The role of leptin within the substantia nigra and lateral hypothalamus in suppression of hyperactivity in activity-based anorexia model.

Student: Tamara Recourt 6458160
Supervisor: Prof. Roger Adan & Nick Papavoine
Department: UMC Utrecht, Translational Neuroscience, Adan lab
Period internship: 26-02-24 till 17-05-24

Abstract

Anorexia nervosa (AN) is an eating disorder characterized by a pursuit for thinness and compulsive thoughts about nutrition, body shape, and weight. Hyperactivity has also been recognized as a key symptom associated with AN, impacting chronicity and complications. Leptin, a hormone produced by white adipocytes, plays a role in regulating appetite and energy homeostasis. Leptin has emerged as a potential therapeutic target for reducing hyperactivity in AN, as leptin has showed to reduce activity levels in animal models for AN. This study aimed to investigate the role of leptin within the substantia nigra (SN) and lateral hypothalamus (LH) in suppressing hyperactivity without impacting food intake using the activity based anorexia model (ABA-model). Limited research time permitted the assessment of only the lateral hypothalamus. The findings indicate that leptin infusions in the LH did not significantly reduce hyperactivity or food intake during the ABA-model, challenging the hypothesized outcomes. The absence of explicit exclusion criteria and histological validation for infusion sites may have influenced the results. Despite the non-significant findings, this study contributes to understanding leptin's role in AN. Previous clinical case studies suggest potential beneficial effects of leptin on reducing inner restlessness, anxiety and depressed mood in AN. These findings underscore leptin's broader impact beyond modulating physical symptoms, highlighting its potential as a therapeutic intervention for addressing both physical and psychological aspects of AN. Further clinical research is warranted to elucidate leptin's effect in AN patients and to develop targeted treatment strategies.

Introduction

Anorexia nervosa (AN) is a severe psychiatric disorder often chronical and with high mortality rates between 5-10%. (1-5) AN is more common among young females then males with a lifetime prevalence up to 4% in women. (1,3) Characteristics for AN are a pursuit for thinness and compulsive thoughts about nutrition, body shape and weight, captured by the DSM-5 (diagnostic and statistical manual of mental disorders). (1) There are two types of AN distinguished, the restrictive type and the binge-eating/purging type. (1,6) Both types have been associated with hyperactivity, which has been recognized as a key symptom in AN, although not included in the DSM-5. (7,8) The prevalence of hyperactivity in AN patients is estimated between 30-80%. (9-11) Hyperactivity has been associated with chronicity, longer hospitalizations, and complications as risk of overuse injuries, bone fractures and cardiovascular complications. (8,12,13)There are challenges in treating individuals with AN, as both psychotherapeutic and pharmacological interventions have demonstrated limited effectiveness. Additionally, there is a lack of treatment modalities specifically designed to address hyperactivity in AN. This indicates a considerable need for improvement and further research. (14)

A possible new pharmacological intervention in reducing hyperactivity among individuals with AN could involve the utilization of leptin, a multifunctional hormone produced by (white) adipocytes. (15,16) There are hypotheses suggesting that leptin may exert different effects depending on the individual's energy status, such as during starvation observed in AN. (25) The physiological effects of leptin, mediated via the arcuate nucleus, are a reduction in appetite and an increasement of thermogenesis, resulting in decreased food consumption and heightened energy expenditure (15,17-19). Patients with AN seem to have extremely low

circulating leptin levels, which has been correlated with heightened levels of activity. (23,24) Therefore the use of leptin could be a novel therapeutic approach which may present a promising avenue for addressing hyperactivity in clinical contexts.

A Specific model to assess hyperactivity in rodents is the ABA-model (activity-based anorexia model). In the ABA-model access to a running wheel combined with restricted food availability, leads to both weight loss and hyperactivity, which is seen as an anorexia phenotype. (28) Pre-clinical tests in rodents including leptin showed that there is a possible connection between hypoleptinemia and hyperactivity making use of the ABA-model. (11,26) Subsequent observations revealed that hyperactivity induced by the ABA-model can be reduced through the administration of exogenous leptin. (9,26,27) In addition, clinically leptin has been used as off label treatment in a small number of AN patients yielding a promising outcome in a study conducted by Milos *et al.* and Gradl-Dietsch *et al..* The results indicated improvement in activity levels, reduction in repetitive thoughts of food and inner restlessness, mitigation of weight phobia and less depression symptoms. Notably, no severe side effects were observed during the course of treatment. (29) Albeit that first results regarding the use of leptin and hyperactivity are positive, it should be kept in mind that administration of leptin can also have negative effects. Most frequent side effects of leptin are development of antibodies against leptin, headache, nausea, hypoglycaemia, decreased weight and increased risk of infections. (30,31) In addition, physiologically leptin reduces appetite and increases thermogenesis, resulting in a negative energy balance. (19,32) Clearly, such effects of leptin in AN patients are deemed undesirable. Therefore, it is crucial to identify underlying neuronal circuits affected by leptin that influence (only) hyperactivity in AN patients, without its negative side effects.

Literature shows that the mesolimbic dopaminergic system may play a role in the interaction between leptin and hyperactivity, while this system is also disturbed in AN patients, this could be a possible therapeutic target. (20,21) Previous research has focussed on the ventral tegmental area (VTA), a component of the mesolimbic midbrain, to explore the impact of leptin on reducing hyperactivity in the VTA. Verhagen *et al.* demonstrated that leptin infusion into the VTA leads to a reduction in running wheel activity in the ABA-model. (25) A theory could be that leptin might attenuate the rewarding effects associated with hyperactivity via the mesolimbic dopamine (DA) system. (25,33) Exploring the impact of leptin on hyperactivity in alternative brain regions, such as the substantia nigra and lateral hypothalamus presents an avenue for research that may offer insights into potential therapeutic interventions devoid of the negative side. Omrani *et al.* concludes that leptin indirectly targets the mesolimbic DA system at various levels. The route Omrani *et al.* describes is that the lateral hypothalamus (LH) exerts inhibitory effects on VTA GABAergic neurons, resulting in reduced activity of DA neurons in the VTA, which then project to the nucleus accumbens (NAc) also decreasing DA activity. (33) In addition, in a study with mice, De Vrind *et al.* demonstrated that chemogenetic activation of LepR expressing neurons in the SN led to a decrease in locomotor activity, without impacting feeding. (20) Therefore, the aim of this study is to investigate where in the brain leptin reduces hyperactivity during the ABA-model without reducing food intake targeting the SN and LH. As previous literature did not target these specific brain areas in the ABA-model. We hypothesize that leptin reduces hyperactivity in the LH and SN, without concomitantly reducing food intake. To test our hypothesis we will equip female rats with guide cannulas aiming at either the SN or LH. After,

recovery animals are exposed to the ABA model, in which they will develop hyperactivity. On day 4 of the ABA model, the animals will be infused with either leptin (300 ng) or saline bilaterally in a between subject design. Food intake as well as the activity (revolutions made in the running wheel) are recorded and analysed. The running wheel data and food intake will be statistically analysed to evaluate the effect of leptin. This research will contribute to a deeper understanding of the etiology of AN and may provide novel insights that are pivotal for advancing medical interventions.

Materials & methods

For this experiment, rats were enrolled in an interventional cohort study. All experimental procedures were conducted with the approval of both the Animal Experimentation Committee and the Animal Ethics Committee of Utrecht University, in strict adherence to Dutch law (Herziene wet op Dierproeven, Art 10.a2, 2014) and European regulations (Guideline 2010/63/EU).

Animals

For this report two similar experiments were performed in two different brain areas, namely the lateral hypothalamus and the substantia nigra. For both brain areas data were collected from two cohorts of wildtype female Wistar rats (n=24) with an average weight of 174,6g (± 1,2g) in the LH cohort and average weight of 175,8g (± 1,4g) in SN cohort at time of surgery. Upon arrival, all the animals were individually housed in type II cages (365x207x140mm, 530 cm^2, Tecniplast, Italy) where they were weighed, handled daily and maintained under a standardized 12-hour day/night cycle, with lights on at 1am, the temperature set at 23 degrees Celsius and humidity controlled between 60-70%. Prior to commencing the experiment, food (3,1 Kcal/g, Standard Diet Service, UK) and water were provided *ad libitum* within the home-cage environment. In adherence to ethical guidelines, humane endpoints necessitating exclusion from the experiment included a total body weight loss exceeding 20% after food intake.

Surgery

To investigate where in the brain leptin reduces hyperactivity without reducing food intake targeting the SN and LH, we administered leptin directly in specific brain areas via cannula's, which had to be surgically placed under anaesthesia ketamine/dexdomitor 0,2ml/100gr i.p. (ketamine 75mg/kg, Narketan, Vetoquinol BV and dexmedetomidine 0.5mg/kg, Dexdomitor, Orion Phrama BV). After induction the animals head was shaved and pain medication carprofen 0,1ml/100gr s.c. (5mg/kg, Rimadyl, Zoetis BV) and a local injection on the skull of lidocaine (max. 7mg/kg, AstraZenica BV) was administered. Eye ointment (20mg chlooramfenicol, 15000IE retinopalmitaat/gr CAG, CEVA Sante Animale BW) was applied. Following the animal's head was prepared for surgery, and it was securely positioned within a sterotactic frame (Kopf Instruments, USA) equipped with oxygen support. A minor incision (1.5cm) was carefully made along the midline of the skull to expose the cranial landmarks, namely Bregma and Lambda and fosforic acid (Astringedent, Ultradent Products GmbH) was applied. Subsequently, the cannulas where inserted in the lateral hypothalamus or substantia nigra. The following coordinates where used for the lateral hypothalamus AP -2.6, ML +2.5 and DV -8.2 angle <5° and for the substantia nigra AP -5.30, ML +2.1 and DV -7.0 angle 0°,

according to the landmarks. Following the insertion of the cannulas, four anchor screws were meticulously inserted to ensure stability. After inserting the screws and the cannula the whole was glued together with dental cement (GC Fuji PLUS, GC Corporation BV). After the surgical procedure, the animals were administered saline s.c. and atipamazole 0,1ml/100gr s.c. (2.5mg/kg, Alzane, Syya BV), and carefully placed on a heating plate set to 40 degrees Celsius until they regained consciousness and started moving. Postoperatively, the animals received pain medication (Carprofen, 0.05mg/mL, Rimadyl, Zoetis BV) via their water bottles for a duration of five days. To facilitate recovery from surgery, the animals were granted a seven-day period to recuperate and acclimate before the commencement of the experiment. During this recovery period, they were provided with *ad libitum* access to food and water and body weight was measured daily.

Experimental set-up: ABA-model

To investigate our hypothesis, animals were exposed to the ABA-model. In this model, animals experience restricted access to food, leading to induced hyperactivity, consequently resulting in a negative energy balance and subsequent weight reduction. This combination of reduced food intake, hyperactivity and weight loss is seen as an Anorexia Nervosa phenotype. (28) Previous studies confirm these characteristics using the ABA model. (34)

An ABA-day is partitioned into four phases; the light phase, during which animals are inactive (i); the food anticipatory phase, occurring 2 hours prior to food access, during which activity strongly increases (ii); the food access phase, commencing in the dark phase and lasting 1.5 hours (iii), and finally, the dark phase, during which the animals exhibit activity (iiii) **(figure 1)**.

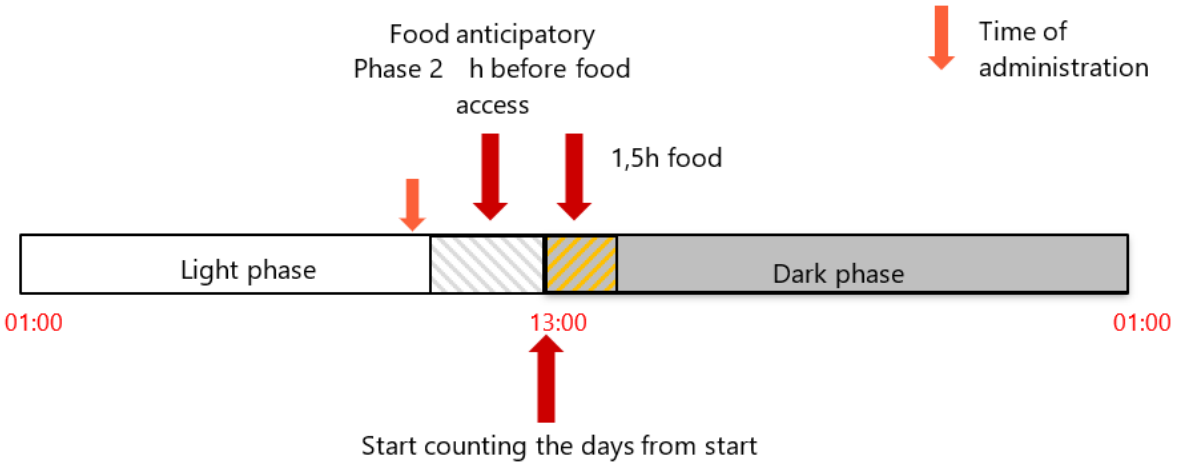

Figure 1. *Overview of an ABA day with different phases: light phase (i); food anticipatory phase (ii); food access phase (iii); dark phase (iiii). Time of administration is indicated with an orange arrow.*

Before the actual start of ABA the animals were exposed to the running wheel for 13 days for training. Also, they were given a test infusion with saline through their cannulas to get familiar at 14 days prior to the start of ABA. Following, 7 days before ABA the animals get the first leptin (1ug/300nl injection) or control (saline, 0,3ul injection) bilateral infusions, during *ad libitum* food access, thus positive energy balance. At day 0 the ABA-model starts and has a duration of 6 days were the animals developed hyperactivity in the food anticipatory phase. Following the onset of hyperactivity on ABA-day 4, the animals were infused with either leptin (leptin concentration 1ug/3nl injection volume, LH;n=13, SN;n=13) or control

(saline, 3nl injection volume, LH;n=9 SN;n=10) directly into the LH or SN before the commencement of the food anticipatory phase on ABA day 4.

At the end of ABA day 6 the animals were sacrificed CO_2 euthanasia. Directly after sacrificing the animals, 0,3ul ink was injected through the cannula and the brains were stored in a freezer with a temperature of -80 degrees Celsius to preserve the brains for microscopy, which has not been included in this article due to time limitation.

Data collection & analysis

To investigate where in the brain leptin reduces hyperactivity without reducing food intake in the SN and LH, the food intake and running wheel activity were measured. The running wheel activity (RWA) was continuously monitored using a Cage Registration Program from the Department Biomedical Engineering, UMC Utrecht, The Netherlands. Food intake was manually measured before and after food access. To establish control groups, the animals were stratified into experimental cohorts using Randomice, ensuring matches based on body weight, food intake, baseline running wheel activity, room and location in the room. Baseline RWA was defined as the average RWA recorded over the four days preceding the initiation of infusion. Given the variation in activity levels and food intake among rats, the impact of the infusions on running wheel activity and food intake is expressed as a fraction of the corresponding values recorded on the day before infusion, presented on an individual rat basis. The results were subjected to statistical analysis, focusing on means and standard errors. Date were processed utilizing Excel (2021) for Windows. Differences were assessed using an independent t-test, with statistical significance established at $p<0.05$.

Results

Due to time limitations the substantia nigra data has not been included in this report. Therefore, we only show the results regarding the lateral hypothalamus.

Characteristics animals

In the LH cohort 3 animals of the n=24 died during or after surgery. Experimental groups were established looking at total body weight, food intake, and running wheel activity before the start of ABA during *ad libitum* food access. Table 1 shows an overview of baseline characteristics of the animals during the *ad libitum* access to food before the start of ABA. The total body weight was not significantly different (T=8, p=0,98) between experimental groups (control, 189.7 ± 7.1g; leptin 189.6 ± 5.5g), baseline food intake was not significantly different (T=8, P=0,96) between experimental groups (control, 14.5 ± 1.6g; leptin 14.5 ± 1.5g) and the running wheel activity also did not show a significant difference (T=8, p=0,94) between groups (control, 2007.1 ± 822.5g; leptin 2034.4 ± 876.8g).

Table 1. Baseline characteristics: ad libitum food access pre-ABA

	Lateral hypothalamus	
	Leptin (n=13)	***Controle (saline; n=9)***
Total body weight (day -1 ABA)	189.6 ± 5.5	189.7 ± 7.1
Food intake (mean day -4 till -1 ABA)	14.5 ± 1.5	14.5 ± 1.6
Total RWA (mean day -3 till -1 ABA)	2034.4 ± 876.8	2007.1 ± 822.5

Leptin in lateral hypothalamus does not effect food intake

To see the effect of leptin on food intake, the food intake was measured during *ad libitum* (positive energy balance) food access after 1 and 3 hours 7 days prior to the start of the ABA-model **(Figure 1A)** and during the ABA-model in a negative energy balance **(Figure 1B)**. There was no significant difference in food intake after 1h (control 2.1g ± 1.0, leptin 2.7g ± 0.9, T=8, p=0.17) and 3h (control 5.3g ± 1.6, leptin 5.2g ± 2.4, T=8, p=0.87) between the control and leptin group. For the negative energy balance over the course of the ABA-model leptin was administered at the end of day 4 **(figure 1B)**. To establish differences between groups and food intake before and after leptin infusion the fraction of day 5 compared to day 4 was calculated. The mean fraction for food intake was not significantly between groups (T= 8, p=0.54) with a mean fraction in the control group of 0.98 ± 0.26 and in the leptin group of 1.1 ± 0.34 **(figure 1D)**. **Figure 1C** displays the mean intake per individual rat before and after leptin infusion.

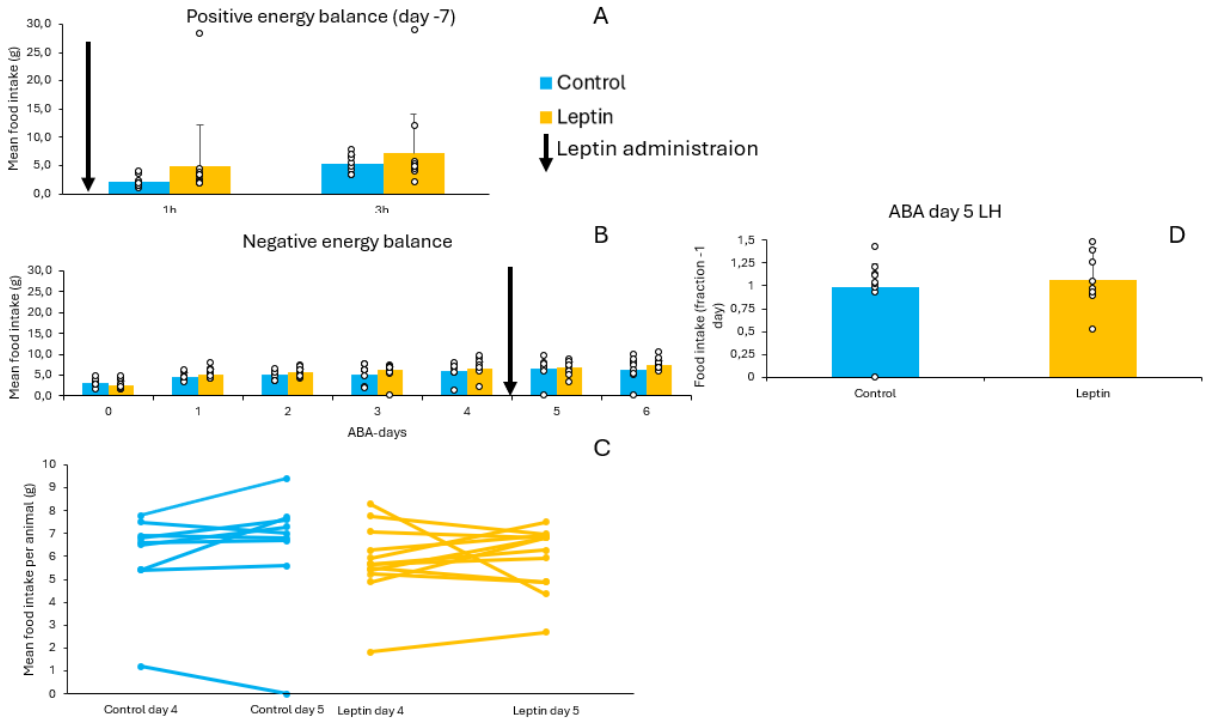

Figure 1. Overview food intake during positive and negative energy balance. Leptin (orange) infusion did not significantly (3h, p=0.87) affect food intake measured after 1h and 3h during a positive energy balance control (blue). **(1A)** The food intake during a negative energy balance and leptin administration at day 4 **(1B)** was displayed as fractions **(1D)** with no significant differences between control and leptin group (p=0.54). No trend was seen amongst the individual rats displayed **(1C)**.

Leptin in lateral hypothalamus does not suppress hyperactivity

To see the effect of running wheel activity, the running wheel activity was measured during *ad libitum* (positive energy balance) 7 days prior to the start of the ABA-model (**Figure 2A)** and during the ABA-model in a negative energy balance **(Figure 2B)**. There was no significant difference in running wheel activity (measured as running wheel revolutions) during the positive energy balance between groups (control 14 ± 3; leptin 15 ± 5, T=8, p= 0.19). For the negative energy balance over the course of the ABA-model the animals start to develop

hyperactivity during the food anticipatory phase **(figure 2B)**. To establish differences between groups, a bar graph **(2C)** shows the mean revolutions during the food anticipatory activity (FAA). The FAA after leptin infusion is not significantly different between groups (T=8, p=0.85). To have less influence of the variety among the rats the fractions are displayed in **figure 2C**. The mean fraction for running wheel revolutions was not significantly between groups (T=8, p=0.72) with a mean fraction in the control group of 1.19 ± 1.20 and in the leptin group of 1.03 ± 0.77. **Figure 1E** displays the mean revolutions per individual rat before and after leptin infusion during the negative energy balance.

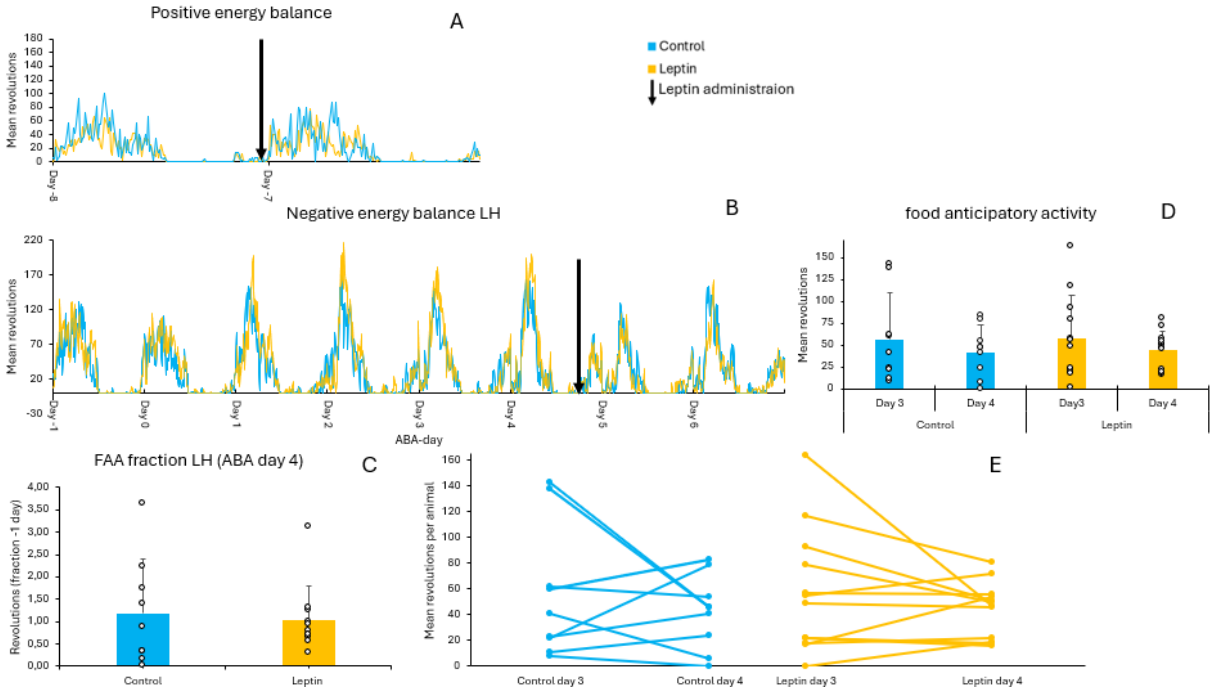

Figure 2. Overview running wheel activity during positive and negative energy balance. Leptin infusion during positive energy balance did not significantly (p=0.19) influence running wheel revolutions **(2A).** The running wheel activity during a negative energy balance and leptin administration at day 4 **(2B)** was displayed as bar graphs during the food anticipatory phase **(2D)** and as fractions **(2C)** with no significant differences between control and leptin group (p=0.85 and p=0.72). No trend was seen amongst the individual rats displayed **(2E)**.

Discussion

In the present study, we aimed to investigate the role of leptin within the substantia nigra and lateral hypothalamus in suppressing hyperactivity in the ABA-model. However, due to limited research time, only the results regarding the lateral hypothalamus have been assessed. Our findings indicate that leptin infusions in the lateral hypothalamus did not result in a significant reduction in hyperactivity or food intake during the ABA-model. This does not correspond with our hypothesis. While the observed effects did not reach statistical significance, it is important to note that the study lacked explicit exclusion criteria, potentially affecting result interpretation. Furthermore, the absence of histological validation for infusion sites raises concerns regarding the precision of targeting specific brain areas.

This study implemented a schedule consisting of 1.5h of food access per day, coupled with continuous access to a running wheel to induce negative energy balance and promote the development of hyperactivity over the duration of the ABA-model. This experimental design aligns with previous research demonstrating that restricted feeding combined with access to a running wheel reliably induces hyperactivity in animal models, mimicking key features observed in individuals with anorexia nervosa. Previous studies have demonstrated that subcutaneous leptin injections or chronic leptin treatment via an intracerebroventricular cannula can reduce hyperactivity during the ABA-model. (27,35) Additionally, direct leptin infusions in the VTA showed a dose-dependent reduction in hyperactivity. (25) Despite previous studies demonstrating such effects in other brain regions, our observations showed no effects of leptin on hyperactivity in the lateral hypothalamus. Firstly, the differential response observed could suggest region-specific roles of leptin signalling within the brain. The lateral hypothalamus is known to play a critical role in regulating feeding behaviour, but its specific involvement in modulating hyperactivity may be more complex then previously assumed. This could imply the existence of distinct neural circuits or mechanisms governing hyperactivity, which may not be directly influenced by leptin signalling in the lateral hypothalamus. This highlights the complexity of neural circuits underlying hyperactivity and underscores the need for comprehensive investigations into the multifaceted mechanisms involved. Expanding the scope of research to explore interactions between leptin signalling in the lateral hypothalamus and other neurochemical systems implicated in hyperactivity could provide valuable insights. Investigating potential crosstalk between leptin and neurotransmitters within the lateral hypothalamus may unveil novel therapeutic targets for addressing hyperactivity in conditions like AN.

However, while hyperactivity is often observed in individuals with AN and is considered a key symptom, it is not included as a diagnostic criterion in the DSM-5. (8,13,36) This omission underscores the complexity of assessing and understanding hyperactivity within the disorder. Moreover, the ABA-model, which primarily mimics patients with hyperactivity, does not encompass those without it, potentially limiting its generalizability to all presentations of AN. Another critical consideration is the multifaceted nature of hyperactivity, which encompasses both physical and mental aspects. (9,11,23) While the ABA-model focusses on physical hyperactivity, it may overlook the mental dimension such as inner restlessness, which patients may also perceive as hyperactivity. (29) This discrepancy underscores the importance of adopting the comprehensive approach to assessing and interpreting hyperactivity in both research and clinical settings.

Clinical case studies have provided insights into the potential therapeutic benefits of leptin administration for individuals with AN, demonstrating its capacity to alleviate not only physical symptoms but also psychological distress. Specifically, these studies have highlighted the potential of leptin to reduce inner restlessness, anxiety, and depressive symptoms in AN patients. (29) Such findings underscore the multifaceted impact of leptin beyond its potential role in decreasing hyperactivity, suggesting its potential as a comprehensive therapeutic intervention capable of addressing both the physiological and psychological dimensions of AN. The observed improvements in mood and anxiety levels following leptin administration hold significant implications for the overall treatment approach in AN. By targeting not only the physical symptoms but also the emotional distress commonly experienced by AN patients, leptin therapy may offer a novel treatment strategy that addresses the complex

interlay between biological, psychological and behavioural factors underlying the disorder. Importantly, these beneficial effects through leptin intervention may contribute to enhancing patient cooperation and engagement in treatment regimes. Enhanced collaboration and adherence to treatment protocols are crucial factors in achieving favourable outcomes in AN management. Greater patient collaboration, facilitated by improvements in mood and anxiety, can enhance treatment adherence, optimize nutritional rehabilitation, and promote psychological interventions aimed at addressing maladaptive eating behaviours and distorted body image perceptions. Despite the promising insights offered by clinical case studies, it is essential to acknowledge the preliminary nature of these findings and the need for further empirical research to corroborate and expand upon them. Rigorous clinical investigations are warranted to elucidate the full spectrum of leptin's therapeutic effects in AN. Moreover, comprehensive assessments encompassing a range of outcome measures, including physical, psychological, and behavioural parameters, are necessary to delineate the precise mechanisms underlying leptin's therapeutic efficacy in AN. By advancing our understanding of leptin's therapeutic potential and its impact on various dimensions of AN pathology, we can inform the development of more targeted and effective treatment strategies tailored to the diverse needs of affected individuals. Integrating leptin therapy into comprehensive treatment regimens may present a promising avenue for improving clinical outcomes and enhancing the overall well-being of individuals with AN and its challenges.

In conclusion, this study reveals that direct infusion of leptin into the lateral hypothalamus of animals subjected to the ABA-model did not yield a significant reduction in either running wheel activity or food intake. Given the intricate role of leptin across various brain regions, further investigation into alternative neuronal circuits is warranted to elucidate its impact on hyperactivity without adverse consequences. This underscores the need for continued research to explore leptin's multifaceted effects within different brain areas and its potential for reducing hyperactivity while minimizing negative side effects.

References

(1) Hengeveld M.W., van Balkom A.J.L.M, van Heeringen C., Sabbe B.G.C. Leerboek psychiatrie. Third ed. Utrecht: De Tijdstroom; 2016.

(2) Hay P, Girosi F, Mond J. Prevalence and sociodemographic correlates of DSM-5 eating disorders in the Australian population. J Eat Disord 2015 -04-25;3(1).

(3) van Eeden Annelies, van Hoeken Daphne, Hoek Hans. Incidence, prevalence and mortality of anorexia nervosa and bulimia nervosa. Curr Opin Psychiatry 2021 Nov;6(34):515-524.

(4) Sullivan PF. Mortality in anorexia nervosa. The American journal of psychiatry 1995 July;152(7):1073-1074.

(5) Arcelus, Mitchell Alex, Wales Jackie, et al.=l. Mortality rates in patients with anorexia nervosa and other eating disorders. A meta-analysis of 36 studies. Archives of general psychiatry 2011 July;68(7):724-731.

(6) Kaye Walter FJ, Paulus Martin. New insights into symptoms and neurocircuit function of anorexia nervosa. Nature reviews neuroscience 2009 July:573-584.

(7) Gull W. Anorexia nervosa (apepsia hysterica, anorexia hysterica). Obesity research 1997 September;5(5):498-502.

(8) Dalle Grave Riccardo, Calugi Simona, Marchesini Giulio. Compulsive exercise to control shape or weight in eating disorders: prevalence, associated features and treatment outcome. Comprehensive psychiatry 2008 July;49(4).

(9) Hebebrand Johannes, Milos Gabriella, Wabitsch Martin, et all. Clinical trials required to assess potential benefits and side effects of treatment of patients with anorexia nervosa with recombinant human leptin. Frontiers in psychology 2019 May.

(10) Alberti Marta, Galvani Christel, Choch El, et all. Assessment of physical activity in anorexia nervosa and treatment outcome. Medicine & science in sports & exercise 2013 September;45(9):1643-1648.

(11) Hebebrand J, Exner C, Hebebrand K, et all. Hyperactivity in patients with anorexia nervosa and in semistarved rats: evidence for a pivotal role of hypoleptinemia. Physiology & behavior 2003 June;79(1):25-37.

(12) Achamrah N, Coëffier M, Déchelotte P. Physical activity in patients with anorexia nervosa. Nutr Rev 2024 -03-11;74(5):301.

(13) Solenberger Susan. Exercise and eating disorders: a 3-year inpatient hospital record analysis. Eating behaviours 2001;2(2):151-168.

(14) Hebebrand Johannes AJ, Herpetz-Dahlmann Beate. New Oxford Textbook of psychiatry . third ed. Oxford: Oxford University Press; 2020.

(15) Zhang Y SC. Leptin function and regulation. Comprehensive physiology 2918 January;8(1):351-369.

(16) Feldman Mark, Friedman Lawrence S, Brandt Lawrence J. Sleisenger and Fordtran's gastrointestinal and liver disease. eleventh ed.: Elsevier; 2020.

(17) Hall Julianne M, Koeppen Bruce M, Swiatecke-Urban A. Stanton Bruce A. Berne & Levy Physiology. 8th ed.: Elsevier.

(18) Kumar Vinay, Abbas Abul K, Aster Jon C, Deyrup Andrea T. Robbins & Kumar Basic Pathology. eleventh ed.: Elsevier; 2022.

(19) Boron Walter F BEL. Medical physiology. Third ed.: Elsevier; 2016.

(20) De Vrind VAJ, Van ‘t Sant LJ, Rozeboom A, Luijendijk-Berg MCM, Omrani A, Adan RAH. Leptin Receptor Expressing Neurons in the Substantia Nigra Regulate Locomotion, and in The Ventral Tegmental Area Motivation and Feeding. Front Endocrinol 2021 -07-01;12.

(21) O'Hara, CB, Campbell IC, Schmidt U. A reward-centred model of anorexia nervosa: a focussed narrative review of the neurological and psychophysiological literature. Neuroscience and biobehavioural reviews 2015 May;52(131).

(22) Hebebrand J, van der Heyden J, Devos R, Kopp W, et al. Plasma concentrations of obese protein in anorexia nervosa. Lancet 1995:1624-1625.

(23) Holtkamp Kristian, Herpertz-Dahlmann Beate, Hebebrand Kathrin, et al. Physical activity and restlessness correlate with leptin levels in patients with adolescent anorexia nervosa . 2006 August;60(3):311-313.

(24) Holtkamp K, Herpertz-Dahlmann B, Mika C, Heer M, Heussen N, Fichter M, et al. Elevated Physical Activity and Low Leptin Levels Co-occur in Patients with Anorexia Nervosa. The Journal of Clinical Endocrinology & Metabolism 2003 -11-01;88(11):5169.

(25) Verhagen LAW, Luijendijk MCM, Adan RAH. Leptin reduces hyperactivity in an animal model for anorexia nervosa via the ventral tegmental area. European Neuropsychopharmacology 2011 -03;21(3):274.

(26) Hebebrand J, Muller TD, Holtkamp K, Herpetz-Dahlmann B. The role of leptin in anorexia nervosa: clinical implications. 2007 January:23-35.

(27) Exner C, Hebebrand J, Hemschmidt H, Wewetzer C, et al. Leptin suppresses semi-starvation induced hyperactivity in rats: implications for anorexia nervosa. 2000 September;5(5).

(28) Schalla Martha SA. Activity based Anorexia as an animal model for Anorexia Nervosa - A systematic review. 2019 May;6(69).

(29) Milos G, Antel J, Kaufmann L, Barth N, Koller A, Tan S, et al. Short-term metreleptin treatment of patients with anorexia nervosa: rapid on-set of beneficial cognitive, emotional, and behavioral effects. Transl Psychiatry 2020 -08-27;10(1).

(30) Ajluni Nevin, Dar Moahad, Xu John et al. Efficacy and safety of metreleptin in patients with partial lipodystrophy lessons from an expanded access program. 2016 March;7(3).

(31) Brown Rebecca, Oral Elif, Cochran Elaine, et al. Long-term effectiveness and safety of metreleptin in the treatment of patients with generalized lipodystrophy. 2018 June;60(3):479-489.

(32) Roger, Adan A.H., Kaye, Walter H. Neurobiology driving hyperactivity in Activity-based anorexia. 2011;6:229-250.

(33) Omrani A, De Vrind VAJ, Lodder B, Stoltenborg I, Kooij K, Wolterink-Donselaar IG, et al. Identification of Novel Neurocircuitry Through Which Leptin Targets Multiple Inputs to the Dopamine System to Reduce Food Reward Seeking. Biological Psychiatry 2021 -12-15;90(12):843.

(34) de Rijke CE, Hillebrand JJG, Verhagen LAW, Roeling TAP, Adan RAH. Hypothalamic neuropeptide expression following chronic food restriction in sedentary and wheel-running rats. 2005 10/;35(2):381-390.

(35) Hillebrand JJG, Kas MJH, van Elburg AA, Hoek HW, Adan RAH. Leptin's effect on hyperactivity: potential downstream effector mechanisms. 2008 August;94(5):689-695.

(36) American Psychiatric association. Diagnostic and statistical manual of mentol disorders . 5th edition ed.; 2013.

Abbreviations

AN = Anorexia Nervosa
DSM-5 = Diagnostic and statistical manual of mental disorders
ABA-model = Activity-Based anorexia model
LepR = leptin receptor
VTA = ventral tegmental area
DA = dopamine
LH = lateral hypothalamus
NAc = nucleus accumbens
SN = substantia nigra
RWA = running wheel activity
FAA = food anticipatory activity