.

Kas MJ, van Dijk G, Scheurink AJ, Adan RA. Agouti-related protein prevents self-starvation. Mol Psychiatry. 2003 Feb;8(2):235-40.

Kask A, Harro J, von Hörsten S, Redrobe JP, Dumont Y, Quirion R. The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. Neurosci Biobehav Rev. 2002 May;26(3):259-83.

Kawashima S, Sakihara S, Kageyama K, Nigawara T, Suda T. Corticotropin-releasing factor (CRF) is involved in the acute anorexic effect of alpha-melanocyte-stimulating hormone: a study using CRF-deficient mice. Peptides. 2008 Dec;29(12):2169-74.

Kaye WH, Berrettini W, Gwirtsman H, George DT. Altered cerebrospinal fluid neuropeptide Y and peptide YY immunoreactivity in anorexia and bulimia nervosa. Arch Gen Psychiatry. 1990 Jun;47(6):548-56.

Kaye WH, Fudge JL, Paulus M. New insights into symptoms and neurocircuit function of anorexia nervosa. Nat Rev Neurosci. 2009 Aug;10(8):573-84.

Kiess W, Reich A, Meyer K, Glasow A, Deutscher J, Klammt J, Yang Y, Müller G, Kratzsch J. A role for leptin in sexual maturation and puberty? Horm Res. 1999;51 Suppl 3:55-63.

Kim JG, Park BS, Yun CH, Kim HJ, Kang SS, D'Elia AV, Damante G, Lee KU, Park JW, Kim ES, Namgoong IS, Kim YI, Lee BJ. Thyroid Transcription Factor-1 Regulates Feeding Behavior via Melanocortin Pathway in the Hypothalamus. Diabetes. 2011 Mar;60(3):710-9.

Kinzig KP, Hargrave SL. Adolescent activity-based anorexia increases anxiety-like behavior in adulthood. Physiol Behav. 2010 Sep 1;101(2):269-76.

Kirchner H, Tong J, Tschöp MH, Pfluger PT. Ghrelin and PYY in the regulation of energy balance and metabolism: lessons from mouse mutants. Am J Physiol Endocrinol Metab. 2010 May;298(5):E909-19.

Kirk, R. E. (1982). Experimental design: Procedures for the behavioral sciences (2nd. ed.). Pacific Grove, CA: Brooks/Cole.

Koda S, Date Y, Murakami N, Shimbara T, Hanada T, Toshinai K, Niijima A, Furuya M, Inomata N, Osuye K, Nakazato M. The role of the vagal nerve in peripheral PYY3-36-induced feeding reduction in rats. Endocrinology. 2005 May;146(5):2369-75.

Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci. 2002 Mar;25(3):154-9.

Krahn DD, Gosnell BA, Majchrzak MJ. The anorectic effects of CRH and restraint stress decrease with repeated exposures. Biol Psychiatry. 1990 May 15;27(10):1094-102.

Kuo LE, Czarnecka M, Kitlinska JB, Tilan JU, Kvetnanský R, Zukowska Z. Chronic stress, combined with a high-fat/high-sugar diet, shifts sympathetic signaling toward neuropeptide Y and leads to obesity and the metabolic syndrome. Ann N Y Acad Sci. 2008 Dec;1148:232-7.

Kushi A, Sasai H, Koizumi H, Takeda N, Yokoyama M, Nakamura M. Therapeutic potential of neuropeptide Y (NPY) receptor ligands. Proc Natl Acad Sci U S A. 1998 Dec 22;95(26):15659-64.

Lawson EA, Eddy KT, Donoho D, Misra M, Miller KK, Meenaghan E, Lydecker J, Herzog D, Klibanski A. Appetite-regulating hormones cortisol and peptide YY are associated with disordered eating psychopathology, independent of body mass index. Eur J Endocrinol. 2011 Feb;164(2):253-61.

LeSauter J, Hoque N, Weintraub M, Pfaff DW, Silver R. Stomach ghrelin-secreting cells as food-entrainable circadian clocks. Proc Natl Acad Sci U S A. 2009 Aug 11;106(32):13582-7.

Lee NJ, Enriquez RF, Boey D, Lin S, Slack K, Baldock PA, Herzog H, Sainsbury A. Synergistic attenuation of obesity by Y2- and Y4-receptor double knockout in ob/ob mice. Nutrition. 2008 Sep;24(9):892-9.

Lesch KP, Selch S, Renner TJ, Jacob C, Nguyen TT, Hahn T, Romanos M, Walitza S, Shoichet S, Dempfle A, Heine M, Boreatti-Hümmer A, Romanos J, Gross-Lesch S, Zerlaut H, Wultsch T, Heinzel S, Fassnacht M, Fallgatter A, Allolio B, Schäfer H, Warnke A, Reif A, Ropers HH, Ullmann R. Genome-wide copy number variation analysis in attention-deficit/hyperactivity disorder:

association with neuropeptide Y gene dosage in an extended pedigree. Mol Psychiatry. 2010 Mar 23.

Lewis DY, Brett RR. Activity-based anorexia in C57/BL6 mice: effects of the phytocannabinoid, Delta9-tetrahydrocannabinol (THC) and the anandamide analogue, OMDM-2. Eur Neuropsychopharmacol. 2010 Sep;20(9):622-31.

Lewis DY, Brett RR. Activity-based anorexia in C57/BL6 mice: effects of the phytocannabinoid, Delta9-tetrahydrocannabinol (THC) and the anandamide analogue, OMDM-2. Eur Neuropsychopharmacol. 2010 Sep;20(9):622-31.

Lloyd B, Ravi P, Mendes N, Klibanski A, Misra M. Peptide YY levels across pubertal stages and associations with growth hormone. J Clin Endocrinol Metab. 2010 Jun;95(6):2957-62.

Luquet S, Perez FA, Hnasko TS, Palmiter RD. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. Science. 2005 Oct 28;310(5748):683-5.

Maejima Y, Sedbazar U, Suyama S, Kohno D, Onaka T, Takano E, Yoshida N, Koike M, Uchiyama Y, Fujiwara K, Yashiro T, Horvath TL, Dietrich MO, Tanaka S, Dezaki K, Oh-I S, Hashimoto K, Shimizu H, Nakata M, Mori M, Yada T. Nesfatin-1-regulated oxytocinergic signaling in the paraventricular nucleus causes anorexia through a leptin-independent melanocortin pathway. Cell Metab. 2009 Nov;10(5):355-65.

Maltais LJ, Lane PW, Beamer WG. Anorexia, a recessive mutation causing starvation in preweanling mice. J Hered. 1984 Nov-Dec;75(6):468-72.

Mantzoros C, Flier JS, Lesem MD, Brewerton TD, Jimerson DC. Cerebrospinal fluid leptin in anorexia nervosa: correlation with nutritional status and potential role in resistance to weight gain. J Clin Endocrinol Metab. 1997 Jun;82(6):1845-51.

Mashiko S, Moriya R, Ishihara A, Gomori A, Matsushita H, Egashira S, Iwaasa H, Takahashi T, Haga Y, Fukami T, Kanatani A. Synergistic interaction between neuropeptide Y1 and Y5 receptor pathways in regulation of energy homeostasis. Eur J Pharmacol. 2009 Aug 1;615(1-3):113-7.

McSherry JA. Was Mary, Queen of Scots, anorexic? Scott Med J. 1985 Oct;30(4):243-5.

Mercader JM, Lozano JJ, Sumoy L, Dierssen M, Visa J, Gratacòs M, Estivill X. High ambient temperature reverses hypothalamic MC4 receptor overexpression in an animal model of anorexia nervosa. Physiol Genomics. 2008 Nov 12;35(3):341-50.

Misra M, Klibanski A. Neuroendocrine consequences of anorexia nervosa in adolescents. Endocr Dev. 2010;17:197-214.

Misra M, Miller KK, Tsai P, Gallagher K, Lin A, Lee N, Herzog DB, Klibanski A. Elevated peptide YY levels in adolescent girls with anorexia nervosa. J Clin Endocrinol Metab. 2006 Mar;91(3):1027-33.

Monteleone P, Bifulco M, Di Filippo C, Gazzerro P, Canestrelli B, Monteleone F, Proto MC, Di Genio M, Grimaldi C, Maj M. Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. Genes Brain Behav. 2009 Oct;8(7):728-32.

Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V. Blood levels of the

endocannabinoid anandamide are increased in anorexia nervosa and in binge-eating disorder, but not in bulimia nervosa. Neuropsychopharmacology. 2005 Jun;30(6):1216-21.

Moriya J, Takimoto Y, Yoshiuchi K, Shimosawa T, Akabayashi A. Plasma agouti-related protein levels in women with anorexia nervosa. Psychoneuroendocrinology. 2006 Oct;31(9):1057-61.

Moriya R, Mashiko S, Ishihara A, Takahashi T, Murai T, Ito J, Mitobe Y, Oda Z, Iwaasa H, Takehiro F, Kanatani A. Comparison of independent and combined chronic anti-obese effects of NPY Y2 receptor agonist, PYY(3-36), and NPY Y5 receptor antagonist in diet-induced obese mice. Peptides. 2009 Jul;30(7):1318-22.

Morley JE, Farr SA, Sell RL, Hileman SM, Banks WA. Nitric oxide is a central component in neuropeptide regulation of appetite. Peptides. 2011 Apr;32(4):776-80.

Müller TD, Tschöp MH, Jarick I, Ehrlich S, Scherag S, Herpertz-Dahlmann B, Zipfel S, Herzog W, de Zwaan M, Burghardt R, Fleischhaker C, Klampfl K, Wewetzer C, Herpertz S, Zeeck A, Tagay S, Burgmer M, Pfluger PT, Scherag A, Hebebrand J, Hinney A. Genetic variation of the ghrelin activator gene ghrelin O-acyltransferase (GOAT) is associated with anorexia nervosa. J Psychiatr Res. 2010 Oct 28.

Nakahara K, Takata S, Ishii A, Nagao K, Bannai M, Takahashi M, Murakami N. Somatostatin is involved in anorexia in mice fed a valine-deficient diet. Amino Acids. 2011 Feb 4.

Naveilhan P, Hassani H, Canals JM, Ekstrand AJ, Larefalk A, Chhajlani V, Arenas E, Gedda K, Svensson L, Thoren P, Ernfors P. Normal feeding behavior, body weight and leptin response require the neuropeptide Y Y2 receptor. Nat Med. 1999 Oct;5(10):1188-93.

Nergårdh R, Ammar A, Brodin U, Bergström J, Scheurink A, Södersten P. Neuropeptide Y facilitates activity-based-anorexia. Psychoneuroendocrinology. 2007 Jun;32(5):493-502.

Olszewski PK, Schiöth HB, Levine AS. Ghrelin in the CNS: from hunger to a rewarding and memorable meal? Brain Res Rev. 2008 Jun;58(1):160-70.

Painsipp E, Herzog H, Holzer P. Implication of neuropeptide-Y Y2 receptors in the effects of immune stress on emotional, locomotor and social behavior of mice. Neuropharmacology. 2008 Jul;55(1):117-26.

Pardo M, Roca-Rivada A, Al-Massadi O, Seoane LM, Camiña JP, Casanueva FF. Peripheral leptin and ghrelin receptors are regulated in a tissue-specific manner in activity-based anorexia. Peptides. 2010 Oct;31(10):1912-9.

Pfluger PT, Kampe J, Castaneda TR, Vahl T, D'Alessio DA, Kruthaupt T, Benoit SC, Cuntz U, Rochlitz HJ, Moehlig M, Pfeiffer AF, Koebnick C, Weickert MO, Otto B, Spranger J, Tschöp MH. Effect of human body weight changes on circulating levels of peptide YY and peptide YY3-36. J Clin Endocrinol Metab. 2007 Feb;92(2):583-8.

Pirke KM, Broocks A, Wilckens T, Marquard R, Schweiger U. Starvation-induced hyperactivity in the rat: the role of endocrine and neurotransmitter changes. Neurosci Biobehav Rev. 1993 Fall;17(3):287-94.

Pralong FP, Gonzales C, Voirol MJ, Palmiter RD, Brunner HR, Gaillard RC, Seydoux J, Pedrazzini

T. The neuropeptide Y Y1 receptor regulates leptin-mediated control of energy homeostasis and reproductive functions. FASEB J. 2002 May;16(7):712-4.

Prince AC, Brooks SJ, Stahl D, Treasure J. Systematic review and meta-analysis of the baseline concentrations and physiologic responses of gut hormones to food in eating disorders. Am J Clin Nutr. 2009 Mar;89(3):755-65.

Pétervári E, Garami A, Soós S, Székely M, Balaskó M. Age-dependence of alpha-MSH-induced anorexia. Neuropeptides. 2010 Aug;44(4):315-22.

Rask-Andersen M, Olszewski PK, Levine AS, Schiöth HB. Molecular mechanisms underlying anorexia nervosa: focus on human gene association studies and systems controlling food intake. Brain Res Rev. 2010 Mar;62(2):147-64.

Rasmusson AM, Schnurr PP, Zukowska Z, Scioli E, Forman DE. Adaptation to extreme stress: post-traumatic stress disorder, neuropeptide Y and metabolic syndrome. Exp Biol Med (Maywood). 2010 Oct 1;235(10):1150-62.

Read NW, McFarlane A, Kinsman RI, Bates TE, Blackhall NW, Farrar GB, Hall JC, Moss G, Morris AP, O'Neill B, et al. Effect of infusion of nutrient solutions into the ileum on gastrointestinal transit and plasma levels of neurotensin and enteroglucagon. Gastroenterology. 1984 Feb;86(2):274-80.

Redrobe JP, Dumont Y, Herzog H, Quirion R. Characterization of neuropeptide Y, Y(2) receptor knockout mice in two animal models of learning and memory processing. J Mol Neurosci. 2004;22(3):159-66.

Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W. Thyroid hormones and their relation to weight status. Horm Res. 2008;70(1):51-7.

Renquist BJ, Lippert RN, Sebag JA, Ellacott KL, Cone RD. Physiological roles of the melanocortin MC(3) receptor. Eur J Pharmacol. 2011 Jan 3.

Riediger T, Cordani C, Potes CS, Lutz TA. Involvement of nitric oxide in lipopolysaccharide induced anorexia. Pharmacol Biochem Behav. 2010 Apr 27.

Riediger T, Giannini P, Erguven E, Lutz T. Nitric oxide directly inhibits ghrelin-activated neurons of the arcuate nucleus. Brain Res. 2006 Dec 13;1125(1):37-45.

Rikke BA, Yerg JE 3rd, Battaglia ME, Nagy TR, Allison DB, Johnson TE. Strain variation in the response of body temperature to dietary restriction. Mech Ageing Dev. 2003 May;124(5):663-78.

Rinaman L, Vollmer RR, Karam J, Phillips D, Li X, Amico JA. Dehydration anorexia is attenuated in oxytocin-deficient mice. Am J Physiol Regul Integr Comp Physiol. 2005 Jun;288(6):R1791-9.

Rothenhäusler Hb, Täschner KL. Kompendium Praktische Psychiatrie. 1.Auflage. Wien: Springer-Verlag; 2007.

Routtenberg A, Kuznesof AW. Self-starvation of rats living in activity wheels on a restricted feeding schedule. J Comp Physiol Psychol. 1967 Dec;64(3):414-21.

Sainsbury A, Bergen HT, Boey D, Bamming D, Cooney GJ, Lin S, Couzens M, Stroth N, Lee NJ, Lindner D, Singewald N, Karl T, Duffy L, Enriquez R, Slack K, Sperk G, Herzog H. Y2Y4 receptor double knockout protects against obesity due to a high-fat diet or Y1 receptor deficiency in mice.

Diabetes. 2006 Jan;55(1):19-26.

Sainsbury A, Schwarzer C, Couzens M, Fetissov S, Furtinger S, Jenkins A, Cox HM, Sperk G, Hökfelt T, Herzog H. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. Proc Natl Acad Sci U S A. 2002 Jun 25;99(13):8938-43.

Sato N, Kageyama S, Chen R, Suzuki M, Mori H, Tanioka F, Yamada H, Kamo T, Tao H, Shinmura K, Nozawa A, Sugimura H. Association between neuropeptide Y receptor 2 polymorphism and the smoking behavior of elderly Japanese. J Hum Genet. 2010 Sep 2.

Schwartz MW, Sipols AJ, Marks JL, Sanacora G, White JD, Scheurink A, Kahn SE, Baskin DG, Woods SC, Figlewicz DP, et al. Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology. 1992 Jun;130(6):3608-16.

Shimada M, Tritos NA, Lowell BB, Flier JS, Maratos-Flier E. Mice lacking melanin-concentrating hormone are hypophagic and lean. Nature. 1998 Dec 17;396(6712):670-4.

Shoblock JR, Welty N, Nepomuceno D, Lord B, Aluisio L, Fraser I, Motley ST, Sutton SW, Morton K, Galici R, Atack JR, Dvorak L, Swanson DM, Carruthers NI, Dvorak C, Lovenberg TW, Bonaventure P. In vitro and in vivo characterization of JNJ-31020028 (N-(4-{4-[2-(diethylamino)-2-oxo-1-phenylethyl]piperazin-1-yl}-3-fluorophenyl)-2-pyridin-3-ylbenzamide), a selective brain penetrant small molecule antagonist of the neuropeptide Y Y(2) receptor. Psychopharmacology (Berl). 2010 Feb;208(2):265-77.

Siegfried Z, Berry EM, Hao S, Avraham Y. Animal models in the investigation of anorexia. Physiol Behav. 2003 Jun;79(1):39-45.

Smith GP. Animal models of human eating disorders. Ann N Y Acad Sci. 1989;575:63-72; discussion 72-4.

Stanley BG, Leibowitz SF. Neuropeptide Y: stimulation of feeding and drinking by injection into the paraventricular nucleus. Life Sci. 1984 Dec 24;35(26):2635-42.

Støving RK, Andries A, Brixen K, Flyvbjerg A, Hørder K, Frystyk J. Leptin, ghrelin, and endocannabinoids: potential therapeutic targets in anorexia nervosa. J Psychiatr Res. 2009 Apr;43(7):671-9.

Szczypka MS, Rainey MA, Kim DS, Alaynick WA, Marck BT, Matsumoto AM, Palmiter RD. Feeding behavior in dopamine-deficient mice. Proc Natl Acad Sci U S A. 1999 Oct 12;96(21):12138-43.

Södersten P, Nergårdh R, Bergh C, Zandian M, Scheurink A. Behavioral neuroendocrinology and treatment of anorexia nervosa. Front Neuroendocrinol. 2008 Oct;29(4):445-62.

Tabarin A, Diz-Chaves Y, Consoli D, Monsaingeon M, Bale TL, Culler MD, Datta R, Drago F, Vale WW, Koob GF, Zorrilla EP, Contarino A. Role of the corticotropin-releasing factor receptor type 2 in the control of food intake in mice: a meal pattern analysis. Eur J Neurosci. 2007 Oct;26(8):2303-14.

Terashi M, Asakawa A, Harada T, Ushikai M, Coquerel Q, Sinno MH, Déchelotte P, Inui A, Fetissov SO. Ghrelin reactive autoantibodies in restrictive anorexia nervosa. Nutrition. 2011 Apr;27(4):407-13.

Thornton JE, Cheung CC, Clifton DK, Steiner RA. Regulation of hypothalamic proopiomelanocortin mRNA by leptin in ob/ob mice. Endocrinology. 1997 Nov;138(11):5063-6.

Thorsell A. Brain neuropeptide Y and corticotropin-releasing hormone in mediating stress and anxiety. Exp Biol Med (Maywood). 2010 Oct 1;235(10):1163-7.

Ueno H, Yamaguchi H, Mizuta M, Nakazato M. The role of PYY in feeding regulation. Regul Pept. 2008 Jan 10;145(1-3):12-6.

Utz AL, Lawson EA, Misra M, Mickley D, Gleysteen S, Herzog DB, Klibanski A, Miller KK. Peptide YY (PYY) levels and bone mineral density (BMD) in women with anorexia nervosa. Bone. 2008 Jul;43(1):135-9.

Vandereycken W, van Deth R. Who was the first to describe anorexia nervosa: Gull or Lasègue? Psychol Med. 1989 Nov;19(4):837-45.

van Leeuwen SD, Bonne OB, Avraham Y, Berry EM. Separation as a new animal model for self-induced weight loss. Physiol Behav. 1997 Jul;62(1):77-81.

Vannacci A, Ravaldi C, Giannini L, Rotella CM, Masini E, Faravelli C, Ricca V. Increased nitric oxide production in eating disorders. Neurosci Lett. 2006 May 22;399(3):230-3.

Verhagen LA, Egecioglu E, Luijendijk MC, Hillebrand JJ, Adan RA, Dickson SL. Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. Eur Neuropsychopharmacol. 2010 Jul 8.

Verhagen LA, Luijendijk MC, Adan RA. Leptin reduces hyperactivity in an animal model for anorexia nervosa via the ventral tegmental area. Eur Neuropsychopharmacol. 2011 Mar;21(3):274-81.

Verty AN, Evetts MJ, Crouch GJ, McGregor IS, Stefanidis A, Oldfield BJ. The Cannabinoid Receptor Agonist THC Attenuates Weight Loss in a Rodent Model of Activity-Based Anorexia. Neuropsychopharmacology. 2011 Mar 16.

Vignini A, Canibus P, Nanetti L, Montecchiani G, Faloia E, Cester AM, Boscaro M, Mazzanti L. Lipoproteins obtained from anorexia nervosa patients induce higher oxidative stress in U373MG astrocytes through nitric oxide production. Neuromolecular Med. 2008;10(1):17-23.

Watts AG, Salter DS, Neuner CM. Neural network interactions and ingestive behavior control during anorexia. Physiol Behav. 2007 Jul 24;91(4):389-96.

Watts AG, Sanchez-Watts G, Kelly AB. Distinct patterns of neuropeptide gene expression in the lateral hypothalamic area and arcuate nucleus are associated with dehydration-induced anorexia. J Neurosci. 1999 Jul 15;19(14):6111-21.

Watts AG. Dehydration-associated anorexia: development and rapid reversal. Physiol Behav. 1999 Jan 1-15;65(4-5):871-8.

Weyermann P, Dallmann R, Magyar J, Anklin C, Hufschmid M, Dubach-Powell J, Courdier-Fruh I, Henneböhle M, Nordhoff S, Mondadori C. Orally available selective melanocortin-4 receptor antagonists stimulate food intake and reduce cancer-induced cachexia in mice. PLoS One. 2009;4(3):e4774.

Winer, B. J. (1971). Statistical principles in experimental design (2nd. ed.). New York: McGraw-Hill.

Wu Q, Palmiter RD. GABAergic signaling by AgRP neurons prevents anorexia via a melanocortin-independent mechanism. Eur J Pharmacol. 2011 Jan 3.

Xu Y, Jones JE, Lauzon DA, Anderson JG, Balthasar N, Heisler LK, Zinn AR, Lowell BB, Elmquist

JK. A serotonin and melanocortin circuit mediates D-fenfluramine anorexia. J Neurosci. 2010 Nov 3;30(44):14630-4.

Yamada M, Miyakawa T, Duttaroy A, Yamanaka A, Moriguchi T, Makita R, Ogawa M, Chou CJ, Xia B, Crawley JN, Felder CC, Deng CX, Wess J.Mice lacking the M3 muscarinic acetylcholine receptor are hypophagic and lean. Nature. 2001 Mar 8;410(6825):207-12.

Zhang L, Riepler SJ, Turner N, Enriquez RF, Lee IC, Baldock PA, Herzog H, Sainsbury A. Y2 and Y4 receptor signaling synergistically act on energy expenditure and physical activity. Am J Physiol Regul Integr Comp Physiol. 2010 Dec;299(6):R1618-28.