**The Effect of Serum BDNF Levels on Central Serotonin Transporter
Availability in Obese Versus Non-Obese Adults:
A [¹¹C]DASB Positron Emission Tomography Study**

Dissertation
zur Erlangung des akademischen Grades
Dr. med.

an der Medizinischen Fakultät
der Universität Leipzig

eingereicht von:

Philipp Hinderberger, B.Sc.

geboren am 8. September 1985
in Bad Säckingen

angefertigt an der:

Klinik und Poliklinik für Nuklearmedizin
Universität Leipzig

Betreuer:

Prof. Dr. med. Swen Hesse

Beschluss über die Verleihung des Doktorgrades vom:

22.11.2016

Table of Contents

Bibliographic Summary	3
Introduction.....	4
Obesity: The Not-So-New Epidemic	4
Regulation of Energy Homeostasis.....	5
Reward Systems and Food Intake.....	7
Neurobiology of Feeding.....	9
Serotonin and the Regulation of Energy Balance	10
Hypothalamic Melanocortin Pathways	12
Reciprocal Interaction between Synaptic Activity and BDNF.....	14
Significance of Genetic Polymorphisms.....	15
Study Hypothesis	16
Publication	17
Summary	24
Works Cited	26
Appendices.....	I
Glossary.....	I
Curriculum Vitae	II
Publications.....	III
Statement on Academic Integrity	IV
Acknowledgments.....	V

Bibliographic Summary

Hinderberger, Philipp

The Effect of Serum BDNF Levels on Central Serotonin Transporter Availability in Obese Versus Non-Obese Adults: A [^{11}C]DASB Positron Emission Tomography Study

Universität Leipzig, Dissertation

39 S., 137 Lit., 2 Abb., 5 Anlagen.

Abstract

Background

Serotonin (5-HT) and its neurotrophic support system, specifically brain-derived neurotrophic factor (BDNF), are thought to modulate energy homeostasis and susceptibility to obesity. Moreover, a polymorphism (5-HTTLPR) in the serotonin reuptake transporter (5-HTT) gene impairs its transcription, thereby altering serotonergic tone and potentially contributing to such susceptibility. This study aims to investigate the effect of BDNF, biallelic 5-HTTLPR, and central *in-vivo* 5-HTT availability in highly obese versus non-obese subjects using positron emission tomography (PET) and 5-HTT selective [^{11}C]DASB.

Methods

Thirty-eight subjects, 24 obese (body mass index, BMI, $>35 \text{ kg/m}^2$), otherwise mentally and physically healthy, and 14 non-obese ($\text{BMI} \leq 25 \text{ kg/m}^2$), age- and sex-matched healthy controls were included in this study. Parametric images of binding potential were generated from PET data. Central 5-HTT availability, 5-HTTLPR genotype, and serum BDNF concentrations were analyzed, first in a volume of interest, then in a voxel-wise manner.

Results

Overall, our results showed an absence of a linear correlation between BDNF, *in-vivo* central 5-HTT availability, and body mass index (BMI). 5-HTTLPR genotyping revealed BDNF and hippocampal 5-HTT availability to be negatively correlated ($r = -0.57$, $p = 0.007$) in long allelic homozygotes. However, obese subjects exhibited opposing effects of BDNF levels on 5-HTT availability in the nucleus accumbens (NAcc) relative to our non-obese controls.

Conclusions

Our data did not confirm an overall correlation between serum BDNF, *in-vivo* central 5-HTT availability, 5-HTTLPR, and BMI. However, there is evidence that serotonergic tone linked to BDNF, specifically in the NAcc, is involved in the pathophysiology of obesity, although this needs further exploration over a wide range of reward-related eating behaviors.

Introduction

Obesity: The Not-So-New Epidemic

The global rates of overweight and obesity have markedly increased over the last few decades.¹ Specifically, in the last two decades, Germany showed an increase in relative prevalence of 27% and 38% for males and females, respectively, which grants it a place in the top 10 countries with the highest number of obese individuals (19 million) in 2013.² In short, obesity is caused by a disturbed balance of energy intake and expenditure. This review will focus on the side of energy intake, although expenditure will be briefly addressed as well. Concluding remarks will elaborate on the rationale of the underlying publication on which this dissertation is based on. Identifying and developing new therapies to counteract this epidemic and its comorbidities are important targets of current research.

Increases in body weight diminish physical stamina, which in turn usually reduces energy spent through exercise. Eating and activity patterns are easily disturbed by lifestyle changes which ultimately can cause accumulation of adipose tissue and obesity. Regulation of food intake has been characterized by two fundamentally different theories proposed throughout the last century.

The depletion/repletion model suggests that available energy supply is kept steady at any given moment by the body sensing an appropriate proxy for energy stores and keeping it in equilibrium. According to this model, food intake begins once energy reserves drop below a threshold level (e.g., by monitoring blood glucose or free fatty acid levels) and stops when they are sufficiently refilled. Models based on this principle can theoretically explain both the beginning and end points of food intake. The glucostatic theory of appetite control is one widely accepted example.³ Introduced in 1961 by Jean Mayer, it suggests slight drops in glucose levels (or its increased usage) are sensed by glucoreceptors and in turn elicit appetite. Indexes of energy depletion and repletion (i.e., blood glucose and free lipid levels) can be used to predict energy intake. However, they are unsuitable in predicting energy expenditure. Thus, depletion/repletion models fail to acknowledge the equilibrium between energy intake and expenditure, which is a fundamental requirement for energy reserves to remain stable over time.

Another approach to modeling energy homeostasis has been postulated by G. C. Kennedy as early as 1953. His lipostatic model suggests that food consumption is connected to the levels of energy reserves (i.e., adipose tissue).⁴ Specifically, secretion levels of certain metabolic signals (amongst other modulators of food intake) stand in relation to the amount of lipid storages.⁵ This effectively uncouples momentary food needs from meal initiation and replenishment of depleted energy reserves from termination of food intake. Instead, feeding can begin because of various external motives (i.e., habitual and acquired reasons, chance, social reasons, and time of day). Consequently, termination of food intake is affected by a multitude of intrinsic aspects (e.g., signals triggered by food intake and signals proportional to fat reserves).⁶ Therefore, feeding occurs in animals when the situation and their lifestyle allows it. Energy homeostasis works by adjusting the intake amount of every meal to keep energy reserves balanced. Permanent yet changing energy requirements are fulfilled by energy provided by momentary food intake, as well as stored energy (i.e., in fatty tissue during food scarcity). Reduction in energy reserves causes feeding behavior which is characterized by increased meal portions. However, animals also resort to higher feeding frequencies to secure their energy requirements and survival when food or stored energy resources become increasingly limited. In conclusion, there is a growing consensus in favor of the hypothesis of a lipostatic model regulating energy equilibrium.^{6,7}

Regulation of Energy Homeostasis

Claude Bernard first proposed the paradigm of an organism's capability to maintain an intrinsic energy balance in the 1800s. Walter Cannon later expanded upon this concept in 1932 and named it homeostasis. Energy and glucose homeostasis is achieved through balancing both (1) energy intake and expenditure and (2) glycolysis and gluconeogenesis. Complex physiological processes are involved in this rudimentary but crucial task.

The term adiposity negative feedback (ANF) was coined by Kennedy. To date, the most widely studied ANF signal has been leptin, a peptide hormone which is released by adipocytes⁸ in relation to the amount of adipose tissue.⁹ Once secreted, leptin can freely cross the blood-brain barrier allowing for equal concentrations in both compartments and interacting with its central receptors to regulate energy

homeostasis.^{10,11} Furthermore, food intake and body weight is decreased after central leptin administration while disruptions in leptin signaling result in hyperphagia and consequent increases in body mass.^{8,12,13} Insulin, another key regulator of energy balance, is secreted by β -cells in the pancreas. Copying the pharmacokinetics of leptin, insulin circulates proportional to the amounts of fat tissue and conveys signals of satiety to the brain. Therefore, accumulation of adipose tissue can be caused by disrupted insulin signaling. Both leptin and insulin are involved in ANF, yet leptin's anorexigenic effects are much more pronounced compared to the anorexigenic effects of insulin.^{14,15}

Besides ANF, there are various other neurochemical pathways, including hormones and feeding-related signals which modulate energy homeostasis. This includes peptides secreted by the intestines to transmit information about satiety and to regulate the end point of a meal. Glucagon-like peptide 1 (GLP1),¹⁶ peptide YY (PYY),¹⁷ and cholecystokinin (CCK)¹⁸ are all neurochemicals conveying information about satiety. Ghrelin, on the other hand, is a peptide hormone secreted by the stomach mucosa before meals to stimulate food intake.¹⁹ Excessive feeding can be attenuated by other endogenous signals, such as brain-derived neurotrophic factor²⁰ and nutrients themselves (e.g., glucose and fatty acids).²¹

The decision to initiate eating is dependent on numerous extrinsic factors, while portion size is mostly regulated by intrinsic signals. CCK and GLP1 are produced by the gastrointestinal tract upon food intake and hold key roles as intrinsic signaling molecules. These neuropeptides, together with signals released upon gastric expansion, convey feedback regarding satiety through afferent vagal fibers that originate from the intestines and project to the hindbrain, specifically to the nucleus of solitary tract (NTS).^{18,22} These satiety-promoting peptides have a profound yet short mode of action. Also, they lack persisting weight loss effects when administered repeatedly. CCK's satiety-promoting effects were studied in a rodent model by its application at meal initiation over several days. Repeated administration led to decreased portion sizes but it lacked a significant effect on body mass. This was due to a compensatory increase in feeding frequency, which counteracts reduced portions to maintain the overall amount of energy intake.²³

These findings are in line with the generally accepted working principle of energy homeostasis which states ANF decreases energy intake through sensitization of the

brain to satiety-promoting signals (i.e., leptin and insulin pathways).^{24–26} Blood concentrations of these peptides drop when weight reduction occurs, in turn diminishing the satiating power of nutrients and ultimately leading to greater meal portions. Studies show that leptin accelerates satiety by improving sensitivity to satiety-promoting signals, which leads to smaller meal portions.^{27–29} Conversely, disruptions in central leptin pathways are linked to lowered sensitivity to CCK and increased meal sizes.³⁰ The neurochemistry of leptin and other satiety promoting peptides integrates leptin receptors located in the hindbrain (i.e., NTS neurons),³¹ and the forebrain (i.e., hypothalamic arcuate nucleus (ARC)),³² which allows energy reserves such as adipose tissue, to distinctly modulate individual meal sizes to maintain energy equilibrium.^{29,33}

Reward Systems and Food Intake

Aside from hormonal control, central reward pathways also play an integral part in regulating feeding behavior. Unsurprisingly, food palatability is an important decision factor regarding food intake because of its ability to modulate sensitivity to satiety signals. This, in turn, facilitates intake of palatable over bland-tasting nutrients and is an essential component of energy homeostasis.³⁴ Rodent and human studies indicate satiety control relies in part on inhibiting brain reward pathways.^{35,36} Moreover, after episodes of dieting, animals exhibit refeeding hyperphagia which is driven by higher motivation for food acquisition and an increased food's capacity to condition place preferences (a measure for the motivational effects of food). Central administration of leptin or insulin was shown to diminish these effects, which drive post-fasting increases in energy intake.^{37,38} Weight loss followed by food constraint causes compensatory hyperphagia partly because of attenuated feedback from fatty tissue, which in turn increases the stimulatory properties of food on brain reward systems. This is supported by clinical studies reporting a decrease in energy intake after leptin administration in congenitally leptin-deficient subjects. According to Farooqi et al., this effect is linked to a diminished subjective reward experience and lower activation of brain regions integral to the mesolimbic reward system (i.e., ventral striatum) after exposure to visual food cues.³⁹

Homeostatic regulation of nutritional reward is the focus of ongoing research. In short, hormonal and metabolic signals conveying information about energy reserves

modulate central neuronal reward circuits, thus regulating hedonic value of food (Figure 1).^{40,41}

Adapted from Nature Reviews Neuroscience

Figure 1 The brain combines information about long- and short-term energy feedback. Leptin levels are indicative of long-term energy availability, as they relate to the amount of adipose tissue. Signals conveying acute food intake (e.g., CCK, GLP1) originate from the intestines as well as signals released by nutrients (e.g., glucose, FFA). Energy intake exceeding the organism's needs reduces food rewards and increases satiety. During times of food scarcity, food reward circuits adapt to increase

food's rewarding potential and reduce the effect of satiety signals. This putative model proposes serotonergic tone and BDNF to also be involved in these regulating pathways. Cumulatively, this leads to increased food intake until energy reserves are refilled. 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; CCK, cholecystokinin; FFA, free fatty acids, GLP1, glucagon-like peptide 1. (Adapted from Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake in health and disease. *Nat Rev Neurosci* **15**; 2014: 367—378)⁴²

The mesolimbic dopaminergic system belongs to such reward circuits. Dopaminergic neurons originate in the ventral tegmental area (VTA) and project to regions in the forebrain (e.g., to the NAcc).⁴³ The lateral hypothalamic area (LHA), together with its projections from other hypothalamic regions, is assumed to integrate signals from both the energy homeostasis and the mesolimbic dopamine system.⁴⁴ On the other hand, neurons originating in the LHA influence rewarding behavior and satiety controls located in the hindbrain (e.g., NTS).⁴⁵

Neurobiology of Feeding

During the past 15 years, a substantial deepening of our insights into the neurocircuitry of energy regulation has occurred. Two distinct types of neurons are of particular importance in regulating both appetite and energy expenditure: AgRP/NPY and POMC/CART neurons. Neurons co-expressing agouti-related protein (AgRP), neuropeptide Y (NPY), and GABA are among the most well-described neuronal regulators in energy homeostasis.^{10,46} AgRP neurons are located in the ARC and increase food intake once activated.^{47,48} Fitting in the context of energy balance, both insulin and leptin, being major anabolic signals, inhibit AgRP activity by binding their respective receptors.^{49–51} This concept is supported by the fact that AgRP neurons show strong activity in transgenic leptin deficient murine models, which results in severe hyperphagia.^{46,52} Conversely, ghrelin stimulates AgRP neurons upon growth hormone secretagogue receptor (GHSR) binding, thereby inducing pro-anabolic effects.⁵³

Besides AgRP neurons, anorexic pro-opiomelanocortin (POMC) expressing neurons are located in the ARC, which release α -melanocyte stimulating hormone (α -MSH) together with cocaine- and amphetamine-related transcript (CART). α -MSH functionally reduces food intake upon binding its melanocortin receptor. In contrast to

AgRP neurons, leptin holds stimulatory value to POMC neurons and an absence of leptin leads to decreased activity.⁵⁴ However, it is important to note that this is a simplified generalization, as distinct POMC populations are reported to have opposing effects.⁵⁵ Significant crosstalk exists between POMC and AgRP. In fact, various metabolic pathways, integral for balancing energy reserves, converge in these neurons of the arcuate nuclei. POMC neurons produce and release α -MSH, which binds to melanocortin receptors located especially in neurons of the paraventricular nuclei of hypothalamus (PVN). Among the five different subtypes, MC3R and MC4R are crucial in the control of energy intake and homeostasis.⁵⁶ Binding of these receptors stimulates energy expenditure and inhibits food intake. Similarly, their inhibition causes decreases in energy expenditure and stimulation of food intake. The ability of MC4R binding to stimulate energy expenditure seems to be mediated by triggering neuronal circuits that originate in the PVN and project to the NTS.⁵⁷ Firing of these pathways leads to increases in sympathetic tonus and energy expenditure.⁵⁸ Unlike energy intake, energy expenditure is not tightly controlled. Basal metabolic rate is determined by thyroid hormones and sympathetic tonus (i.e., catecholamine levels). The majority of energy expenditure, however, is caused by activity, which is less influenced by physiological than behavioral factors.

In conclusion, leptin and insulin activate distinct metabolic pathways in POMC and AgRP neurons, although their exact effect on membrane potential remains elusive. Intriguingly, another POMC neuronal subset is the target of ascending serotonergic projections, providing an interface between serotonergic tone and energy homeostasis.⁵⁹

Serotonin and the Regulation of Energy Balance

5-hydroxytryptamine (5-HT, serotonin) has been shown to play a key role in integrating behavioral and physiological functions to control energy balance.⁶⁰ This complex task is achieved through modifying the activity of distinct neuropeptide systems and autonomic pathways, affecting natural patterns of eating, nutrient selection, and body weight.^{61,62} In animal models, administration of serotonergic agents modulates feeding habits by accelerating satiety and decreasing carbohydrate breakdown.⁶³ Additionally, some serotonin reuptake inhibitors were shown to increase energy expenditure and

metabolic rate.⁶⁴ For this reason, it has been postulated that a link exists between serotonergic regulation and the incidence of obesity.

5-HT is expressed in the earliest life forms and has been existing since then, which makes it a suitable nominee as a key player in energy homeostasis. In humans, its synthesis from L-tryptophan is mediated by two enzymes, tryptophan hydroxylase (TPH) and tryptophan decarboxylase. Once secreted, its action begins by binding at one of seven subtypes of 5-HT receptors and ends by neuronal reuptake through the 5-HT transporter (5-HTT). Serotonergic neurons of the raphe nuclei located in the hindbrain innervate most CNS regions and thereby modulate behavioral and biological functions, such as circadian rhythm,⁶⁵ mood,⁶⁶ and energy intake.⁶⁷ However, an estimated 90% of 5-HT has its source in the periphery, where it is synthesized by enterochromaffin cells of the gut to influence intestinal activity. This gut-derived serotonin is stored in blood platelets and functions in hemodynamic control once activated.⁶⁸ 5-HT can also be found in various other tissues (e.g., hepatic,⁶⁹ bone,⁷⁰ and pancreatic β -cells).⁷¹

At least 14 distinct 5-HT receptors have been described in mammals. They are classified into 7 subgroups based on their signaling mechanisms. This diversity enables 5-HT to produce various effects at target neurons. Actually, the effects of 5-HT on energy balance range from direct control of peripheral fatty tissue activity to central food intake regulation in the hypothalamus.⁷² Efforts to target brain 5-HT as an obesity treatment began after an inverse relation between food intake and central 5-HT levels was described.⁷³ The prototype for this kind of pharmaceutical agent is fenfluramine, which stimulates 5-HT release and thereby suppresses food intake.⁶² Similarly, intraventricular application of p-chlorophenylalanine has been shown to decrease central 5-HT production by irreversibly inhibiting TPH, thus causing hyperphagia in rodent models.⁷⁴ Reduced food intake is caused by various 5-HTT inhibitors (i.e., fluoxetine and sibutramine) as well as monoamine oxidase inhibitors (i.e., pargyline and clorgyline).⁷⁵⁻⁷⁷ Therefore, brain 5-HT likely exhibits anorexigenic effects in humans.

Transgenic murine models and receptor-specific ligands were utilized to shed light on the impact of distinct 5-HT receptors on appetite suppression. At first, subtypes 5-HT_{2C} and 5-HT_{1B} were of particular interest because of the anorectic effect induced by meta-chlorophenylpiperazine (mCPP) when acting at these receptors.⁷⁸ Similarly, the

satiety-inducing effects of fenfluramine were weakened by an antagonist of the 5-HT_{2C} receptor.⁷⁹ Conversely, agonists acting at this receptor subtype possess anorectic properties and enhance insulin sensitivity and glucose tolerance.^{80,81} Understanding of the 5-HT_{2C} receptor's role in feeding regulation was significantly improved by a transgenic rodent model. mCPP lost its anorectic properties in 5-HT_{2C} receptor knockout mice, which exhibited severe hyperphagia and consequent obesity.⁸² Studies focusing on the 5-HT_{1B} receptor subtype reported similar results.^{83,84} In 2012, lorcaserin was approved as a novel obesity treatment option. By selectively acting at the 5-HT_{2C} receptor, it reduces body weight without affecting energy expenditure.^{85,86} Studies on the influence of 5-HT on hypothalamic feeding circuits showed 5-HT's ability to inhibit the synthesis and release of AgRP via 5-HT_{1B} receptor activation.⁸⁷ On POMC neurons, 5-HT binds to the 5-HT_{2C} receptor and stimulates α -MSH production and release (Figure 2).⁸⁸ In summary, brain 5-HT modulates feeding circuits in the arcuate nuclei of the hypothalamus, thereby inhibiting energy intake.

Hypothalamic Melanocortin Pathways

The hypothalamic melanocortin pathways play an important role in maintaining energy balance, and disturbed signaling is linked to severe obesity. Mutations in the MC4R gene is the most widely known monogenic cause for obesity, accounting for nearly 5 to 6 percent of early-onset obesity in children.⁸⁹ Conversely, anorexia-cachexia syndrome triggered by infections, uremia, or cancer can in part be attributed to disproportionate activation of melanocortin pathways.⁹⁰ AgRP is an endogenous antagonist at the MC3R and MC4R receptors and stimulates food intake by suppressing α -MSH induced effects. Increased production of AgRP caused by genetic mutations is linked to excessive food intake and obesity, although its exact role in physiological energy homeostasis remains elusive.

Orexigenic neurons of the arcuate nuclei also produce NPY, which is released to stimulate appetite when the body's energy reserves drop. Simultaneously, POMC and melanocortin pathway activity is inhibited to further increase food intake. Moreover, binding of MC4R causes rapid release of BDNF in the hypothalamus (Figure 2).⁹¹ It is likely that MC4R activation in the NTS has similar effects on BDNF release and ultimately, energy intake. This is supported by the fact that administration of BDNF into the PVN suppresses food intake and increases energy expenditure.²⁰ This finding

nominates the hypothalamus as an important site for BDNF to modulate energy homeostasis and feeding behavior.

Adapted from Guyton and Hall Textbook of Medical Physiology

Figure 2 Two crucial neuronal subsets in the arcuate nuclei regulate energy homeostasis: (1) neurons producing agouti-related peptide (AgRP) as well as neuropeptide Y (NPY) and (2) pro-opiomelanocortin (POMC) neurons which synthesize α -melanocyte-stimulating hormone (α -MSH) and cocaine- and amphetamine-regulated transcript (CART). AgRP and NPY stimulate energy intake and decrease energy expenditure. POMC and CART attenuate energy intake and raise energy expenditure. α -MSH is a neurotransmitter that binds to subtype 4 melanocortin receptors (MC4R) in the paraventricular nuclei of the hypothalamus (PVN) and activates projections to the nucleus tractus solitarius (NTS) thereby raising energy expenditure and sympathetic tonus. AgRP, in turn, suppresses neuronal

activity when binding to MC4R. Cholecystokinin (CCK), leptin, and insulin are metabolic signals that stimulate POMC/CART neurons, whereas they suppress AgRP/NPY neurons. Ghrelin is produced by stomach endothelium and increases AgRP/NPY activity upon binding its specific GHSR ultimately stimulating energy intake. Serotonin suppresses appetite via activation of serotonin receptors 1B and 2C (HTR1B, HTR2C) on NPY/AgRP and POMC/CART neurons, respectively. This provides a putative mechanism for hippocampal integration of short-term meal pattern with long-term energy balance signals. 5-HT, serotonin; BDNF, brain-derived neurotrophic factor; HTR1B, serotonin receptor 1B; HTR2C, serotonin receptor 2C; LEPR, leptin receptor; NPY1R, neuropeptide Y1 receptor. (Adapted from Hall JE. Dietary Balances; Regulation of Feeding; Obesity and Starvation; Vitamins and Minerals. In: *Guyton and Hall Textbook of Medical Physiology*. 13th ed. Philadelphia, PA: Elsevier; 2016:887-902)⁹²

Reciprocal Interaction between Synaptic Activity and BDNF

Intriguingly, studies show that synaptic activity can influence energy metabolism through neurochemical pathways. An ideal candidate for such neuronal modulator is represented by BDNF, which has been linked to synaptic plasticity and fine-tuning of energy homeostasis. More specifically, BDNF can alter cognitive function through metabolic signals.⁹³ Animal models showed BDNF to modulate various aspects of energy metabolism (i.e., insulin sensitivity and satiety,⁹⁴ glucose metabolism,⁹⁵ and lipid metabolism).⁹⁶ MC4R located in the hypothalamus regulate expression of BDNF, which further supports the hypothesis of an interaction between energy metabolism and neuronal plasticity.⁹⁷

Along with BDNF, nerve growth factor, neurotrophin-3 (NT3), and neurotrophin-4 (NT4) all belong to a family of structurally and functionally related proteins. BDNF and its receptor tropomyosin receptor kinase B (trkB) are synthesized ubiquitously throughout the central nervous system.⁹⁸ However, smaller amounts can also be found in non-neurogenic cells, including leukocytes,⁹⁹ endothelial cells,¹⁰⁰ and skeletal muscle cells.¹⁰¹ Once BDNF is released, it is able to bi-directionally cross the blood brain barrier,¹⁰² which suggests a linear relationship between central and plasma BDNF levels.¹⁰³ BDNF acts primarily through binding with its high affinity trkB. After binding to its receptor, activated receptor-ligand complexes are internalized and

transported in a retrograde manner to the cell soma, where they induce various survival-promoting effects inside the nucleus.¹⁰⁴ Through this process, BDNF is able to modulate a variety of neural functions, including neuronal development, synaptic plasticity, neurogenesis, and neuronal survival.^{105,106} Interestingly, a *de-novo* mutation in *trkB* was linked with severe obesity as well as developmental delay in humans.¹⁰⁷

Several lines of evidence suggest that 5-HT and BDNF have varied influences on neurogenesis, neuronal survival, and synaptic plasticity. Both signals are thought to co-adjust each other in a manner in which BDNF aids survival and growth of serotonergic neurons, while 5-HT up-regulates transcription of BDNF.¹⁰⁸ Meanwhile, a putative model has been proposed that also links impaired BDNF production and 5-HT signaling to a positive energy balance and an overall increased obesity risk.¹⁰⁹

Significance of Genetic Polymorphisms

Various genetic factors have been postulated to influence susceptibility for hyperphagia and obesity.¹¹⁰ Specifically, genes encoding the serotonergic system are being investigated because of their important role in central nervous control of energy homeostasis. Serotonergic activity is tightly coupled to the activity of the 5-HT reuptake transporter (5-HTT). 5-HTT is an integral membrane protein located on serotonergic neuron membranes and acts by removing and recycling 5-HT from the extracellular space, thereby modifying the duration and intensity of postsynaptic receptor-mediated (wired) as well as extra-synaptic (volume) transmission. This makes it a crucial control point in the fine-tuning of 5-HT neurotransmission and extracellular 5-HT levels.¹¹¹ There is a 44 base pair insertion/deletion polymorphism in its promoter region, precisely where the 5-HTT gene-linked polymorphic region (5-HTTLPR) is located. Prevalent in most samples studied so far,¹¹² 5-HTTLPR is a repeat polymorphism commonly subdivided into two alleles, which correspond to 14 and 16 repeat units or a short (S) and long (L) version, respectively.¹¹³ Substantial evidence suggests that the S-allelic carrier is connected to impaired transcription, resulting in diminished 5-HTT activity, while the L-allelic carrier exhibits nearly a 2-fold increase in reuptake capacity.¹¹⁴ More recently, another functional allele has been identified. An adenosine to guanine polymorphism at position 6 of the 16 repeat long allele causes a third variant, L_G. First proposed by Nakamura et al., L_G matches genetic expression of the

S-variant.^{112,115} In summary, several studies suggest an association between the 5-HTTLPR genotype and BMI.^{61,116,117}

Another genetic variant has been associated with an increased risk for obesity. A single nucleotide polymorphism in the BDNF gene, namely val66met, causes a valine to methionine substitution at codon 66. This results in perturbed intracellular pro-BDNF trafficking and secretion of mature BDNF.¹¹⁸ However, current literature renders ambiguous as an increased obesity risk has been associated with the met allele,^{119,120} the val allele,^{121,122} while some studies failed to detect a significant correlation between val66met genotype and BMI altogether.^{123,124} This lack of a clear consensus has prompted our team to extend future investigations by including val66met genotype status.

Study Hypothesis

To date, the exact pathogenesis of obesity still remains elusive despite being a global health concern. The following publication aims at shedding light on the intricate and usually well-balanced energy homeostasis by quantifying cerebral 5-HTT availability and serum BDNF levels in highly obese and non-obese subjects. This was accomplished through a compilation of relevant PET study data, their statistical evaluation and integration of current literature.

Based on previous findings, we postulated attenuated BDNF and 5-HTT availability in brain regions relevant to energy homeostasis and appetite control associated with increases in body mass index.¹²⁵ Moreover, we anticipated a significant difference in serum BDNF in obese subjects, in particular in individuals with lower expressing 5-HTT genotype, compared to their non-obese controls. These findings will hopefully improve our understanding of obesity and help combat this epidemic by identifying new targets for therapeutic strategies.

The effect of serum BDNF levels on central serotonin transporter availability in obese versus non-obese adults: A [¹¹C]DASB positron emission tomography study

Philipp Hinderberger^{a, b}, Michael Rullmann^{a, b}, Mandy Drabe^b, Julia Luthardt^a, Georg-Alexander Becker^a, Matthias Blüher^b, Ralf Regenthal^c, Osama Sabri^{a, b}, Swen Hesse^{a, b, *}

^a Department of Nuclear Medicine, University of Leipzig, Leipzig, Germany

^b Integrated Research and Treatment Center (IFB) Adiposity Diseases, University of Leipzig, Leipzig, Germany

^c Division of Clinical Pharmacology, Rudolf-Boehm-Institute of Pharmacology and Toxicology, University of Leipzig, Leipzig, Germany

ARTICLE INFO

Article history:

Received 22 July 2015

Received in revised form

26 February 2016

Accepted 20 April 2016

Available online 22 April 2016

Keywords:

Brain-derived neurotrophic factor

Neurotrophin

Serotonin

Serotonin transporter

Serotonin-transporter-linked polymorphic region

Serotonergic tone

Positron emission tomography

Energy homeostasis

Obesity

ABSTRACT

Background: Serotonin (5-HT) and its neurotrophic support system, specifically brain-derived neurotrophic factor (BDNF), are thought to modulate energy homeostasis and susceptibility to obesity. Moreover, a polymorphism (5-HTTLPR) in the serotonin reuptake transporter (5-HTT) gene impairs its transcription, thereby altering serotonergic tone and potentially contributing to such susceptibility. This study aims to investigate the effect of BDNF, biallelic 5-HTTLPR, and central *in-vivo* 5-HTT availability in highly obese versus non-obese subjects using positron emission tomography (PET) and 5-HTT selective [¹¹C]DASB.

Methods: Thirty-eight subjects, 24 obese, otherwise mentally and physically healthy, and 14 non-obese healthy controls were included in this study. Parametric images of binding potential were generated from PET data. Central 5-HTT availability, 5-HTTLPR genotype, and serum BDNF concentrations were analyzed, first in a volume of interest, then in a voxel-wise manner.

Results: Overall, our results showed an absence of a linear correlation between BDNF, *in-vivo* central 5-HTT availability, and body mass index (BMI). 5-HTTLPR genotyping revealed BDNF and hippocampal 5-HTT availability to be negatively correlated ($r = -0.57$, $p = 0.007$) in long allelic homozygotes. However, obese subjects exhibited opposing effects of BDNF levels on 5-HTT availability in the nucleus accumbens (NAcc) relative to our non-obese controls.

Conclusions: Our data did not confirm an overall correlation between serum BDNF, *in-vivo* central 5-HTT availability, 5-HTTLPR, and BMI. However, there is evidence that serotonergic tone linked to BDNF, specifically in the NAcc, is involved in the pathophysiology of obesity, although this needs further exploration over a wide range of reward-related eating behaviors.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

The global rates of being overweight and obesity have markedly increased over the past few decades (Nguyen and El-Serag, 2010). Specifically, in the last two decades, Germany showed an increase in relative prevalence by 27% and 38% for males and females,

respectively, which grants it a place in the top 10 countries with the highest number of obese individuals (19 million) in 2013 (Mensink et al., 2013). Identifying and developing new therapies to counteract this epidemic and its comorbidities is an important target of current research.

Several lines of evidence suggest serotonin or 5-hydroxytryptamine (5-HT) and brain-derived neurotrophic factor (BDNF) have various influences on neurogenesis, neuronal survival, and synaptic plasticity. Both signals are thought to co-adjust each other in a manner that BDNF aids survival and growth of serotonergic neurons, while 5-HT up-regulates transcription of BDNF

* Corresponding author. Department of Nuclear Medicine, University of Leipzig, Liebigstrasse 18, 04103, Leipzig, Germany.

E-mail address: Swen.Hesse@medizin.uni-leipzig.de (S. Hesse).

(Mattson et al., 2004). Meanwhile, a putative model has been proposed which links impaired BDNF production and 5-HT signaling to a positive energy balance and an increased obesity risk over an individual's life span (Ooi et al., 2012). Haahr et al. (2015) have recently reported a link between serotonergic neurotransmission and potential weight changes in obese individuals by assessing serotonin 5-HT_{2A} receptors and 5-HTT availability before and after gastric bypass surgery.

5-HT plays a key role in integrating behavioral and physiological functions to control energy balance (Leibowitz, 1990). This complex task is achieved through modifying activity of distinct neuropeptide systems and autonomic pathways, thereby affecting natural patterns of eating, nutrient selection, and body weight (Lam and Heisler, 2007; Sookoian et al., 2007). Administration of serotonergic agents may modulate eating habits by accelerating satiety and decreasing carbohydrate breakdown in animals (Donovan and Tecott, 2013). Additionally, several 5-HT reuptake inhibitors were shown to increase energy expenditure and metabolic rate (Halford et al., 2005). For this reason, it has been postulated that there exists a connection between serotonergic regulation and the incidence of obesity.

Serotonergic tone is tightly coupled to the activity of the 5-HT transporter (5-HTT). This integral membrane protein is located on the presynapses of serotonergic nerve terminals, axons, and cell bodies where it attenuates synaptic and extrasynaptic (volume) neurotransmission (Zhou et al., 1998). 5-HTT binding is a marker for both synaptic 5-HT, serotonergic neurons, and their projections. 5-HTT acts by extracellular 5-HT reuptake, thereby modifying the duration and intensity of postsynaptic receptor-mediated signaling. This makes it a crucial control point in the fine-tuning of 5-HT neurotransmission and extracellular 5-HT levels (Lesch, 2000).

Genetic variants in the 5-HTT-coding gene, namely SLC6A4, a 44 base pair insertion/deletion polymorphism in its promoter region (5-HTTLPR) may also alter 5-HTT gene expression and modulate serotonergic tone. This polymorphism is commonly subdivided into a short (S) and long (L) allele (Lesch et al., 1996). The less frequent short allelic version is connected to impaired transcription of 5-HTT and limited 5-HT reuptake, while carriers of the long allele exhibit nearly a 2-fold increase in 5-HTT activity (Lesch et al., 1993).

Studies show synaptic activity can influence energy metabolism by enabling mental processes to alter somatic activity at the molecular level. Specifically, BDNF modulates neurochemistry in the hippocampus through (1) 5-HT signaling (Thierry Deltheil et al., 2008a,b) and (2) exercise induced plasticity which in turn affects cognitive function (Vaynman et al., 2006). This neurotrophin acts primarily through binding with its high affinity receptor tropomyosine receptor kinase B (TrkB). Activated receptor-ligand complexes induce various neuronal-promoting effects (Curtis et al., 1995) such as development and survival of neurons, synaptic plasticity, and neurogenesis (Salehi et al., 2003; Waterhouse and Xu, 2009). Interestingly, a *de novo* mutation in TrkB was linked to severe obesity (Yeo et al., 2004) supporting its importance in energy homeostasis and body weight control. Animal studies have shown that BDNF affects various aspects of energy metabolism, i.e. insulin sensitivity and satiety (Kernie et al., 2000), glucose metabolism (Tonra et al., 1999), and lipid metabolism (Tsuchida et al., 2002). In rodents, central administration of BDNF resulted in increased satiety and attenuated weight gain (Pellemounter et al., 1995), while animals heterozygous for a BDNF mutation exhibited hyperphagia and consequent obesity due to lower BDNF levels (Fox and Byerly, 2004). In humans, decreased satiety and increased food intake have been postulated as characteristics of chronic BDNF deficiency (Rosas-Vargas et al., 2011). Lommatzsch et al. (2005) demonstrated a negative correlation between serum BDNF and body mass index (BMI) supporting its pivotal role in the regulation

of eating behavior. Although current literature indicates an involvement of BDNF in the pathophysiology of eating disorders and obesity, this has not been addressed in *in-vivo* imaging so far (Ooi et al., 2012; Wisse and Schwartz, 2003).

The aim of the present study was to investigate the effect between BDNF and cerebral 5-HTT availability in a carefully selected cohort. 5-HTT selective radiotracer [¹¹C]DASB and serum BDNF, a central BDNF proxy, were employed for this purpose. This study sample consisted of highly obese, non-depressed, and physically healthy participants as well as non-obese, healthy controls. Based on previous findings (Mueller et al., 2015), we postulated lower serum BDNF and 5-HTT availability in brain regions relevant to energy homeostasis, appetite control, and memory formation associated with increases in body mass index (BMI). Moreover, we expected a significant difference in serum BDNF in obese subjects, in particular in individuals with lower expressing 5-HTT genotype, compared to their non-obese controls.

2. Material and methods

2.1. Subjects

Imaging data and blood samples were obtained from 24 obese individuals categorized by a BMI >35 kg/m² (mean BMI, 40.7 ± 4.3 kg/m²; 5 men; mean age, 36.9 ± 10.7 years; range, 21–59 years) and 14 non-obese controls (mean BMI, 22.5 ± 2.6 kg/m²; 5 men; mean age, 36.1 ± 7.2 years; range, 21–49 years) after obtaining informed consent and performing a medical screen. Exclusion criteria were neurologic or psychiatric disorders, recent substance abuse or consumption of psychotropic medication, and illegal drugs. Specifically, any medical condition that may alter brain function, any medication acting centrally or altering body weight, over the counter medication, nutritional supplements (within the past 2 months), pregnancy and breast-feeding, or history of substance abuse resulted in exclusion from this study. Control subjects were thoroughly matched for sex and age and screened for glucose tolerance and eating disorders. Peripheral blood samples from all subjects were collected shortly before the PET scan, immediately refrigerated and partly processed to blood serum. Whole blood samples were used for preparation of genomic DNA and genotyping was performed by amplification with polymerase chain reaction (PCR) and a standardized procedure with primers synthesized by Invitrogen (UK), according to primers described by Heils et al. (1996). PCR products were analyzed by gel electrophoresis in 2% agarose gels (peq Gold Universal agarose, peqLab, Germany) stained with ethidium bromide. A 100-bp ladder (Amersham Biosciences Europe, Germany) was employed for size definition. Due to the accidental malfunctioning of cooling equipment and consequent damage of blood samples, four individuals had to be removed from the analyses.

2.2. Imaging

All subjects were PET scanned as previously described (Hesse et al., 2015, 2011). Briefly, each study participant underwent a 90-min dynamic scan (23 frames: 4 × 15 s, 4 × 1 min, 5 × 2 min, 5 × 5 min, 5 × 10 min) on the ECAT EXACT HR + tomograph (Siemens, Erlangen, Germany, intrinsic resolution at the centre: 4.3 mm, axial resolution: 5–6 mm field of view 15.5 cm, 3–4 mm full width at half maximum) in a 3-dimensional acquisition mode. The radiotracer [¹¹C]-(3-amino-4-(2-dimethylaminoethyl)-phenylsulfanyl)-benzonitrile([¹¹C]DASB), was synthesized according to procedures reported by Wilson et al. (2000). With the start of each scan, 484.1 ± 9.9 [mean ± standard deviation (s.d.)] MBq [¹¹C] DASB was intravenous injected as a continuous bolus over 90 s. A

10-min transmission scan (from three $^{68}\text{Ga}/^{68}\text{Ge}$ sources) prior to PET acquisition was used for attenuation correction and iterative reconstruction (10 iterations, 16 subsets) in transverse image series (63 slices, 128×128 matrix, voxel size $2.6 \times 2.6 \times 2.4 \text{ mm}^3$) with a Hann-filter (cut-off 4.9 mm) for post-processing. Magnetic resonance imaging (MRI) was performed on all study participants for imaging data co-registration. For this purpose, magnetization prepared rapid gradient-echo T1-weighted sequences were acquired either on a Siemens Verio or TimTrio 3T scanner (Siemens, Erlangen, Germany).

2.3. Imaging analysis

Parametric images of 5-HTT non-displaceable binding potential (BP_{ND} ; Innis et al., 2007) were generated from the PET data by using the multi-linear reference tissue model with two parameters and the cerebellar cortex as reference tissue (Ichise et al., 2003; Kim et al., 2006). Regional analyses of BP_{ND} values took place by manual delineation of the volumes of interest (VOI; frontal areas: medial prefrontal, dorsolateral prefrontal, orbitofrontal; insula, nucleus accumbens (NAcc), head of the caudate nucleus, putamen, thalamus, hypothalamus, anterior cingulate cortex, raphe nuclei, amygdala, hippocampus, pons and midbrain including substantia nigra and ventral tegmental area) after co-registering BP_{ND} images with 3D MRI data using PMOD software (Version 3.4). They were realigned and stereo-tactically normalized according to the anterior commissure-posterior commissure line. Voxel-based analyses were performed using SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, UK) and Matlab 7.3.0 (The MathWorks Inc., USA). We spatially normalized the parametric BP_{ND} images by applying the transformation matrix, which was estimated when the co-registered MRI was spatially normalized to the T1 template integrated in SPM8. Smoothing was done using a Gaussian filter kernel with 8 mm full width at half maximum.

2.4. Brain-derived neurotrophic factor measurements

Blood samples were collected during two timeframes: the first being between 8 and 10 a.m. (morning group) and the second being between 2 and 4 p.m. (afternoon group). The serum was separated, aliquoted and stored before further use. The clotting duration was a minimum of 2 h (Maffioletti et al., 2014). Serum samples were assayed for BDNF levels with a commercially available immunoassay kit distributed by R&D Systems, Inc (Minneapolis, USA). In short, 96 microwell plates coated with anti-BDNF monoclonal antibody were incubated at 21°C for 1 h. Finally, photometry was employed to obtain BDNF concentrations through interpolation of a reference curve.

2.5. Statistical analysis

Statistical computations involved primary analyses based on VOI data followed by voxel-wise whole brain analyses in order to identify small clusters in brain regions relevant for the study purposes. Initially, we tested our data for normal distribution using the Shapiro-Wilks test and the Levene test for homogeneity of variance. The Mann-Whitney-Wilcoxon test was performed as a non-parametric test whenever variables did not appear to be normally distributed. Following careful examination and detection of asymmetries between corresponding left and right hemispheres, VOIs were averaged side-by-side to reduce the number of dependent variables within the models. From 30 measured VOIs, 15 (averaged or unique) were used in further analyses. Pearson's chi-squared test was used to check for deviations from

Hardy–Weinberg Equilibrium (HWE).

Intergroup comparisons (obese vs non-obese, L/L vs S+) of 5-HTT BP_{ND} and serum BDNF were assessed using a general linear model (GLM) controlling for age, sex, sunshine duration on the test day (h), and scan time. Sunshine durations were retrieved from German Weather Service (DWD, Deutscher Wetterdienst). Previous studies suggested this to be a significant confounder for brain 5-HTT binding and BDNF levels (Bus et al., 2011; Praschak-Rieder et al., 2008). Correlations between 5-HTT BP_{ND} , serum BDNF, and BMI were evaluated using a multiple linear regression model. When the variances derived from ANOVA were significant, the effects of age, sex, sunshine duration, and scan time were added as confounders. Regression analyses included a comparison of beta estimates between obese and non-obese controls. Here, the aim was to assess interactions between 5-HTT BP_{ND} , conveying information regarding serotonergic tonus, and conditions of brain BDNF, indexed by peripheral BDNF through serum analyses. Data are presented as mean $\pm$ SD. Differences at $p < 0.05$ were considered as significant. Graphical presentations were created with Graphpad Prism 7.0.

2.6. Ethics statement

Ethical approval for this study was obtained from the ethics committee of the Medical Faculty of the University of Leipzig (registered under no. 206-10-08032010). All subjects gave their written informed consent. The study conformed to all regulations set forth by the ICH Guideline for Good Clinical Practice (GCP), the declaration of Helsinki, and the German Federal Office for Radiation Protection (registered under no. Z5-22461-2-2011-002). The study was registered at the European clinical trial database (EudraCT 2012-000568-32) and the German Clinical Trials Register (DRKS).

3. Results

Table 1 depicts demographic and clinical parameters of study participants. Stratification based on BMI did not reflect significant differences in serum BDNF ($p = 0.62$; Table 1 and Fig. 1). 5-HTT BP_{ND} in NAcc significantly correlated with BDNF in non-obese ($r = -0.64$, $p = 0.01$), but not in obese subjects ($r = 0.21$, $p = 0.27$).

5-HTTLPR genotype distributions are summarized in Table 2. No differences were observed in overall cerebral 5-HTT availability between homozygous L and homozygous as well as heterozygous S genotyped (S+) individuals (group VOI BP_{ND} 0.60 ± 0.22 versus 0.62 ± 0.16 , respectively; $p = 0.7$). Student's t -test returned equal means for 5-HTT BP_{ND} (Table 3 and Fig. 2). This includes regions that were previously linked to obesity (i.e., hypothalamus, insula,

Table 1
Data on study sample scanned with [^{11}C]DASB stratified by BMI.

	Obese ^a	Non-obese ^b	p -value ^c
n	24	14	
Age (years)	36.9 ± 10.7	36.1 ± 7.2	0.85
BMI (kg/m^2)	40.7 ± 4.3	22.5 ± 2.6	<0.0001
Sunshine (h)	5.8 ± 5.5	3.5 ± 4.0	0.10
BDNF (ng/ml)	28.1 ± 6.6	27.1 ± 5.3	0.62

BMI, body mass index; BDNF, brain-derived neurotrophic factor.

A total of 38 subjects scanned with [^{11}C]DASB. Possible confounders are age, BMI, and sunshine duration, but no intergroup differences were found between obese and non-obese controls (Student's t -test). Values represent mean $\pm$ standard deviation (s.d.), while bold values indicate $p < 0.05$.

^a BMI $> 35 \text{ kg}/\text{m}^2$.

^b BMI $< 25 \text{ kg}/\text{m}^2$.

^c Student's t -test.

Table 2Data on study sample scanned with [¹¹C]DASB stratified by 5-HTT genotype.

	L/L	S+	p-value ^a
n	19	19 (2)	
Age (years)	35.3 ± 8.8	37.4 ± 9.6	0.45
BMI (kg/m ²)	35.5 ± 8.8	34.5 ± 10.9	0.75
Sunshine (h)	5.1 ± 4.9	5.0 ± 5.1	0.96
BDNF (ng/ml)	27.8 ± 5.7	27.6 ± 6.5	0.93

BMI, body mass index; BDNF, brain-derived neurotrophic factor; L/L, homozygous L carriers; S+, homozygous and heterozygous S carriers.

5-HTTLPR allele distribution in scanned subjects. Allele distribution was in Hardy–Weinberg equilibrium (Pearson chi-squared test). The number in brackets in S+ indicates the number of homozygous S/S individuals. Values represent mean ± s.d.

^a Student's *t*-test.

Table 3Serotonin transporter (5-HTT) binding potential BP_{ND} in subjects with L/L genotype compared to S-carriers.

Volume of interest	L/L	S+	p-value ^a
mPFC	0.23 ± 0.12	0.22 ± 0.11	0.79
OFC	0.46 ± 0.16	0.45 ± 0.14	0.78
DLPFC	0.25 ± 0.14	0.24 ± 0.11	0.75
ACC	0.40 ± 0.16	0.42 ± 0.11	0.57
Insula	0.65 ± 0.22	0.68 ± 0.16	0.56
Hippocampus	0.53 ± 0.22	0.61 ± 0.25	0.26
Amygdala	1.29 ± 0.45	1.35 ± 0.33	0.65
NAcc	1.58 ± 0.47	1.62 ± 0.37	0.70
HCN	1.41 ± 0.41	1.51 ± 0.31	0.36
Putamen	1.27 ± 0.40	1.30 ± 0.23	0.82
Thalamus	1.48 ± 0.51	1.53 ± 0.37	0.75
Hypothalamus	1.93 ± 0.72	2.11 ± 0.38	0.29
SN/VTA	1.52 ± 0.62	1.66 ± 0.41	0.36
Raphe	3.26 ± 1.10	3.42 ± 0.45	0.51
Group ^b	0.60 ± 0.22	0.62 ± 0.16	0.70

mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; NAcc, nucleus accumbens; HCN, head of the caudate nucleus; SN/VTA, substantia nigra/ventral tegmental area; values represent mean ± s.d.

^a Student's *t*-test.

^b Represents the mean of all investigated volumes of interests (VOIs).

orbitofrontal cortex, and amygdala). Both subgroups exhibited equal means for BDNF levels (Student's *t*-test; $p = 0.93$). Serum BDNF and hippocampal 5-HTT BP_{ND} were negatively correlated in long-allelic homozygotes ($r = -0.57$, $p = 0.007$; Fig. 3). In S-carriers,

Fig. 1. Boxplot of serum BDNF comparing obese and non-obese subjects. F-Test was used to examine variances ($p = 0.43$) and the Student's paired *t*-Test (two-tailed, equal variances) was used to show there is no significant difference between both groups with regards to serum BDNF ($p = 0.62$).

the Pearson correlation coefficient lacked significance ($r = 0.24$, $p = 0.26$). These results survived adjusting for the number of genotypes and multiple regression analysis to account for covariates (i.e. age, sex, BMI, scan time, and sunshine duration). Voxel-based computations revealed no additional findings.

Interaction analyses including comparisons of beta estimates of regression coefficients showed a significant difference ($p = 0.02$, $F = 5.54$) between 5-HTT BP_{ND} in NAcc and serum BDNF between obese ($r = 0.21$, $p = 0.27$) and non-obese ($r = -0.64$, $p = 0.01$) subjects (Fig. 4). Pooled error variance was applied to increase statistical power after verifying matching variances between groups.

4. Discussion

To the best of our knowledge, no *in-vivo* studies have been undertaken to examine the influence of peripheral BDNF levels on 5-HTT availability in human obesity. Using [¹¹C]DASB and PET, this is the first study investigating the aforementioned parameters in a sample of obese individuals and comparing those findings with non-obese controls, to further extend our understanding of the intricate interaction of the 5-HT network and its neurotrophic support system in obesity. Contrary to our initial hypothesis, there is no statistically significant correlation between any of these parameters in our obese subgroup.

However, interaction analyses revealed opposing effects of serum BDNF on 5-HTT binding in the NAcc in obese versus non-obese individuals. Considering 5-HTT availability holds information about serotonergic tone, this finding suggests conditions of brain BDNF (indexed by peripheral BDNF levels) play a different role in obese and non-obese individuals in predicting serotonergic tone in the NAcc. This brain area belongs to the mesolimbic dopamine system widely known for its role in reward seeking behavior. There is evidence that BDNF and TrkB are particularly expressed (Numan and Seroogy, 1999) and involved in the neurophysiology of drug dependence (Graham et al., 2007) in these regions. Although these studies are related to illicit drug consumption, palatable food intake has been shown to elicit similar dopaminergic reward pathways (Volkow et al., 2008). Moreover, dopamine and serotonin neurotransmission seem to reciprocally modulate each other in the NAcc (De Deurwaerdère et al., 1998). Although exact working principles remain unknown, this could be an indication that BDNF is affecting energy homeostasis through distinct neuronal pathways in the NAcc integrating both the dopamine and serotonin system.

We further assessed the interaction between BDNF and 5-HTT by genotyping the 5-HTT promoter polymorphism. Long allelic homozygotes showed a negative correlation in the hippocampus, i.e. higher peripheral BDNF levels predicted lower 5-HTT density in the hippocampus. This contradicts animal models which previously reported positive correlations (Deltheil et al., 2008b; Homberg et al., 2014). Hippocampal function has been extensively studied regarding long-term potentiation and memory formation. Recently, this brain region has received increased attention for its potential role in energy metabolism. Specifically, hypotheses linking high-caloric intake to hippocampal disruption and altered BDNF secretion. A vicious circle of excess food intake causing disruption of hippocampal function and reducing its inhibitory effects over food intake might contribute to the pathogenesis of obesity.

Val66met, a polymorphism of the BDNF-coding gene, holds predictive value of serotonergic tone (Fisher et al., 2014) in addition to the functional activity of its mature protein (Egan et al., 2003). Our team has ongoing efforts assessing the influence of BDNF genotype as a link between impaired BDNF expression and an increased risk for obesity. Further carefully designed studies will be

Fig. 2. Comparing 5-HTT BP_{ND} for various regions of interest between L/L and S+ carriers of 5-HTTLPR. Bars display mean and s.d. of 5-HTT BP_{ND}. mPFC, medial prefrontal cortex; OFC, orbitofrontal cortex; DLPFC, dorsolateral prefrontal cortex; ACC, anterior cingulate cortex; NAcc, nucleus accumbens; HCN, head of the caudate nucleus; SN/VTA, substantia nigra/ventral tegmental area.

Fig. 3. Scatterplot showing Pearson correlation ($r = -0.57$, $p = 0.007$) between BDNF levels and 5-HTT BP_{ND} in hippocampus in subjects with long 5-HTT allele.

Fig. 4. Scatterplot showing Pearson correlation between BDNF levels and 5-HTT BP_{ND} in the nucleus accumbens (NAcc) for obese ($r = 0.21$, $p = 0.27$) and non-obese ($r = -0.64$, $p = 0.01$) subjects. Comparison of beta estimates of regression coefficients revealed a significant intergroup difference ($p = 0.02$).

necessary to conclude causal interactions between BDNF, its genotype, and the prevalence of obesity.

Two possible limitations of the present study should be noted. BDNF can bi-directionally cross the blood brain barrier, suggesting a linear relationship between both compartments. However, not all BDNF is produced by the central nervous system. Distinct peripheral tissues also secrete BDNF in smaller amounts, e.g. endothelial and smooth muscle cells. Thus, serum assessments always reflect the sum of BDNF originating from cerebral and peripheral tissue. Additionally, serum concentrations exhibit more variance compared to measures of whole blood (Karege et al., 2005). To date there is no clear consensus whether serum, plasma, or whole blood reflect the best proxy for central BDNF levels. A second limitation stems from the fact that we have not performed a tri-allelic genotyping of the 5-HTT promoter gene. This allows grouping the high expressing genotype L_A/L_A against all other genotypes and the possibility to reveal further genotype effects in target parameters when comparing obese and non-obese adults.

In conclusion, BDNF and 5-HTT availability do not qualify as suitable measures for predicting BMI in our subjects. Contrary to our hypothesis, we were unable to detect any significant intergroup differences regarding 5-HTT density and BDNF levels.

However, there was evidence for opposing effects of serum BDNF on 5-HTT availability in the NAcc in obese versus non-obese subjects. Genotyping for variants of the 5-HTT gene promoter revealed a negative correlation between BDNF and 5-HTT binding in the hippocampus in long allelic homozygotes. Corroborating previous studies, we suggest that allelic variations of the 5-HTT polymorphism have a minor (if present at all) impact on 5-HTT availability in obese and non-obese individuals (Lim et al., 2006; Murthy et al., 2010; Shioe et al., 2003; Willeit et al., 2001). We advocate that concentration of serum BDNF does not significantly change with respect to sex, age, sunshine exposure, scan time, and body weight, which complements previous reports by Gajewska et al. (2014). While this remains a topic of scientific debate, additional studies will be necessary to define the exact impact and neurophysiology of BDNF on energy homeostasis.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by the German Federal Ministry of Education and Research (FKZ: 01EO1001).

References

- Bus, B.A.A., Molendijk, M.L., Penninx, B.J.W.H., Buitelaar, J.K., Kenis, G., Prickaerts, J., Elzinga, B.M., Voshaar, R.C.O., 2011. Determinants of serum brain-derived neurotrophic factor. *Psychoneuroendocrinology* 36, 228–239. <http://dx.doi.org/10.1016/j.psyneuen.2010.07.013>.
- Curtis, R., Adryan, K.M., Stark, J.L., Park, J.S., Compton, D.L., Weskamp, G., Huber, L.J., Chao, M.V., Jaenisch, R., Lee, K.F., 1995. Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron* 14, 1201–1211.
- De Deurwaerdere, P., Stinus, L., Spampinato, U., 1998. Opposite change of in vivo dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT₃ receptors. *J. Neurosci.* 18, 6528–6538.
- Deltheil, T., Guiard, B.P., Cerdan, J., David, D.J., Tanaka, K.F., Repérant, C., Guilloux, J.P., Coudoré, F., Hen, R., Gardier, a. M., 2008a. Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. *Neuropharmacology* 55, 1006–1014. <http://dx.doi.org/10.1016/j.neuropharm.2008.08.001>.
- Deltheil, T., Guiard, B.P., Guilloux, J.P., Nicolas, L., Deloménie, C., Repérant, C., Le, Maitre, E., Leroux-Nicollet, I., Benmansour, S., Coudoré, F., David, D.J., Gardier, A.M., 2008b. Consequences of changes in BDNF levels on serotonin neurotransmission, 5-HT transporter expression and function: studies in adult mice hippocampus. *Pharmacol. Biochem. Behav.* 90, 174–183. <http://dx.doi.org/10.1016/j.pbb.2007.09.018>.
- Donovan, M.H., Tecott, L.H., 2013. Serotonin and the regulation of mammalian energy balance. *Front. Neurosci.* 7, 36. <http://dx.doi.org/10.3389/fnins.2013.00036>.
- Egan, M.F., Kojima, M., Callicott, J.H., Goldberg, T.E., Kolachana, B.S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., Weinberger, D.R., 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell* 112, 257–269. [http://dx.doi.org/10.1016/S0092-8674\(03\)00035-7](http://dx.doi.org/10.1016/S0092-8674(03)00035-7).
- Fisher, P.M., Holst, K.K., Adamsen, D., Klein, A.B., Frokjaer, V.G., Jensen, P.S., Svarer, C., Gillings, N., Baare, W.F.C., Mikkelsen, J.D., Knudsen, G.M., 2014. BDNF Val66met and 5-HTTLPR polymorphisms predict a human in vivo marker for brain serotonin levels. *Hum. Brain Mapp.* <http://dx.doi.org/10.1002/hbm.22630>.
- Fox, E. a., Byerly, M.S., 2004. A mechanism underlying mature-onset obesity: evidence from the hyperphagic phenotype of brain-derived neurotrophic factor mutants. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R994–R1004. <http://dx.doi.org/10.1152/ajpregu.00727.2003>.
- Gajewska, E., Sobieska, M., Suwalska, A., 2014. Obesity itself does not influence BDNF serum levels in adults. *Eur. Rev. Med. Pharmacol. Sci.* 18, 3246–3250.
- Graham, D.L., Edwards, S., Bachtell, R.K., DiLeone, R.J., Rios, M., Self, D.W., 2007. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat. Neurosci.* 10, 1029–1037. <http://dx.doi.org/10.1038/nn1929>.
- Haahr, M.E., Hansen, D.L., Fisher, P.M., Svarer, C., Stenbaek, D.S., Madsen, K., Madsen, J., Holst, J.J., Baare, W.F.C., Hojgaard, L., Almdal, T., Knudsen, G.M., 2015. Central 5-HT neurotransmission modulates weight loss following gastric bypass surgery in obese individuals. *J. Neurosci.* 35, 5884–5889. <http://dx.doi.org/10.1523/JNEUROSCI.3348-14.2015>.
- Halford, J.C.G., Harrold, J.A., Lawton, C.L., Blundell, J.E., 2005. Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. *Curr. Drug Targets* 6, 201–213.
- Heils, A., Teufel, A., Petri, S., Stöber, G., Riederer, P., Bengel, D., Lesch, K.P., 1996. Allelic variation of human serotonin transporter gene expression. *J. Neurochem.* 66, 2621–2624.
- Hesse, S., Rullmann, M., Luthardt, J., Winter, K., Hankir, M., Becker, G.-A., Zientek, F., Reissig, G., Regenthal, R., Drabe, M., Schinke, C., Bresch, A., Arelin, K., Lobsien, D., Patt, M., Meyer, P.M., Fasshauer, M., Fenske, W.K., Blüher, M., Stumvoll, M., Sabri, O., 2015. Central serotonin transporter availability in highly obese individuals compared with non-obese controls – a [¹¹C]DASB positron emission tomography study. *Eur. J. Nucl. Med. Mol. Imaging.*
- Hesse, S., Stengler, K., Regenthal, R., Patt, M., Becker, G.-A., Franke, A., Knüpfer, H., Meyer, P.M., Luthardt, J., Jahn, I., Lobsien, D., Heinke, W., Brust, P., Hegerl, U., Sabri, O., 2011. The serotonin transporter availability in untreated early-onset and late-onset patients with obsessive-compulsive disorder. *Int. J. Neuropsychopharmacol.* 14, 606–617. <http://dx.doi.org/10.1017/S1461145710001604>.
- Homberg, J.R., Molteni, R., Calabrese, F., Riva, M.a., 2014. The serotonin-BDNF duo: developmental implications for the vulnerability to psychopathology. *Neurosci. Biobehav. Rev.* 43, 35–47. <http://dx.doi.org/10.1016/j.neubiorev.2014.03.012>.
- Ichise, M., Liow, J.-S., Lu, J.-Q., Takano, A., Model, K., Toyama, H., Suhara, T., Suzuki, K., Innis, R.B., Carson, R.E., 2003. Linearized reference tissue parametric imaging methods: application to [¹¹C]DASB positron emission tomography studies of the serotonin transporter in human brain. *J. Cereb. Blood Flow. Metab.* 23, 1096–1112. <http://dx.doi.org/10.1097/01.WCB.0000085441.37552.CA>.
- Innis, R.B., Cunningham, V.J., Delforge, J., Fujita, M., Gjedde, A., Gunn, R.N., Holden, J., Houle, S., Huang, S.-C., Ichise, M., Iida, H., Ito, H., Kimura, Y., Koeppe, R.A., Knudsen, G.M., Knuuti, J., Lammertsma, A.A., Laruelle, M., Logan, J., Maguire, R.P., Mintun, M.A., Morris, E.D., Parsey, R., Price, J.C., Slifstein, M., Sossi, V., Suhara, T., Votaw, J.R., Wong, D.F., Carson, R.E., 2007. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. *J. Cereb. Blood Flow. Metab.* 27, 1533–1539. <http://dx.doi.org/10.1038/sj.jcbfm.9600493>.
- Karege, F., Bondolfi, G., Gervasoni, N., Schwald, M., Aubry, J.-M., Bertschy, G., 2005. Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol. Psychiatry* 57 (9), 1068–1072. <http://dx.doi.org/10.1016/j.biopsych.2005.01.008>.
- Kernie, S.G., Liebl, D.J., Parada, L.F., 2000. BDNF regulates eating behavior and locomotor activity in mice. *EMBO J.* 19, 1290–1300. <http://dx.doi.org/10.1093/emboj/19.6.1290>.
- Kim, J.S., Ichise, M., Sangare, J., Innis, R.B., 2006. PET imaging of serotonin transporters with [¹¹C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. *J. Nucl. Med.* 47, 208–214.
- Lam, D.D., Heisler, L.K., 2007. Serotonin and energy balance: molecular mechanisms and implications for type 2 diabetes. *Expert Rev. Mol. Med.* 9, 1–24. <http://dx.doi.org/10.1017/S1462399407000245>.
- Leibowitz, S.F., 1990. The role of serotonin in eating disorders. *Drugs* 39 (Suppl. 3), 33–48.
- Lesch, K.-P., 2000. Molecular biology, pharmacology, and genetics of the serotonin transporter: psychobiological and clinical implications. In: Baumgarten, H.G., Göthert, M. (Eds.), *Serotonergic Neurons and 5-HT Receptors in the CNS* SE - 26, Handbook of Experimental Pharmacology. Springer, Berlin Heidelberg, pp. 671–705. http://dx.doi.org/10.1007/978-3-642-60921-3_26.
- Lesch, K.P., Aulakh, C.S., Wolozin, B.L., Tolliver, T.J., Hill, J.L., Murphy, D.L., 1993. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Brain Res. Mol. Brain Res.* 17, 31–35.
- Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Müller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531.
- Lim, J.-E., Papp, a., Pinsonneault, J., Sadée, W., Saffen, D., 2006. Allelic expression of serotonin transporter (SERT) mRNA in human pons: lack of correlation with the polymorphism SERTLR. *Mol. Psychiatry* 11, 649–662. <http://dx.doi.org/10.1038/sj.mp.4001797>.
- Lommatzsch, M., Zingler, D., Schubbaeck, K., Schloetcke, K., Zingler, C., Schuff-Werner, P., Virchow, J.C., 2005. The impact of age, weight and gender on BDNF levels in human platelets and plasma. *Neurobiol. Aging* 26, 115–123. <http://dx.doi.org/10.1016/j.neurobiolaging.2004.03.002>.
- Maffioletti, E., Zanardini, R., Gennarelli, M., Bocchio-Chiavetto, L., 2014. Influence of clotting duration on brain-derived neurotrophic factor (BDNF) dosage in serum. *Biotechniques* 57, 111–114.
- Mattson, M.P., Maudsley, S., Martin, B., 2004. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci.* 27, 589–594. <http://dx.doi.org/10.1016/j.tins.2004.08.001>.
- Mensink, G.B.M., Schienkiewitz, A., Haftenberger, M., Lampert, T., Ziese, T., Scheidt-Nave, C., 2013. Overweight and obesity in Germany: results of the german health interview and examination survey for adults (DEGS1). *Bundesgesundheitsblatt. Gesundheitsforsch. Gesundheitsschutz* 56, 786–794. <http://dx.doi.org/10.1007/s00103-012-1656-3>.
- Mueller, K., Möller, H.E., Horstmann, A., Busse, F., Lepsien, J., Blüher, M., Stumvoll, M., Villingr, A., Plegier, B., 2015. Physical exercise in overweight to obese individuals induces metabolic- and neurotrophic-related structural brain plasticity. *Front. Hum. Neurosci.* 9, 372. <http://dx.doi.org/10.3389/fnhum.2015.00372>.
- Murthy, N.V., Selvaraj, S., Cowen, P.J., Bhagwagar, Z., Riedel, W.J., Peers, P., Kennedy, J.L., Sahakian, B.J., Laruelle, M.A., Rabiner, E.A., Grasby, P.M., 2010. Serotonin transporter polymorphisms (SLC6A4 insertion/deletion and rs25531) do not affect the availability of 5-HTT to [¹¹C] DASB binding in the living human brain. *Neuroimage* 52, 50–54. <http://dx.doi.org/10.1016/j.neuroimage.2010.04.032>.
- Nguyen, D.M., El-Serag, H.B., 2010. The epidemiology of obesity. *Gastroenterol. Clin. North Am.* 39, 1–7. <http://dx.doi.org/10.1016/j.gtc.2009.12.014>.
- Numan, S., Seroogy, K.B., 1999. Expression of trkB and trkC mRNAs by adult midbrain dopamine neurons: a double-label in situ hybridization study. *J. Comp. Neurol.* 403, 295–308. [http://dx.doi.org/10.1002/\(SICI\)1096-9861\(19990118\)403:3<295::AID-CNE2>3.0.CO;2-L](http://dx.doi.org/10.1002/(SICI)1096-9861(19990118)403:3<295::AID-CNE2>3.0.CO;2-L).
- Ooi, C.L., Kennedy, J.L., Levitan, R.D., 2012. A putative model of overeating and obesity based on brain-derived neurotrophic factor: direct and indirect effects. *Behav. Neurosci.* <http://dx.doi.org/10.1037/a0028600>.
- Pelleymounter, M.A., Cullen, M.J., Wellman, C.L., 1995. Characteristics of BDNF-induced weight loss. *Exp. Neurol.* 131, 229–238.
- Praschak-Rieder, N., Willeit, M., Wilson, A.A., Houle, S., Meyer, J.H., 2008. Seasonal variation in human brain serotonin transporter binding. *Arch. Gen. Psychiatry* 65, 1072. <http://dx.doi.org/10.1001/archpsyc.65.9.1072>.
- Rosas-Vargas, H., Martínez-Ezquerro, J.D., Bienvenu, T., 2011. Brain-derived neurotrophic factor, food intake regulation, and obesity. *Arch. Med. Res.* 42, 482–494. <http://dx.doi.org/10.1016/j.arcmed.2011.09.005>.
- Salehi, A., Delcroix, J.-D., Mobley, W.C., 2003. Traffic at the intersection of neurotrophic factor signaling and neurodegeneration. *Trends Neurosci.* [http://dx.doi.org/10.1016/S0166-2236\(02\)00038-3](http://dx.doi.org/10.1016/S0166-2236(02)00038-3).
- Shioe, K., Ichimiya, T., Suhara, T., Takano, A., Sudo, Y., Yasuno, F., Hirano, M.,

- Shinohara, M., Kagami, M., Okubo, Y., Nankai, M., Kanba, S., 2003. No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by pet. *Synapse* 48, 184–188. <http://dx.doi.org/10.1002/syn.10204>.
- Sookoian, S., Gemma, C., García, S.I., Gianotti, T.F., Dieuzeide, G., Roussos, A., Tonietti, M., Trifone, L., Kanevsky, D., González, C.D., Pirola, C.J., 2007. Short allele of serotonin transporter gene promoter is a risk factor for obesity in adolescents. *Obes. Silver Spring* 15, 271–276. <http://dx.doi.org/10.1038/oby.2007.519>.
- Tonra, J.R., Ono, M., Liu, X., Garcia, K., Jackson, C., Yancopoulos, G.D., Wiegand, S.J., Wong, V., 1999. Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. *Diabetes* 48, 588–594.
- Tsuchida, a, Nonomura, T., Nakagawa, T., Itakura, Y., Ono-Kishino, M., Yamanaka, M., Sugaru, E., Taiji, M., Noguchi, H., 2002. Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice. *Diabetes. Obes. Metab.* 4, 262–269.
- Vaynman, S., Ying, Z., Wu, a, Gomez-Pinilla, F., 2006. Coupling energy metabolism with a mechanism to support brain-derived neurotrophic factor-mediated synaptic plasticity. *Neuroscience* 139, 1221–1234. <http://dx.doi.org/10.1016/j.neuroscience.2006.01.062>.
- Volkow, N.D., Wang, G.-J., Fowler, J.S., Telang, F., 2008. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 363, 3191–3200. <http://dx.doi.org/10.1098/rstb.2008.0107>.
- Waterhouse, E.G., Xu, B., 2009. New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. *Mol. Cell. Neurosci.* 42, 81–89. <http://dx.doi.org/10.1016/j.mcn.2009.06.009>.
- Willeit, M., Stastny, J., Pirker, W., Praschak-Rieder, N., Neumeister, A., Asenbaum, S., Tauscher, J., Fuchs, K., Sieghart, W., Hornik, K., Aschauer, H.N., Brücke, T., Kasper, S., 2001. No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biol. Psychiatry* 50, 8–12. [http://dx.doi.org/10.1016/S0006-3223\(00\)01123-9](http://dx.doi.org/10.1016/S0006-3223(00)01123-9).
- Wilson, A.A., Ginovart, N., Schmidt, M., Meyer, J.H., Threlkeld, P.G., Houle, S., 2000. Novel radiotracers for imaging the serotonin transporter by positron emission tomography: synthesis, radiosynthesis, and in vitro and ex vivo evaluation of (11)C-labeled 2-(phenylthio)araalkylamines. *J. Med. Chem.* 43, 3103–3110.
- Wisse, B.E., Schwartz, M.W., 2003. The skinny on neurotrophins. *Nat. Neurosci.* 6, 655–656. <http://dx.doi.org/10.1038/nn0703-655>.
- Yeo, G.S.H., Connie Hung, C.-C., Rochford, J., Keogh, J., Gray, J., Sivaramakrishnan, S., O'Rahilly, S., Farooqi, I.S., 2004. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat. Neurosci.* 7, 1187–1189. <http://dx.doi.org/10.1038/nn1336>.
- Zhou, F.C., Tao-Cheng, J.-H., Segu, L., Patel, T., Wang, Y., 1998. Serotonin transporters are located on the axons beyond the synaptic junctions: anatomical and functional evidence. *Brain Res.* 805, 241–254. [http://dx.doi.org/10.1016/S0006-8993\(98\)00691-X](http://dx.doi.org/10.1016/S0006-8993(98)00691-X).

Summary

Dissertation zur Erlangung des akademischen Grades Dr. med.

The Effect of Serum BDNF Levels on Central Serotonin Transporter Availability in Obese Versus Non-Obese Adults: A [^{11}C]DASB Positron Emission Tomography Study

Eingereicht von Philipp Hinderberger, B.Sc.

Angefertigt an der Universität Leipzig

Betreut von Prof. Dr. med. Swen Hesse

Mai 2016

To the best of our knowledge, no *in-vivo* studies have been undertaken to examine the influence of peripheral BDNF levels on 5-HTT availability in human obesity. Using [^{11}C]DASB and PET, this is the first study investigating the aforementioned parameters in a sample of obese individuals and comparing those findings with non-obese controls, to further elucidate the connection between 5-HT structures and its neurotrophic support system in obesity. Contrary to our initial hypothesis, there is no statistically significant correlation between any of these parameters in our obese subgroup.

However, interaction analyses revealed opposing effects of serum BDNF on 5-HTT binding in the NAcc in obese versus non-obese individuals. While they are positively correlated in the former, the latter shows a negative correlation. Considering 5-HTT availability holds information about serotonergic tone, this finding suggests conditions of brain BDNF (indexed by peripheral BDNF levels) play a different role in obese and non-obese individuals in predicting serotonergic tonus in the NAcc. This brain area belongs to the mesolimbic dopamine system widely known for its role in reward seeking behavior. There is evidence that BDNF and TrkB are particularly expressed¹²⁶ and involved in the neurophysiology of drug dependence in these regions.¹²⁷ Although these studies are related to illicit drug consumption, palatable food intake has been shown to elicit similar dopaminergic reward pathways.¹²⁸ Moreover, dopamine and serotonin neurotransmission seem to reciprocally modulate each other in the NAcc.¹²⁹

Although exact working principles remain unknown, this could be an indication that BDNF is affecting energy homeostasis through distinct neuronal pathways in the NAcc integrating both the dopamine and serotonin system.

We further assessed the interaction between BDNF and 5-HTT by genotyping the 5-HTT promotor polymorphism. Long allelic homozygotes showed a negative correlation in the hippocampus (i.e., higher peripheral BDNF levels predicted lower 5-HTT density). This contradicts animal models which previously reported a positive correlation.^{130,131} Hippocampal function has been extensively studied regarding long-term potentiation and memory formation. Recently, this brain region has received increased attention for its potential role in energy metabolism. Specifically, hypotheses linking high-caloric intake to hippocampal disruption and attenuated BDNF secretion are under investigation.¹⁰⁹ A vicious circle of excess food intake distributing hippocampal function thereby further reducing its inhibitory effects over food intake might contribute to the pathogenesis of obesity.

Val66met, a polymorphism of the BDNF-coding gene, holds predictive value of serotonergic tone¹³² in addition to the functional activity of its mature protein.¹¹⁸ Our team has ongoing efforts assessing the influence of BDNF genotype as a link between impaired BDNF expression and an increased risk for obesity.

In conclusion, BDNF and 5-HTT availability do not qualify as suitable measures for predicting BMI in this study sample. Contrary to our hypothesis, we were unable to detect any significant intergroup differences regarding 5-HTT density and BDNF levels. However, there was evidence for opposing effects of serum BDNF on 5-HTT availability in the NAcc in obese versus non-obese subjects. Corroborating previous studies, we suggest that allelic variations of the 5-HTT polymorphism have a minor (if present at all) impact on 5-HTT availability in obese and non-obese individuals.^{133–136} We advocate that concentration of serum BDNF does not significantly change with respect to sex, age, sunshine exposure, scan time, and body weight, which also complements previous reports.¹³⁷ While this remains a topic of scientific debate, additional studies will be necessary to define the exact impact and neurophysiology of BDNF on energy homeostasis.

Works Cited

1. Nguyen DM, El-Serag HB. The epidemiology of obesity. *Gastroenterol Clin North Am.* 2010;39(1):1-7. doi:10.1016/j.gtc.2009.12.014.
2. Mensink GBM, Schienkiewitz A, Haftenberger M, Lampert T, Ziese T, Scheidt-Nave C. [Overweight and obesity in Germany: results of the German Health Interview and Examination Survey for Adults (DEGS1)]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz.* 2013;56(5-6):786-794. doi:10.1007/s00103-012-1656-3.
3. Mayer J, Thomas DW. Regulation of Food Intake and Obesity. *Science (80-).* 1967;156:328-337.
4. Kennedy GC. The Role of Depot Fat in the Hypothalamic Control of Food Intake in the Rat. *Proc R Soc B Biol Sci.* 1953;140(901):578-592. doi:10.1098/rspb.1953.0009.
5. Gao Q, Horvath TL. Neurobiology of feeding and energy expenditure. *Annu Rev Neurosci.* 2007;30:367-398. doi:10.1146/annurev.neuro.30.051606.094324.
6. Woods SC, Strubbe JH. The psychobiology of meals. *Psychon Bull Rev.* 1994;1(2):141-155. doi:10.3758/BF03200770.
7. Woods SC. The eating paradox: how we tolerate food. *Psychol Rev.* 1991;98(4):488-505.
8. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature.* 1994;372(6505):425-432. doi:10.1038/372425a0.
9. Considine R V, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med.* 1996;334(5):292-295. doi:10.1056/NEJM199602013340503.
10. Baskin DG, Breininger JF, Schwartz MW. Leptin receptor mRNA identifies a subpopulation of neuropeptide Y neurons activated by fasting in rat hypothalamus. *Diabetes.* 1999;48(4):828-833.
11. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med.* 1996;2(5):589-593.
12. Lee GH, Proenca R, Montez JM, et al. Abnormal splicing of the leptin receptor in diabetic mice. *Nature.* 1996;379(6566):632-635. doi:10.1038/379632a0.
13. Halaas JL, Boozer C, Blair-West J, Fidathusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proc Natl Acad Sci U S A.* 1997;94(16):8878-8883.
14. Woods SC, Lotter EC, McKay LD, Porte D. Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature.* 1979;282(5738):503-505.
15. Bagdade JD, Bierman EL, Porte D. The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J Clin Invest.* 1967;46(10):1549-1557. doi:10.1172/JCI105646.
16. Turton MD, O'Shea D, Gunn I, et al. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature.* 1996;379(6560):69-72. doi:10.1038/379069a0.
17. Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature.* 2002;418(6898):650-654. doi:10.1038/nature02666.
18. Gibbs J, Young RC, Smith GP. Cholecystokinin decreases food intake in rats. *J*

- Comp Physiol Psychol.* 1973;84(3):488-495.
19. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature.* 2000;407(6806):908-913. doi:10.1038/35038090.
 20. Wang C, Bomberg E, Levine A, Billington C, Kotz CM. Brain-derived neurotrophic factor in the ventromedial nucleus of the hypothalamus reduces energy intake. *Am J Physiol Regul Integr Comp Physiol.* 2007;293(151):R1037-R1045. doi:10.1152/ajpregu.00125.2007.
 21. Lam TKT. Neuronal regulation of homeostasis by nutrient sensing. *Nat Med.* 2010;16(4):392-395. doi:10.1038/nm0410-392.
 22. Strubbe JH, Woods SC. The timing of meals. *Psychol Rev.* 2004;111(1):128-141. doi:10.1037/0033-295X.111.1.128.
 23. West DB, Fey D, Woods SC. Cholecystokinin persistently suppresses meal size but not food intake in free-feeding rats. *Am J Physiol.* 1984;246(5 Pt 2):R776-R787.
 24. Barrachina MD, Martínez V, Wang L, Wei JY, Taché Y. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A.* 1997;94(19):10455-10460.
 25. Figlewicz DP, Stein LJ, West D, Porte D, Woods SC. Intracisternal insulin alters sensitivity to CCK-induced meal suppression in baboons. *Am J Physiol.* 1986;250(5 Pt 2):R856-R860.
 26. McMinn JE, Sindelar DK, Havel PJ, Schwartz MW. Leptin deficiency induced by fasting impairs the satiety response to cholecystokinin. *Endocrinology.* 2000;141(12):4442-4448. doi:10.1210/endo.141.12.7815.
 27. Hulsey MG, Lu H, Wang T, Martin RJ, Baile CA. Intracerebroventricular (i.c.v.) administration of mouse leptin in rats: behavioral specificity and effects on meal patterns. *Physiol Behav.* 1998;65(3):445-455.
 28. Emond M, Schwartz GJ, Ladenheim EE, Moran TH. Central leptin modulates behavioral and neural responsivity to CCK. *Am J Physiol.* 1999;276(5 Pt 2):R1545-R1549.
 29. Williams DL, Baskin DG, Schwartz MW. Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. *Endocrinology.* 2009;150(4):1680-1687. doi:10.1210/en.2008-1045.
 30. McLaughlin CL, Baile CA. Decreased sensitivity of Zucker obese rats to the putative satiety agent cholecystokinin. *Physiol Behav.* 1980;25(4):543-548.
 31. Grill HJ, Schwartz MW, Kaplan JM, Foxhall JS, Breininger J, Baskin DG. Evidence that the caudal brainstem is a target for the inhibitory effect of leptin on food intake. *Endocrinology.* 2002;143(1):239-246. doi:10.1210/endo.143.1.8589.
 32. Morton GJ, Blevins JE, Williams DL, et al. Leptin action in the forebrain regulates the hindbrain response to satiety signals. *J Clin Invest.* 2005;115(3):703-710. doi:10.1172/JCI22081.
 33. Barrera JG, Sandoval DA, D'Alessio DA, Seeley RJ. GLP-1 and energy balance: an integrated model of short-term and long-term control. *Nat Rev Endocrinol.* 2011;7(9):507-516. doi:10.1038/nrendo.2011.77.
 34. Figlewicz DP. Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. *Am J Physiol Regul Integr Comp Physiol.* 2003;284(4):R882-R892. doi:10.1152/ajpregu.00602.2002.
 35. Dossat AM, Lilly N, Kay K, Williams DL. Glucagon-like peptide 1 receptors in nucleus accumbens affect food intake. *J Neurosci.* 2011;31(41):14453-14457. doi:10.1523/JNEUROSCI.3262-11.2011.

36. Mehta S, Melhorn SJ, Smeraglio A, et al. Regional brain response to visual food cues is a marker of satiety that predicts food choice. *Am J Clin Nutr.* 2012;96(5):989-999. doi:10.3945/ajcn.112.042341.
37. Figlewicz DP, Sipols AJ. Energy regulatory signals and food reward. *Pharmacol Biochem Behav.* 2010;97(1):15-24. doi:10.1016/j.pbb.2010.03.002.
38. Fulton S, Woodside B, Shizgal P. Modulation of brain reward circuitry by leptin. *Science.* 2000;287(5450):125-128.
39. Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC. Leptin regulates striatal regions and human eating behavior. *Science.* 2007;317(5843):1355. doi:10.1126/science.1144599.
40. Berthoud H-R, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. *Am J Physiol Regul Integr Comp Physiol.* 2011;300(6):R1266-R1277. doi:10.1152/ajpregu.00028.2011.
41. Stice E, Figlewicz DP, Gosnell BA, Levine AS, Pratt WE. The contribution of brain reward circuits to the obesity epidemic. *Neurosci Biobehav Rev.* 2013;37(9 Pt A):2047-2058. doi:10.1016/j.neubiorev.2012.12.001.
42. Morton GJ, Meek TH, Schwartz MW. Neurobiology of food intake in health and disease. *Nat Rev Neurosci.* 2014;15(6):367-378. doi:10.1038/nrn3745.
43. Kenny PJ. Common cellular and molecular mechanisms in obesity and drug addiction. *Nat Rev Neurosci.* 2011;12(11):638-651. doi:10.1038/nrn3105.
44. Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav.* 2005;86(5):773-795. doi:10.1016/j.physbeh.2005.08.066.
45. Leininger GM, Jo Y-H, Leshan RL, et al. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metab.* 2009;10(2):89-98. doi:10.1016/j.cmet.2009.06.011.
46. Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of AgRP and NPY in fasting-activated hypothalamic neurons. *Nat Neurosci.* 1998;1(4):271-272. doi:10.1038/1082.
47. Hagan MM, Rushing PA, Pritchard LM, et al. Long-term orexigenic effects of AgRP-(83---132) involve mechanisms other than melanocortin receptor blockade. *Am J Physiol Regul Integr Comp Physiol.* 2000;279(1):R47-R52.
48. Sohn J-W, Elmquist JK, Williams KW. Neuronal circuits that regulate feeding behavior and metabolism. *Trends Neurosci.* 2013;36(9):504-512. doi:10.1016/j.tins.2013.05.003.
49. Cowley MA, Smart JL, Rubinstein M, et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature.* 2001;411(6836):480-484. doi:10.1038/35078085.
50. Spanswick D, Smith MA, Groppi VE, Logan SD, Ashford ML. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature.* 1997;390(6659):521-525. doi:10.1038/37379.
51. Spanswick D, Smith MA, Mirshamsi S, Routh VH, Ashford ML. Insulin activates ATP-sensitive K⁺ channels in hypothalamic neurons of lean, but not obese rats. *Nat Neurosci.* 2000;3(8):757-758. doi:10.1038/77660.
52. Schwartz MW, Baskin DG, Bukowski TR, et al. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes.* 1996;45(4):531-535.
53. Cowley MA, Smith RG, Diano S, et al. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating

- energy homeostasis. *Neuron*. 2003;37(4):649-661.
54. Schwartz MW, Seeley RJ, Woods SC, et al. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes*. 1997;46(12):2119-2123.
 55. Williams KW, Margatho LO, Lee CE, et al. Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J Neurosci*. 2010;30(7):2472-2479. doi:10.1523/JNEUROSCI.3118-09.2010.
 56. MacNeil DJ, Howard AD, Guan X, et al. The role of melanocortins in body weight regulation: opportunities for the treatment of obesity. *Eur J Pharmacol*. 2002;450(1):93-109.
 57. Geerling JC, Shin J-W, Chimenti PC, Loewy AD. Paraventricular hypothalamic nucleus: axonal projections to the brainstem. *J Comp Neurol*. 2010;518(9):1460-1499. doi:10.1002/cne.22283.
 58. Hall JE, da Silva AA, do Carmo JM, et al. Obesity-induced hypertension: role of sympathetic nervous system, leptin, and melanocortins. *J Biol Chem*. 2010;285(23):17271-17276. doi:10.1074/jbc.R110.113175.
 59. Sohn J-W, Xu Y, Jones JE, Wickman K, Williams KW, Elmquist JK. Serotonin 2C receptor activates a distinct population of arcuate pro-opiomelanocortin neurons via TRPC channels. *Neuron*. 2011;71(3):488-497. doi:10.1016/j.neuron.2011.06.012.
 60. Leibowitz SF. The role of serotonin in eating disorders. *Drugs*. 1990;39 Suppl 3:33-48.
 61. Sookoian S, Gemma C, García SI, et al. Short allele of serotonin transporter gene promoter is a risk factor for obesity in adolescents. *Obesity (Silver Spring)*. 2007;15(2):271-276. doi:10.1038/oby.2007.519.
 62. Lam DD, Heisler LK. Serotonin and energy balance: molecular mechanisms and implications for type 2 diabetes. *Expert Rev Mol Med*. 2007;9(5):1-24. doi:10.1017/S1462399407000245.
 63. Donovan MH, Tecott LH. Serotonin and the regulation of mammalian energy balance. *Front Neurosci*. 2013;7:36. doi:10.3389/fnins.2013.00036.
 64. Halford JCG, Harrold JA, Lawton CL, Blundell JE. Serotonin (5-HT) drugs: effects on appetite expression and use for the treatment of obesity. *Curr Drug Targets*. 2005;6(2):201-213.
 65. Monti JM. Serotonin control of sleep-wake behavior. *Sleep Med Rev*. 2011;15(4):269-281. doi:10.1016/j.smrv.2010.11.003.
 66. Canli T, Lesch K-P. Long story short: the serotonin transporter in emotion regulation and social cognition. *Nat Neurosci*. 2007;10(9):1103-1109. doi:10.1038/nn1964.
 67. Lam DD, Garfield AS, Marston OJ, Shaw J, Heisler LK. Brain serotonin system in the coordination of food intake and body weight. *Pharmacol Biochem Behav*. 2010;97(1):84-91. doi:10.1016/j.pbb.2010.09.003.
 68. Keszthelyi D, Troost FJ, Masclee AAM. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. *Neurogastroenterol Motil*. 2009;21(12):1239-1249. doi:10.1111/j.1365-2982.2009.01370.x.
 69. Lesurtel M, Graf R, Aleil B, et al. Platelet-derived serotonin mediates liver regeneration. *Science*. 2006;312(5770):104-107. doi:10.1126/science.1123842.
 70. Yadav VK, Ryu J-H, Suda N, et al. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell*. 2008;135(5):825-837. doi:10.1016/j.cell.2008.09.059.

71. Kim K, Oh C-M, Ohara-Imaizumi M, et al. Functional role of serotonin in insulin secretion in a diet-induced insulin-resistant state. *Endocrinology*. 2015;156(2):444-452. doi:10.1210/en.2014-1687.
72. Hannon J, Hoyer D. Molecular biology of 5-HT receptors. *Behav Brain Res*. 2008;195(1):198-213. doi:10.1016/j.bbr.2008.03.020.
73. Blundell JE. Serotonin and the biology of feeding. *Am J Clin Nutr*. 1992;55(1 Suppl):155S - 159S.
74. Breisch ST, Zemlan FP, Hoebel BG. Hyperphagia and obesity following serotonin depletion by intraventricular p-chlorophenylalanine. *Science*. 1976;192(4237):382-385.
75. Feldman JM. Effect of the monoamine oxidase inhibitors clorgyline and pargyline on the hyperphagia of obese mice. *Behav Brain Res*. 1988;29(1-2):147-158.
76. Heal DJ, Aspley S, Prow MR, Jackson HC, Martin KF, Cheetham SC. Sibutramine: a novel anti-obesity drug. A review of the pharmacological evidence to differentiate it from d-amphetamine and d-fenfluramine. *Int J Obes Relat Metab Disord*. 1998;22 Suppl 1:S18-S28; discussion S29.
77. Heisler LK, Kanarek RB, Gerstein A. Fluoxetine decreases fat and protein intakes but not carbohydrate intake in male rats. *Pharmacol Biochem Behav*. 1997;58(3):767-773.
78. Kennett GA, Curzon G. Evidence that hypophagia induced by mCPP and TFMPD requires 5-HT_{1C} and 5-HT_{1B} receptors; hypophagia induced by RU 24969 only requires 5-HT_{1B} receptors. *Psychopharmacology (Berl)*. 1988;96(1):93-100.
79. Vickers SP, Clifton PG, Dourish CT, Tecott LH. Reduced satiating effect of d-fenfluramine in serotonin 5-HT_{2C} receptor mutant mice. *Psychopharmacology (Berl)*. 1999;143(3):309-314.
80. Lam DD, Przydzial MJ, Ridley SH, et al. Serotonin 5-HT_{2C} receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology*. 2008;149(3):1323-1328. doi:10.1210/en.2007-1321.
81. Zhou L, Sutton GM, Rochford JJ, et al. Serotonin 2C receptor agonists improve type 2 diabetes via melanocortin-4 receptor signaling pathways. *Cell Metab*. 2007;6(5):398-405. doi:10.1016/j.cmet.2007.10.008.
82. Nonogaki K, Strack AM, Dallman MF, Tecott LH. Leptin-independent hyperphagia and type 2 diabetes in mice with a mutated serotonin 5-HT_{2C} receptor gene. *Nat Med*. 1998;4(10):1152-1156. doi:10.1038/2647.
83. Bouwknecht JA, van der Gugten J, Hijzen TH, Maes RA, Hen R, Olivier B. Male and female 5-HT_{1B} receptor knockout mice have higher body weights than wildtypes. *Physiol Behav*. 74(4-5):507-516.
84. Halford JC, Blundell JE. The 5-HT_{1B} receptor agonist CP-94,253 reduces food intake and preserves the behavioural satiety sequence. *Physiol Behav*. 1996;60(3):933-939.
85. Colman E, Golden J, Roberts M, Egan A, Weaver J, Rosebraugh C. The FDA's assessment of two drugs for chronic weight management. *N Engl J Med*. 2012;367(17):1577-1579. doi:10.1056/NEJMp1211277.
86. Martin CK, Redman LM, Zhang J, et al. Lorcaserin, a 5-HT_{2C} receptor agonist, reduces body weight by decreasing energy intake without influencing energy expenditure. *J Clin Endocrinol Metab*. 2011;96(3):837-845. doi:10.1210/jc.2010-1848.
87. Heisler LK, Jobst EE, Sutton GM, et al. Serotonin reciprocally regulates

- melanocortin neurons to modulate food intake. *Neuron*. 2006;51(2):239-249. doi:10.1016/j.neuron.2006.06.004.
88. Heisler LK, Cowley MA, Tecott LH, et al. Activation of central melanocortin pathways by fenfluramine. *Science*. 2002;297(5581):609-611. doi:10.1126/science.1072327.
 89. Farooqi IS, O'Rahilly S. Mutations in ligands and receptors of the leptin-melanocortin pathway that lead to obesity. *Nat Clin Pract Endocrinol Metab*. 2008;4(10):569-577. doi:10.1038/ncpendmet0966.
 90. Laviano A, Inui A, Marks DL, et al. Neural control of the anorexia-cachexia syndrome. *Am J Physiol Endocrinol Metab*. 2008;295(5):E1000-E1008. doi:10.1152/ajpendo.90252.2008.
 91. Nicholson JR, Peter JC, Lecourt AC, Barde YA, Hofbauer KG. Melanocortin-4 receptor activation stimulates hypothalamic brain-derived neurotrophic factor release to regulate food intake, body temperature and cardiovascular function. *J Neuroendocrinol*. 2007;19(12):974-982. doi:10.1111/j.1365-2826.2007.01610.x.
 92. Hall JE. Dietary Balances; Regulation of Feeding; Obesity and Starvation; Vitamins and Minerals. In: *Guyton and Hall Textbook of Medical Physiology*. 13th ed. Philadelphia, PA: Elsevier; 2016:887-902.
 93. Vaynman S, Ying Z, Wu a., Gomez-Pinilla F. Coupling energy metabolism with a mechanism to support brain-derived neurotrophic factor-mediated synaptic plasticity. *Neuroscience*. 2006;139(4):1221-1234. doi:10.1016/j.neuroscience.2006.01.062.
 94. Kernie SG, Liebl DJ, Parada LF. BDNF regulates eating behavior and locomotor activity in mice. *EMBO J*. 2000;19(6):1290-1300. doi:10.1093/emboj/19.6.1290.
 95. Tonra JR, Ono M, Liu X, et al. Brain-derived neurotrophic factor improves blood glucose control and alleviates fasting hyperglycemia in C57BLKS-Lepr(db)/lepr(db) mice. *Diabetes*. 1999;48(3):588-594.
 96. Tsuchida a, Nonomura T, Nakagawa T, et al. Brain-derived neurotrophic factor ameliorates lipid metabolism in diabetic mice. *Diabetes Obes Metab*. 2002;4(4):262-269.
 97. Xu B, Goulding EH, Zang K, et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci*. 2003;6(7):736-742. doi:10.1038/nn1073.
 98. Huang EJ, Reichardt LF. Neurotrophins: roles in neuronal development and function. *Annu Rev Neurosci*. 2001;24:677-736. doi:10.1146/annurev.neuro.24.1.677.
 99. Kerschensteiner M, Gallmeier E, Behrens L, et al. Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation? *J Exp Med*. 1999;189(5):865-870.
 100. Nakahashi T, Fujimura H, Altar CA, et al. Vascular endothelial cells synthesize and secrete brain-derived neurotrophic factor. *FEBS Lett*. 2000;470(2):113-117.
 101. Matthews VB, Aström M-B, Chan MHS, et al. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia*. 2009;52(7):1409-1418. doi:10.1007/s00125-009-1364-1.
 102. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology*. 1998;37(12):1553-1561.

103. Klein AB, Williamson R, Santini M a, et al. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol*. 2011;14(3):347-353. doi:10.1017/S1461145710000738.
104. Curtis R, Adryan KM, Stark JL, et al. Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron*. 1995;14(6):1201-1211.
105. Waterhouse EG, Xu B. New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. *Mol Cell Neurosci*. 2009;42(2):81-89. doi:10.1016/j.mcn.2009.06.009.
106. Salehi A, Delcroix J-D, Mobley WC. Traffic at the intersection of neurotrophic factor signaling and neurodegeneration. *Trends Neurosci*. 2003;26(2):73-80. doi:10.1016/S0166-2236(02)00038-3.
107. Yeo GSH, Connie Hung C-C, Rochford J, et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. *Nat Neurosci*. 2004;7(11):1187-1189. doi:10.1038/nn1336.
108. Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci*. 2004;27(10):589-594. doi:10.1016/j.tins.2004.08.001.
109. Ooi CL, Kennedy JL, Levitan RD. A putative model of overeating and obesity based on brain-derived neurotrophic factor: Direct and indirect effects. *Behav Neurosci*. 2012;126(4):505-514. doi:10.1037/a0028600.
110. Faith MS, Kral TVE. Social Environmental and Genetic Influences on Obesity and Obesity-Promoting Behaviors: Fostering Research Integration. 2006.
111. Lesch KP. Molecular Biology, Pharmacology, and Genetics of the Serotonin Transporter: Psychobiological and Clinical Implications. In: Baumgarten HG, Göthert M, eds. *Serotonergic Neurons and 5-HT Receptors in the CNS SE - 26*. Vol 129. Handbook of Experimental Pharmacology. Springer Berlin Heidelberg; 2000:671-705. doi:10.1007/978-3-642-60921-3_26.
112. Nakamura M, Ueno S, Sano A, Tanabe H. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry*. 2000;5(1):32-38.
113. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996;274(5292):1527-1531.
114. Lesch KP, Aulakh CS, Wolozin BL, Tolliver TJ, Hill JL, Murphy DL. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Brain Res Mol Brain Res*. 1993;17(1-2):31-35.
115. Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. *Alcohol Clin Exp Res*. 2005;29(1):8-16.
116. Fuemmeler BF, Agurs-Collins TD, McClernon FJ, et al. Genes implicated in serotonergic and dopaminergic functioning predict BMI categories. *Obesity (Silver Spring)*. 2008;16(2):348-355. doi:10.1038/oby.2007.65.
117. Sookoian S, Gianotti TF, Gemma C, Burgueño A, Pirola CJ. Contribution of the functional 5-HTTLPR variant of the SLC6A4 gene to obesity risk in male adults. *Obesity (Silver Spring)*. 2008;16(2):488-491. doi:10.1038/oby.2007.64.
118. Egan MF, Kojima M, Callicott JH, et al. The BDNF val66met Polymorphism Affects Activity-Dependent Secretion of BDNF and Human Memory and Hippocampal Function. *Cell*. 2003;112(2):257-269. doi:10.1016/S0092-8674(03)00035-7.

119. Skledar M, Nikolac M, Dodig-Curkovic K, Curkovic M, Borovecki F, Pivac N. Association between brain-derived neurotrophic factor Val66Met and obesity in children and adolescents. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 2012;36(1):136-140. doi:10.1016/j.pnpbp.2011.08.003.
120. Beckers S, Peeters A, Zegers D, Mertens I, Van Gaal L, Van Hul W. Association of the BDNF Val66Met variation with obesity in women. *Mol Genet Metab*. 95(1-2):110-112. doi:10.1016/j.ymgme.2008.06.008.
121. Gunstad J, Schofield P, Paul RH, et al. BDNF Val66Met polymorphism is associated with body mass index in healthy adults. *Neuropsychobiology*. 2006;53(3):153-156. doi:10.1159/000093341.
122. Shugart YY, Chen L, Day INM, et al. Two British women studies replicated the association between the Val66Met polymorphism in the brain-derived neurotrophic factor (BDNF) and BMI. *Eur J Hum Genet*. 2009;17(8):1050-1055. doi:10.1038/ejhg.2008.272.
123. Nikolac Perkovic M, Mustapic M, Pavlovic M, et al. Lack of association between brain-derived neurotrophic factor Val66Met polymorphism and body mass index change over time in healthy adults. *Neurosci Lett*. 2013;545:127-131. doi:10.1016/j.neulet.2013.04.036.
124. Arijia V, Ferrer-Barcala M, Aranda N, Canals J. BDNF Val66Met polymorphism, energy intake and BMI: a follow-up study in schoolchildren at risk of eating disorders. *BMC Public Health*. 2010;10(1):363. doi:10.1186/1471-2458-10-363.
125. Mueller K, Möller HE, Horstmann A, et al. Physical exercise in overweight to obese individuals induces metabolic- and neurotrophic-related structural brain plasticity. *Front Hum Neurosci*. 2015;9:372. doi:10.3389/fnhum.2015.00372.
126. Numan S, Seroogy KB. Expression of trkB and trkC mRNAs by adult midbrain dopamine neurons: A double-label in situ hybridization study. *J Comp Neurol*. 1999;403(3):295-308. doi:10.1002/(SICI)1096-9861(19990118)403:3<295::AID-CNE2>3.0.CO;2-L.
127. Graham DL, Edwards S, Bachtell RK, DiLeone RJ, Rios M, Self DW. Dynamic BDNF activity in nucleus accumbens with cocaine use increases self-administration and relapse. *Nat Neurosci*. 2007;10(8):1029-1037. doi:10.1038/nn1929.
128. Volkow ND, Wang G-J, Fowler JS, Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos Trans R Soc Lond B Biol Sci*. 2008;363(1507):3191-3200. doi:10.1098/rstb.2008.0107.
129. De Deurwaerdère P, Stinus L, Spampinato U. Opposite change of in vivo dopamine release in the rat nucleus accumbens and striatum that follows electrical stimulation of dorsal raphe nucleus: role of 5-HT3 receptors. *J Neurosci*. 1998;18(16):6528-6538.
130. Deltheil T, Guiard BP, Cerdan J, et al. Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. *Neuropharmacology*. 2008;55(6):1006-1014. doi:10.1016/j.neuropharm.2008.08.001.
131. Homberg JR, Molteni R, Calabrese F, Riva M a. The serotonin-BDNF duo: Developmental implications for the vulnerability to psychopathology. *Neurosci Biobehav Rev*. 2014;43:35-47. doi:10.1016/j.neubiorev.2014.03.012.
132. Fisher PM, Holst KK, Adamsen D, et al. BDNF Val66met and 5-HTTLPR polymorphisms predict a human in vivo marker for brain serotonin levels. *Hum Brain Mapp*. September 2014. doi:10.1002/hbm.22630.
133. Lim J-E, Papp a, Pinsonneault J, Sadée W, Saffen D. Allelic expression of

- serotonin transporter (SERT) mRNA in human pons: lack of correlation with the polymorphism SERTLPR. *Mol Psychiatry*. 2006;11(7):649-662. doi:10.1038/sj.mp.4001797.
134. Shioe K, Ichimiya T, Suhara T, et al. No association between genotype of the promoter region of serotonin transporter gene and serotonin transporter binding in human brain measured by pet. *Synapse*. 2003;48(4):184-188. doi:10.1002/syn.10204.
 135. Willeit M, Stastny J, Pirker W, et al. No evidence for in vivo regulation of midbrain serotonin transporter availability by serotonin transporter promoter gene polymorphism. *Biol Psychiatry*. 2001;50(1):8-12. doi:10.1016/S0006-3223(00)01123-9.
 136. Murthy N V, Selvaraj S, Cowen PJ, et al. Serotonin transporter polymorphisms (SLC6A4 insertion/deletion and rs25531) do not affect the availability of 5-HTT to [11C] DASB binding in the living human brain. *Neuroimage*. 2010;52(1):50-54. doi:10.1016/j.neuroimage.2010.04.032.
 137. Gajewska E, Sobieska M, Suwalska A. Obesity itself does not influence BDNF serum levels in adults. *Eur Rev Med Pharmacol Sci*. 2014;18(21):3246-3250.

Appendices

Glossary

[¹¹C]DASB [¹¹C]-3-Amino-4-(2-Dimethylaminomethylphenylsulfanyl)-Benzonitrile

α-MSH α-Melanocyte-Stimulating Hormone

5-HT 5-Hydroxytryptamine, Serotonin

5-HTT Serotonin Reuptake Transporter, SERT

5-HTTLPR Serotonin-Transporter-Linked Polymorphic Region

ACC Anterior Cingulate Cortex

AgRP Agouti-Related Peptide

ANF Adiposity Negative Feedback

ARC Arcuate Nucleus of the Hypothalamus

BDNF Brain-Derived Neurotrophic Factor

BMI Body Mass Index

BP_{ND} Non-Displaceable Binding Potential (i.e., Radioligand Uptake)

CART Cocaine- and Amphetamine-Regulated Transcript

CCK Cholecystokinin

DLPFC Dorsolateral Prefrontal Cortex

FFA Free Fatty Acids

GHSR Growth Hormone Secretagogue Receptor

GLM General Linear Model

GLP1 Glucagon-Like Peptide-1

HCN Head of the Caudate Nucleus

HTR1B 5-Hydroxytryptamine Receptor 1B

HTR2C 5-Hydroxytryptamine Receptor 2C

HWE Hardy-Weinberg Equilibrium

LEPR Leptin Receptor

LHA Lateral Hypothalamic Area

MC3R Melanocortin 3 Receptor

MC4R Melanocortin 4 Receptor

mPFC Medial Prefrontal Cortex

MRI Magnetic Resonance Imaging

MWW Mann-Whitney-Wilcoxon Test

NAcc Nucleus Accumbens

NPY1R Neuropeptide Y Receptor Y1

NTS Nucleus of Solitary Tract

NPY Neuropeptide Y

OFC Orbitofrontal Cortex

PCR Polymerase Chain Reaction

PET Positron Emission Tomography

POMC Proopiomelanocortin

PVN Paraventricular Nucleus of Hypothalamus

SERT Serotonin Reuptake Transporter, 5-HTT

SN/VTA Substantia Nigra/Ventral Tegmental Area

TrkB Tropomyosine Receptor Kinase B

VOI Volume of Interest

VTA Ventral Tegmental Area

Curriculum Vitae

(Auszüge)

Name: Hinderberger
Vorname: Philipp Norbert Heinz-Johann
geboren: 08.09.1985
in: Bad Säckingen

Schul- und Berufsausbildung: 1996–2005 Klettgau Gymnasium-Tiengen
2005–2009 Biomedical Engineering, Nebenfach Biophysik, The George Washington University, Washington D.C., USA
2009–2016 Humanmedizin, Universität Leipzig
2015–2016 Praktisches Jahr:
Universitätsklinikum Leipzig, Klinik für Viszeral-, Transplantations-, Thorax- und Gefäßchirurgie
Universität Leipzig, Institut für Rechtsmedizin
Universitätsklinikum Leipzig, Klinik und Poliklinik für Urologie
Universitätsklinikum UAB, Birmingham, USA, Innere Medizin

Abschlüsse: 2005 Allgemeine Hochschulreife
2009 Bachelor of Science
2016 Ärztliche Prüfung und Approbation als Arzt

Promotion: 2014–2016 Thema: «The Effect of Serum BDNF Levels on Central Serotonin Transporter Availability in Obese Versus Non-Obese Adults: A [¹¹C]DASB Positron Emission Tomography Study», Universität Leipzig, Klinik und Poliklinik für Nuklearmedizin, Betreuer: Prof. Dr. med. Swen Hesse

Zürich, 28. November 2016

Publications

Hinderberger P, Rullmann M, Drabe M, et al. The Effect of Serum BDNF Levels on Central Serotonin Transporter Availability in Obese Versus Non-Obese Adults: A [¹¹C]DASB Positron Emission Tomography Study. *Neuropharmacology*. 2016 Nov;110(Pt A):530-6. doi: 10.1016/j.neuropharm.2016.04.030.

Beier HM, Daele W van den, Diedrich K, Dudenhausen JW, Felberbaum R, Gigerenzer G, Gille G, Habenicht FU, **Hinderberger P**, Holzgreve W, Ledger W, Nieschlag E, Ritzinger P, Taupitz J, Velde ER te. (2012). Medizinische und biologische Aspekte der Fertilität. In Stock G, Bertram H, Fürnkranz-Prskawetz A, Holzgreve W, Kohli M, & Staudinger UM (Eds.) *Zukunft mit Kindern: Fertilität und gesellschaftliche Entwicklung in Deutschland, Österreich und der Schweiz* (pp. 294–390). Frankfurt am Main: Campus Verlag.

Statement on Academic Integrity

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren.

9. Mai 2016

Datum

Unterschrift

Acknowledgments

Die vorliegende Arbeit entstand während meiner Tätigkeit als Doktorand an der Klinik und Poliklinik für Nuklearmedizin am Universitätsklinikum Leipzig. Nach dem erfolgreichen Abschluss meiner Tätigkeit möchte ich die Gelegenheit nutzen, um mich an dieser Stelle bei denjenigen Menschen zu bedanken, die maßgeblich zum Gelingen dieser Dissertation beigetragen haben.

Ich danke meinem Doktorvater Professor Dr. Swen Hesse für seine Unterstützung während meiner Promotionszeit. Von ihm bekam ich die notwendige Weisung, um diese Arbeit erfolgreich zu vollenden und gleichzeitig die nötige Freiheit, um meine eigenen Ideen umsetzen zu können. Ein großer Dank gilt den Mitarbeitern der Klinik und Poliklinik für Nuklearmedizin mit denen ich über mehrere Jahre zusammenarbeiten durfte. Besonders möchte ich Dr. Michael Rullmann danken. Mit seiner Erfahrung, Hilfsbereitschaft und seinen flexiblen Sprechzeiten hat er maßgeblich zu dem Erfolg dieser Arbeit beigetragen.

Privat möchte ich meinen Eltern Barbara und Norbert danken. Ihr habt mir mit eurer kontinuierlichen Unterstützung einen bedeutsamen Rückhalt geboten, die es mir stets ermöglicht hat, mich auf momentane Zielsetzungen zu konzentrieren. Ein großer Dank geht an meinen Freundeskreis, insbesondere an meine langjährigen Schulfreunde Karsten und Thorsten, sowie meine Studienfreunde Anne, Gawad, Jakob, Jens und Rami. Unsere gemeinsamen Dialoge und Unternehmungen waren ein bereichernder Ausgleich zu meinen akademischen Bestrebungen.