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NEURORECEPTOR AVAILABILITY AND CEREBRAL MORPHOLOGY IN HUMAN OBESITY

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Were I a physician with a diploma, I would have written a whole book on obesity; thus I would have acquired a domicile in the domain of science, and would have had the double satisfaction of having, as patients, persons who were perfectly well, and of being besieged by the fairer portion of humanity. ——What I have not done, some other person will do, and if he be learned and prudent (and at the same time a good-fellow), I foretell that he will have wonderful success.

-Jean Anthelme Brillat-Savarin,
La Physiologie du Goût, translated by Fayette Robinson

Isn't it funny
How a bear likes honey?
Buzz! Buzz! Buzz!
I wonder why he does?

-A. A. Milne, *Winnie the Pooh*

To Kerttu and Väinö

ABSTRACT

Henry Karlsson

Neuroreceptor availability and cerebral morphology in human obesity

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Obesity is a major challenge to human health worldwide. Little is known about the brain mechanisms that are associated with overeating and obesity in humans. In this project, multimodal neuroimaging techniques were utilized to study brain neurotransmission and anatomy in obesity. Bariatric surgery was used as an experimental method for assessing whether the possible differences between obese and non-obese individuals change following the weight loss. This could indicate whether obesity-related altered neurotransmission and cerebral atrophy are recoverable or whether they represent stable individual characteristics.

Morbidly obese subjects (BMI $\geq$ 35 kg/m^2) and non-obese control subjects (mean BMI 23 kg/m^2) were studied with positron emission tomography (PET) and magnetic resonance imaging (MRI). In the PET studies, focus was put on dopaminergic and opioidergic systems, both of which are crucial in the reward processing. Brain dopamine D_2 receptor (D_2R) availability was measured using [^{11}C]raclopride and μ-opioid receptor (MOR) availability using [^{11}C]carfentanil. In the MRI studies, voxel-based morphometry (VBM) of T1-weighted MRI images was used, coupled with diffusion tensor imaging (DTI). Obese subjects underwent bariatric surgery as their standard clinical treatment during the study.

Preoperatively, morbidly obese subjects had significantly lower MOR availability but unaltered D_2R availability in several brain regions involved in reward processing, including striatum, insula, and thalamus. Moreover, obesity disrupted the interaction between the MOR and D_2R systems in ventral striatum. Bariatric surgery and concomitant weight loss normalized MOR availability in the obese, but did not influence D_2R availability in any brain region.

Morbidly obese subjects had also significantly lower grey and white matter densities globally in the brain, but more focal changes were located in the areas associated with inhibitory control, reward processing, and appetite. DTI revealed also signs of axonal damage in the obese in corticospinal tracts and occipito-frontal fascicles. Surgery-induced weight loss resulted in global recovery of white matter density as well as more focal recovery of grey matter density among obese subjects.

Altogether these results show that the endogenous opioid system is fundamentally linked to obesity. Lowered MOR availability is likely a consequence of obesity and may mediate maintenance of excessive energy uptake. In addition, obesity has adverse effects on brain structure. Bariatric surgery however reverses MOR dysfunction and recovers cerebral atrophy. Understanding the opioidergic contribution to overeating and obesity is critical for developing new psychological or pharmacological treatments for obesity. The actual molecular mechanisms behind the positive change in structure and neurotransmitter function still remain unclear and should be addressed in the future research.

Key words: bariatric surgery, diffusion tensor imaging, dopamine, magnetic resonance imaging, obesity, opioids, positron emission tomography, reward circuit, voxel-based morphometry

TIIVISTELMÄ

Henry Karlsson

Neuroreseptorit ja aivojen rakenne lihavuudessa

Turun yliopisto, Lääketieteellinen tiedekunta, Neurologian laitos; Turun yliopiston kliininen tohtoriohjelma ja Valtakunnallinen kliininen tutkijakoulu; Valtakunnallinen PET-keskus, Turun yliopisto ja Turun yliopistollinen keskussairaala

Lihavuudesta on tullut yksi maailman suurimmista terveysongelmista. Lihavuuteen liittyvistä aivojen toiminnan ja rakenteen muutoksista tiedetään kuitenkin toistaiseksi melko vähän. Tässä tutkimuksessa selvitettiin aivojen välittäjäainetoiminnan ja rakenteen eroja lihavien ja normaalipainoisten henkilöiden välillä. Lihavuusleikkauksen ja sitä seuraavan laihtumisen aiheuttamia aivojen välittäjäainetoiminnan ja tiheyden muutoksia arvioimalla voitiin päätellä, ovatko aivomuutokset jo olemassa ennen lihavuuden syntyä vai ovatko ne lihavuuden aiheuttamia.

Vaikeasti lihavien henkilöiden (BMI $\geq$ 35 kg/m^2) aivoja verrattiin normaalipainoisten henkilöiden (BMI:n keskiarvo 23 kg/m^2) aivoihin käyttämällä positroniemissiotomografiaa (PET) ja magneettiresonanssikuvantamismenetelmiä (MRI). PET-tutkimuksissa käytettiin kahta radioisotoopilla leimattua merkkiainetta, joista [^{11}C]raklopriidi sitoutuu dopamiinin D_2-reseptoreihin ja [^{11}C]karfentaniili μ-opioidireseptoreihin. Kummatkin reseptorit ovat keskeisessä asemassa aivojen mielihyväjärjestelmän toiminnassa. MRI-tutkimuksissa käytettiin analyysimenetelminä vokselipohjaista morfometriaa (*voxel-based morphometry*, VBM) sekä diffuusiotensorikuvantamista (*diffusion tensor imaging*, DTI). Lihaville tutkittaville tehtiin tutkimuksen aikana lihavuusleikkaus aiemman hoitosuunnitelman mukaisesti.

Ennen lihavuusleikkausta lihavilla tutkittavilla oli selvästi vähemmän μ-opioidireseptoreja mielihyväjärjestelmän keskeisissä osissa, kuten tyvitumakkeissa, insulassa ja talamuksessa, mutta dopamiinin D_2-reseptorien määrä oli sama kuin normaalipainoisilla tutkittavilla. Lisäksi näiden reseptorijärjestelmien välinen yhteys oli häiriintynyt lihavilla tutkittavilla aivojuovion etuosassa. Lihavuusleikkauksen aiheuttaman laihtumisen jälkeen μ-opioidireseptorien määrä palautui samalle tasolle kuin normaalipainoisilla. Dopamiinireseptorien määrässä ei tapahtunut muutosta.

Magneettitutkimuksissa kävi ilmi, että lihavilla tutkittavilla aivojen harmaan ja valkean aineen tiheydet olivat pienemmät kuin normaalipainoisilla tutkittavilla. Eroa löytyi erityisesti mielihyvään ja ruokahaluun liittyvillä alueilla. Myös valkean aineen radastoissa oli vaurion merkkejä. Leikkauksen jälkeen tapahtui palautumista laajasti valkean aineen alueilla mutta myös selvästi pienemmillä harmaan aineen alueilla.

Tulokset osoittavat, että opioidijärjestelmä liittyy keskeisesti lihavuuteen ja liikasyömiseen. Opioidijärjestelmän poikkeava toiminta on todennäköisesti lihavuuden aiheuttamaa ja saattaa ylläpitää haitallista syömiskäyttäytymistä. Lisäksi lihavuudella on haitallisia vaikutuksia aivojen rakenteeseen. Lihavuusleikkaus palauttaa opioidijärjestelmän ennalleen ja korjaa lihavuuden aiheuttamaa aivokudoksen harventumaa. Opioidijärjestelmän merkityksen ymmärtäminen on välttämätöntä lihavuuden uusien psykologisten ja farmakologisten hoitomuotojen kehittämisessä. Solutason mekanismit leikkauksen aiheuttamien muutosten taustalla ovat kuitenkin edelleen epäselvät, ja niihin tulisi keskittyä jatkotutkimuksissa.

Avainsanat: diffuusiotensorikuvantaminen, dopamiini, lihavuus, lihavuusleikkaus, magneettiresonanssikuvantaminen, mielihyväjärjestelmä, opioidit, positroniemissiotomografia, vokselipohjainen morfometria

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ABBREVIATIONS

BDI-II = Beck Depression Inventory II

BMI = body mass index

BP_{ND} = binding potential (non-displaceable uptake)

CCK = cholecystokinin

CT = computed tomography

D_2R = dopamine D_2 receptor

DAT = dopamine transporter

DEBQ = Dutch Eating Behaviour Questionnaire

DTI = diffusion tensor imaging

DW = diffusion-weighted

EPI = echo-planar imaging

FA = fractional anisotrophy

FCQ = Food Craving Questionnaire

FDR = false discovery rate

fMRI = functional magnetic resonance imaging

FWE = family wise error

FWHM = full width at half maximum

GLM = general linear model

GLP-1 = glucagon like peptide 1

GM = gray matter

HDL = high density lipoprotein

LOR = line of response

MD = mean diffusivity

MNI = Montreal Neurological Institute

MOR = μ-opioid receptor

MR = magnetic resonance

MRI = magnetic resonance imaging

OGTT = oral glucose tolerance test

PET = positron emission tomography

PYY = peptide-YY

ROI = region of interest

RYGB = Roux-en-Y gastric bypass operation

SD = standard deviation

SAT = subcutaneous adipose tissue

SPECT = single-photon emission computed tomography

SPM = statistical parametric mapping

SRTM = simplified reference tissue model

STAI = State-Trait Anxiety Inventory

TAC = time-activity curve

TE = echo time

TR = repetition time

VAT = visceral adipose tissue

VBM = voxel based morphometry

VTA = ventral tegmental area

WM = white matter

YFAS = Yale Food Addiction Scale

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-V:

I **Karlsson HK**, Tuominen L, Tuulari JJ, Hirvonen J, Parkkola R, Helin S, Salminen P, Nuutila P and Nummenmaa L. 2015. Obesity is associated with decreased μ-opioid but unaltered dopamine D2 receptor availability in the brain. The Journal of Neuroscience 35:3959-65.

II Tuominen L, Tuulari JJ, **Karlsson HK**, Hirvonen J, Helin S, Parkkola R, Salminen P, Hietala J, Nuutila P and Nummenmaa L. 2015. Aberrant mesolimbic dopamine-opiate interaction in obesity. Neuroimage 122:80-86.

III **Karlsson HK**, Tuulari JJ, Tuominen L, Hirvonen J, Honka H, Parkkola R, Helin S, Salminen P, Nuutila P and Nummenmaa L. 2015. Weight loss after bariatric surgery normalizes brain opioid receptors in morbid obesity. Molecular Psychiatry (epub Oct 13).

IV **Karlsson HK**, Tuulari JJ, Hirvonen J, Lepomäki V, Parkkola R, Hiltunen J, Hannukainen JH, Soinio M, Pham T, Salminen P, Nuutila P and Nummenmaa L. 2013. Obesity is associated with white matter atrophy: a combined diffusion tensor imaging and voxel-based morphometric study. Obesity 21:2530-7.

V Tuulari JJ, **Karlsson HK**, Antikainen O, Hirvonen J, Pham T, Salminen P, Helmiö M, Parkkola R, Nuutila P and Nummenmaa L. Bariatric surgery induces white and grey matter density recovery in the morbidly obese: A voxel-based morphometric study. Human Brain Mapping (accepted).

1 INTRODUCTION

Obesity is an immense burden to human health and economy worldwide. The prevalence of obesity has increased steeply over the last decades. In Finland, 20 % of the adult population is obese (Männistö et al., 2012), while in the United States percentage is over 30 % (Ogden et al., 2014). There are no signs of termination of the obesity epidemic. Quite the contrary: we live in a highly "obesogenic" environment, where physical, social, and cultural factors make many people to eat more than they need to in order to survive. Therefore, the enormous public and personal health costs caused by obesity and its co-morbidities will rise in the future. According to the recent statistics of World Health Organization, more than 2.8 million people die each year because of overweight and obesity (WHO, 2015). Increase of mortality originates from the numerous harmful metabolic effects associated with increased body weight, including elevated blood pressure and insulin resistance. Thus, obesity is linked to numerous co-morbidities such as hypertension, type 2 diabetes, and coronary heart disease.

Several studies have tried to reveal the causes of obesity. It is now obvious that common obesity is a complex, multifactorial, and heterogenous condition, which cannot be explained by, for example, simple genetic factors, although few exceptions may exist (Albuquerque et al., 2015). While the adverse effects of obesity to internal organs are well described, growing evidence shows that obesity is also associated with changes in brain morphology and function. Moreover, it is possible that individual differences in brain networks subserving reward processing and appetite control may make some individuals prone to weight gain. Studies have shown reductions in gray matter density in obese versus normal-weight individuals, and some studies have pointed out changes in white matter as well. There are also studies that have found neurochemical alterations in obesity. Some researchers have even drawn parallels between obesity and addictive disorders, mainly because the hallmark of many addictive disorders – lowered dopamine D_2 receptor (D_2R) density in the striatum – has been observed in obese individuals. Animal studies also highlight the importance of endogenous opioid system, particularly the μ-opioid receptor (MOR), in eating and obesity, but currently its role in human obesity remains unresolved. Furthermore, it is currently unknown whether cerebral atrophy and altered neurotransmission are causes or consequences of obesity.

Identifying the neurochemical mechanisms that make some individuals prone to overeating as well as pinpointing how obesity changes the functioning and the anatomy of the human brain is critical for understanding the current high prevalence of obesity and designing appropriate therapies for obesity. In the experimental studies presented in this thesis, a multimodal neuroimaging techniques including positron emission tomography (PET), diffusion tensor imaging (DTI), and voxel-based morphometry (VBM), were used to study brain function and anatomy in morbidly obese and normal-weight individuals. Obese subjects underwent bariatric surgery, which is so far the most efficient way of losing weight and remitting co-morbid diseases such as diabetes. This allowed evaluating whether the observed differences between obese and normal-weight individuals are recoverable by weight loss, or whether they represent stable traits in obesity.

2 REVIEW OF THE LITERATURE

2.1 Cerebral regulation of appetite

Animals depend solely on food as their source of energy. Consequently, feeding has to be secured to offer optimal possibilities for survival. In normal conditions, food intake and the choice of food is controlled by the mesocorticolimbic areas of the brain, including hypothalamus, striatum, amygdala, and prefrontal cortices. Hypothalamus constitutes the key part of physiological appetite regulation in the brain. It receives neural and humoral signals from the other parts of body, and integrates these signals together with insula in order to keep the body weight in balance (Saper et al., 2002; Berthoud, 2012). Most important gut hormones associated with appetite control are glucagon-like peptide 1 (GLP-1), peptide-YY (PYY), ghrelin, and cholecystokinin (CCK). Hypothalamus also sensors body adiposity via leptin, a hormone produced by adipose cells (Keen-Rhinehart et al., 2013) and an important factor in the control of feeding and energy expenditure in humans (Ahima, 2008).

Homeostatic mechanisms form the basis of appetite control, yet they are supported by brain's reward circuit, which guides appetitive motivation and generation of pleasurable sensations upon food consumption. Common obesity does not originate from dysfunctional homeostatic mechanisms only, and the brain systems controlling pleasure and reward are also critical for overeating and obesity (Kringelbach, 2004; Berthoud, 2012; Keen-Rhinehart et al., 2013; Alonso-Alonso et al., 2015; Yu et al., 2015). Feeding has a strong hedonic aspect, and both humans and animals can eat for the sake of pleasure regardless of current energy balance (Palmiter, 2007; Kenny, 2011; Johnson, 2013). Moreover, volitional appetite control is partly impaired among obese subjects, which may cause unhealthy eating patterns (Batterink et al., 2010; Tuulari et al., 2015).

In the modern western society where continuous flow of food and food product imagery are served to people, the role of actual physiological control of appetite may become less important. It is likely that the dysfunction of reward circuit and volitional appetite control are possible culprits behind the obesity epidemic. Additionally, integration of homeostatic control and reward functions play a pivotal role in the onset of pathological eating habits. For example, animal studies have shown a direct link between leptin and dopaminergic system, as leptin seems to inhibit the reward circuit through dopaminergic neurons (Fulton et al., 2006; Hommel et al., 2006). Also insulin and ghrelin can directly interact with dopaminergic system (Kenny, 2011; Dunn et al., 2012).

2.2 Obesity and the reward circuit

2.2.1 Reward systems in the human brain

Multiple reward systems are located in the mammalian brain (Kringelbach, 2004). Based on wide range of studies, the distinction between neuronal circuits for reward *wanting* and *liking* has been made. Wanting is a process of incentive motivation, which directs behaviour towards obtaining certain goals, whereas liking refers to the hedonic reaction to the pleasure associated with reward consumption. Although these processes are highly related, they remain dissociable. (Berridge, 2009; Castro and Berridge, 2014; Berridge and Kringelbach, 2015). For a long time, dopamine was regarded as the major neurotransmitter in reward and pleasure, but recent evidence has shown that mesolimbic dopamine neurons may be more important in motivational drive, such as wanting and craving, and the actual pleasure response may have other contributors. In contrast to dopaminergic system, the opioidergic system has been more consistently linked with hedonic responses and 'liking' responses. Both wanting and liking functions as well as the systems subserving them – dopaminergic and opioidergic systems, respectively – are needed for normal reward (Berridge, 2009; Berridge et al., 2010; Berridge and Kringelbach, 2015).

Several animal and human studies have confirmed that the reward system is dysfunctional in obesity. Overeating is considered to originate from the imbalance between the reward circuit involving ventral striatum, amygdala, and several other interconnected brain regions, and the regions that inhibit reward-seeking such as dorsolateral prefrontal and orbitofrontal cortices, insula, and dorsal striatum (Volkow et al., 2008b; Verdejo-Garcia and Bechara, 2009; Koob and Volkow, 2010; Volkow et al., 2012). Especially functional imaging studies in obese human subjects suggest that the reward circuit is overactive to reward anticipation, and that inhibitory networks cannot control the reward circuit sufficiently (Kelley and Berridge, 2002; Volkow and Wise, 2005; Stoeckel et al., 2008; Volkow et al., 2008a; Stice et al., 2011; Nummenmaa et al., 2012). Also altered reward system connectivity has been proposed to play a significant role in human obesity (Gupta et al., 2015; Marques-Iturria et al., 2015). However, the role of the opioidergic 'liking' and dopaminergic 'wanting' neuroreceptor systems in human obesity remains vague.

2.2.2 Dopaminergic system in reward functions and obesity

Olds and Milner observed more than 60 years ago that the dopaminergic system plays an important role in reward (Olds and Milner, 1954). This has been confirmed in many subsequent studies. For instance, blockade of dopaminergic system resulted in attenuation of reward properties of food in rats (Wise et al., 1978). Dopamine receptors D_1 and D_2 are the most abundant dopamine receptor types in the human brain, and they have specific roles in the reward learning and feeding (Nakanishi et al., 2014). D_1 and D_2 receptors have own distinct signalling pathways with different downstream signalling molecules, and thus their responses to appetite control are different. Consequently, D_2 receptor stimulation causes

decrease of palatable food intake in rats, but stimulation of D_1 receptors increases it (Cooper and Al-Naser, 2006). The balance between D_1 and D_2 receptors is thus important in feeding behaviour, but the role of different subtypes still remains elusive.

Mesolimbic dopamine system is crucial in learning how natural reward such as food can be found, and it also directs animal behaviour towards the desired reward (Palmiter, 2007; Kenny, 2011). Mice unable to synthesize dopamine are hypoactive and have no motivation to eat, but feeding and moving can be restored by L-dihdroxyphenylalanine (L-DOPA) injections (Zhou and Palmiter, 1995). D_2R density correlates negatively with body weight in rats (Michaelides et al., 2012), and mice with a lower density of D_2R in the putamen exhibit more weight gain than mice with a higher density of D_2R after a high-fat diet (Huang et al., 2006). Thus, low expression of D_2R may lead to reward deficiency and increased motivation to eat.

Furthermore, rats on energy-dense cafeteria diet have decreased D_2R expression as well as depressed dopamine release in ventral striatum, and D_2R knockout mice soon develop compulsive eating habit (Geiger et al., 2009; Johnson and Kenny, 2010). Interestingly, cafeteria diet stimulates dopamine release in obese animals, whereas normal laboratory chow does not (Geiger et al., 2009). This supports the hyporeactivity theory in obesity, in which blunted dopamine release urges obese animals to eat palatable food in order to get the normal stimulus.

Human studies have also suggested the importance of mesolimbic D_2R in obesity. There are clear changes in D_2R expression and function in obese subjects (Stice et al., 2011; Salamone and Correa, 2013). PET studies also point towards the role of D_2R in obesity and feeding. A PET study using D_2R antagonist [^{11}C]raclopride with non-obese human subjects shows that food intake leads to dopamine release in dorsal striatum, and this is correlated with the experienced pleasure from the food. However, no correlation with desire to eat or satiety was observed (Small et al., 2003).

Some PET studies have also demonstrated that reduced baseline D_2R availability in striatum may contribute to overeating in humans (Wang et al., 2001; Volkow et al., 2008b; de Weijer et al., 2011). Moreover, both amphetamine (van de Giessen et al., 2014) and calorie-intake (Wang et al., 2014) triggered dopamine release is lowered among obese. However, contradicting evidence on lowered D_2R availability in obese human subjects has emerged lately (Dunn et al., 2012; Eisenstein et al., 2013; Guo et al., 2014). Overall, the impact of some of these studies is somewhat elusive, partly due to quite small sample sizes and different radioligands. In addition, new evidence links altered D_2R signalling to lowered physical activity rather than compulsive overeating (Beeler et al., 2015).

Presynaptic dopamine transporters (DAT) may play a significant role in obesity, regulating the amount of extracellular dopamine in the synaptic cleft. DAT-deficient mice have more extracellular dopamine, which leads to excessive feeding (Pecina et al., 2003). In addition, high fat diet reduces striatal DAT availability in mice (South and Huang, 2008), but lowered dopamine reuptake may occur without altering the amount of DAT protein (Cone et al., 2013). Few human studies exist, and these have yielded conflicting results. One SPECT study showed that striatal DAT availability has a negative correlation with BMI (Chen et al., 2008), while two SPECT studies with different radioligands could not find any association between BMI and DAT availability (Thomsen et al., 2013; van de Giessen et al., 2013), which makes the role of DAT in human obesity debatable.

The genetic data support the importance of the dopaminergic system in obesity. D_2 receptor variants are likely important for weight gain. Obesity and blunted striatal response to food is moderated by D_2R TaqI A1 allele (Stice et al., 2008). Carpenter and colleagues have recently showed that TaqI A1 allele was significantly associated with BMI (Carpenter et al., 2013). More recently, no association between TaqI A1 allele and adiposity was observed in a large population-based sample (Hardman et al., 2013). Thus, although contribution of dopaminergic system is presumable in human obesity, the actual mechanisms how D_2Rs affect overeating remain unresolved.

2.2.3 Endogenous opioid system in human reward and obesity

Animal studies have demonstrated that the endogenous opioid system is closely involved in the mammalian appetite control. It was initially found that opioid antagonist naloxone suppress appetite in rats (Holtzman, 1979). Subsequent animal studies have confirmed that MOR agonists increase and antagonists decrease liking of palatable foods and food intake (Yeomans and Gray, 2002; Bodnar, 2008; Gosnell and Levine, 2009; Pecina and Smith, 2010; Nogueiras et al., 2012). Long-lasting MOR antagonism reduces hyperphagia in rats, showing that endogenous MOR signalling in the nucleus accumbens is necessary for hedonic eating processes (Shin et al., 2010). Recent work with rats indicates that non-selective opioid antagonism decreases food intake and BMI (Ibrahim et al., 2015). Selective MOR antagonism reduces also food-seeking and binge eating in rats (Giuliano et al., 2012). While opioid antagonism reduces feeding, it also lowers dopamine release in ventral striatum (Sahr et al., 2008), proposing a close interaction between these two receptor systems.

Animal work suggests that there are apparent changes in MOR expression and function in obesity. MOR expression decreases after long-term access to palatable food, but increases after withdrawal (Pitman and Borgland, 2015). However conflicting data also exists. For instance, one study found that rats susceptible to obesity have increased expression of MOR compared to rats that are resistant to diet-induced obesity (Barnes et al., 2006). Moreover, obesity increases MOR binding assessed with autoradiography in some parts of the rat brain, which was thought to be due to decreased release of endogenous opioid peptides (Smith et al., 2002). MOR knockout mice have significantly decreased consumption of sucrose solution compared to wild type mice (Ostlund et al., 2013), while MOR deficient mice have decreased motivation to eat (Papaleo et al., 2007) and resistance to diet-induced obesity (Tabarin et al., 2005; Zuberi et al., 2008).

Genetic data also points to the contribution of MOR in obesity. The MOR gene *OPRM1* modulates the intake of fat and possibly risk for gaining weight in humans (Haghighi et al., 2013). Obese patients with binge-eating disorder have more often gain-of-function G allele in *OPRM1* but obese without binge-eating have the "loss-of-function" A1 allele in D2 receptor gene (Davis et al., 2009). However, also studies with no association with *OPRM1* and obesity exists (Hardman et al., 2013).

Human studies concentrating on endogenous system and obesity are however sparse. Bulimia nervosa (with normal BMI) has been associated with decreased MOR availability in the left insular cortex, and MOR availability is negatively correlated with recent fasting

behaviour (Bencherif et al., 2005). Another study using fMRI showed that MOR antagonism leads to significant reduction in responses to high-calorie food images among binge-eating obese subjects (Cambridge et al., 2013). It is still unclear how endogenous opioid system regulates human appetite, and what is the role of MOR system in human obesity and weight loss.

2.2.4 Interaction between dopaminergic and opioidergic systems in obesity

There is growing evidence that MORs control the release of dopamine through inhibiting GABAergic interneurons in ventral tegmental area (VTA) (Spanagel et al., 1992; Kalivas, 1993; Volkow and Wise, 2005; Rada et al., 2010; Jalabert et al., 2011; Chartoff and Connery, 2014). Furthermore, VTA dopamine neurons express MOR postsynaptically, and there seems to be direct inhibition between MOR and dopamine neurons without GABAergic signalling (Margolis and Hjelmstad, 2014). Tight interaction between dopaminergic and opioidergic systems has been proposed in human reward functions (Le Merrer et al., 2009), but only a few studies have actually investigated this. Data comes mainly from research on drugs of abuse (Volkow, 2010). Dopamine-stimulating drugs such as cocaine and amphetamine cause release of endogenous opioids (Soderman and Unterwald, 2009; Colasanti et al., 2012), which further suggests the interaction of these two systems in reward processing.

The cross-talk between opioidergic and dopaminergic system may underlie aberrant reward-related behaviour, such as excessive feeding pattern. Animal studies have indeed confirmed the interaction between these two systems in food intake. Intravenous administration of MOR agonists triggers dopamine release but also induces eating (Yeomans and Gray, 2002), and MOR antagonists block dopamine release and reduce the amount of food that rats consume (Taber et al., 1998). The interaction between MOR and D_2R receptor systems is important in regulating appetite, but the actual mechanisms are still uncertain. Data comes from preclinical studies, and practically no data on human subjects exist. The role of interaction in human obesity is completely unknown.

2.2.5 Is obesity an addictive disorder?

Because of the apparent changes in the reward circuit in obesity, parallels have been drawn between obesity and addictive disorders such as drug addiction. However, most evidence supporting this view comes from animal studies (Ziauddeen and Fletcher, 2013). As reviewed above, dopaminergic pathways – especially the mesolimbic pathway from ventral tegmental area to nucleus accumbens – regulate reward-related behaviour. Animal studies have shown that feeding increases extracellular dopamine concentration in ventral striatum (Bassareo and Di Chiara, 1997), which resembles the effect that drugs of abuse have. Both drugs of abuse and food can cause synaptic modifications in the mesolimbic dopaminergic system (DiLeone et al., 2012; Baik, 2013). Exposure to drugs of abuse,

such as cocaine or amphetamine, causes a phenomenon known as behavioural sensitization, which leads to enhanced dopamine release in ventral striatum after drug intake, but there are changes in the dopamine receptor availabilities as well (Steketee and Kalivas, 2011). These drug-induced changes may have similarities with the reactions caused by natural reward such as food.

So-called food addiction theory became popular when similar patterns of altered neurotransmission were observed in human subjects with addictive disorders and obesity. Both food and drug-related cues activate same areas of the reward circuit in fMRI studies (Wang et al., 2009; Gearhardt et al., 2011). Moreover, obese subjects and individuals with substance abuse show reduced expression of D_2R in striatal regions of the brain. Cocaine use decreases D_2R density in monkeys, and lowered levels may persist even up to one year of abstinence (Nader et al., 2006). Human studies indicate that alcohol and drug dependence lower D_2R availability in the striatum (Volkow et al., 1996; Volkow et al., 2001; Martinez et al., 2012), and PET imaging has revealed lower baseline D_2R density in the striatum both in addictive disorders and obesity (Wang et al., 2001). In this study, D_2R density was shown to be proportional to BMI. This was taken to suggest that lowered D_2R density would to make individuals prone to overeating because of food-induced reward deficiency. Other PET studies in morbidly obese human subjects have also showed lower striatal D_2R availability (Volkow et al., 2008b; de Weijer et al., 2011), but there are also many conflicting studies showing unaltered availability in the striatum (Haltia et al., 2007b; Haltia et al., 2008; Steele et al., 2010; Eisenstein et al., 2013; Guo et al., 2014).

The role of opioid system in addictive disorders is less well understood. Animal studies show that persistent MOR upregulation occurs in rats both during the use of cocaine and after withdrawal. This may be due to chronic reduction in endogenous opioid release (Bailey et al., 2005). Similarly, cocaine dependence in human subjects is linked to increased MOR availability in large areas, including anterior cingulate and frontal cortex (Gorelick et al., 2005). Alcohol dependence is associated with higher MOR availability in ventral striatum (Heinz et al., 2005; Weerts et al., 2011). On the other hand, opioid addiction may lead to decreased MOR availability, which may subsequently lead to opioid tolerance (Koch and Hollt, 2008). However, the effects of opioids to MOR protein levels are diverse and possibly substance specific (Le Merrer et al., 2009; Whistler, 2012).

Altogether, drug addictions seem to be a neurochemically and behaviourally different state compared with obesity. In contrast to eating, drug addictions are not affected by natural homeostatic mechanisms. Even from a pharmacological point of view, withdrawal symptoms for obesity and drug addiction are different. Moreover, food-related environmental factors begin to affect us already at perinatal stage (Carnell et al., 2012; Grissom et al., 2014). It is thus possible that the basic processes that direct one's motivation towards certain goal – may it be drug or food – can be quite similar, although neurobiologically complex (Salamone and Correa, 2013). However, other processes that maintain the pathological eating habits seem to be different than those supporting drug addiction. Obesity is a also very heterogenous disorder, and a simple model such as food addiction would just appear as a hindrance to future obesity research (Ziauddeen et al., 2012).

2.3 Obesity and brain atrophy

Obesity can cause severe damage to internal organs, for example heart, skeletal muscle, and liver (Unger, 2003). In addition, obesity has been associated with many co-morbidities such as hypertension (Brown et al., 2000), coronary disease (Jousilahti et al., 1996), and type 2 diabetes (Kopelman, 2000). Obesity causes adverse effects to central nervous system based on animal studies (Mattson et al., 2002), but obesity is a notable risk factor to neurodegenerative disorders such as the Alzheimer's disease in humans (Gustafson et al., 2003; Jagust et al., 2005; Kivipelto et al., 2005).

Hypertension and diabetes are associated with decreased brain volume and damage (Raz et al., 2003; Taki et al., 2004; Korf et al., 2007; Gons et al., 2010; Yau et al., 2010; Debette et al., 2011; Hsu et al., 2012; Climie et al., 2015), but also obesity decreases global brain volume (Ward et al., 2005). Several studies using voxel-based morphometry (VBM) approach have demonstrated that obese people have global and focal decreases in brain grey matter (GM) density (Taki et al., 2008; Walther et al., 2010; Horstmann et al., 2011; Brooks et al., 2013; Bobb et al., 2014; Lou et al., 2014; Janowitz et al., 2015). Moreover, lower GM densities in obese subjects have been observed in many focal areas (Pannacciulli et al., 2006; Raji et al., 2010). Frontal and limbic areas seem to be affected already in obese children and adolescents (Alosco et al., 2014a).

It is not clear how weight gain affects the brain, and many physiological processes might be involved. Increased adipose tissue causes a chronic, low-grade inflammation, which is initiated by excess of nutrients in metabolic cells (Gregor and Hotamisligil, 2011; Lumeng and Saltiel, 2011), and which in turn deceases brain GM density (Debette et al., 2010; Cazettes et al., 2011). A high level of C-reactive protein is linked to brain alterations and impaired cognitive functions among obese subjects (van Dijk et al., 2005; Sweat et al., 2008). Also adipokines such as leptin can affect brain structure, leading to decreased local and focal GM density (Pannacciulli et al., 2007; Gunstad et al., 2008; Mueller et al., 2014). Chronic metabolic inflammation may indeed be the key factor behind observed brain density alterations.

Obesity also causes atrophy in brain's white matter (WM). Atrophy in the basal ganglia and corona radiata has been observed among obese subjects (Raji et al., 2010), although one conflicting study has reported increased WM density in obesity (Haltia et al., 2007a). Two tensor-based morphometry studies have observed reductions in both GM and WM densities in obese subjects (Ho et al., 2010; Cole et al., 2013). There is also growing evidence that obesity has a negative effect on the structural integrity of WM, as measured with Diffusion Tensor Imaging (DTI) (Cazettes et al., 2011; Marks et al., 2011; Stanek et al., 2011; Verstynen et al., 2012; Xu et al., 2013; Lou et al., 2014; Mueller et al., 2014; Marques-Iturria et al., 2015; Kullmann et al., 2016). Some of these alterations may be sex-dependent (Mueller et al., 2011). Hypothalamic damage measured with DTI has been linked to poor cognitive performance in obese subjects (Puig et al., 2015). Factors behind WM changes are probably the same than with GM changes. Low-grade inflammation is an important factor behind WM alteration (Puig et al., 2015), while type 2 diabetes (Yau et al., 2009; Yau et al., 2010) and hypertension (Maclullich et al., 2009; Gons et al., 2010) are associated with damage to WM tracts. Abnormal cholesterol profile can also have a negative effect on WM integrity in obesity (Cohen et al., 2011). Among children and

adolescents, obesity has no clear effect on WM integrity (Alosco et al., 2014a). It is possible that changes to WM appear later on life, and GM is more susceptible to damage even at the earlier stages of life.

It is still elusive whether aforementioned changes in brain precede or follow weight gain. Few prospective studies exist. Yokum and colleagues observed reduced brain GM and WM densities in the obese subjects, and at one-year follow-up they found that low GM density in the areas implicated especially in inhibitory functions are associated with future weight gain. Furthermore, reductions in WM density seem to be secondary to weight gain (Yokum et al., 2012). In a recent 5-year follow-up study, higher baseline BMI was associated with greater decline in temporal and occipital GM density (Bobb et al., 2014). Although it seems likely that metabolic factors negatively affect brain structure, it is possible that some of the observed alterations especially in the brain areas associated with reward and behavioural control have preceded obesity and led to pathological eating behaviour (Pannacciulli et al., 2006; Yokum et al., 2012).

2.4 Does weight loss alter brain function and structure?

Many weight loss strategies have been developed to tackle the obesity epidemic. Behavioural interventions include cognitive therapies, dietary modifications, and increased exercise, which all focus on supporting more favourable energy balance. Unfortunately, these therapies have not been very successful. A meta-analysis on behavioural interventions resulted only in mean weight loss from 5 to 9 % at six months, after which regain of weight was observed in many subjects (Franz et al., 2007).

Bariatric surgery is the most effective method for weight loss in obesity (Gloy et al., 2013; Maggard-Gibbons et al., 2013). Bariatric surgery procedures are much more effective than intensive medical therapy to reach weight loss and glycaemic control (Schauer et al., 2014). Systematic reviews show that bariatric surgery leads to substantial weight loss and remission of diabetes (Buchwald et al., 2009), but it also prevents from future type 2 diabetes (Carlsson et al., 2012) and cardiovascular deaths (Sjostrom et al., 2012). Surgery also affects the appetite. For instance, studies with fMRI have shown reduced neural responsivity as well as desire for eating and liking of high-calorie foods after bariatric surgery (Ochner et al., 2012; Frank et al., 2014). One fMRI study showed small differences in reactions to visual food cues between subjects who underwent surgical versus behavioural weight loss (Bruce et al., 2014).

The actual molecular mechanisms of how bariatric surgery affects the appetite control in the brain are still unknown. Gut hormones are affected by bariatric surgery. Elevated PYY levels and decreased ghrelin levels are observed among the subjects who underwent a bariatric surgery (Karamanakos et al., 2008; Munzberg et al., 2015). Moreover, significant improvement of GLP-1 response to food intake has also been observed (Morinigo et al., 2006). Moreover, enteroendocrine melanocortin pathway may have importance in the postsurgical suppression of appetite (Manning et al., 2015), but also psychological factors such as aversive conditioning (arising from the fear of unpleasant feelings like dumping after the surgery) may be involved (Shin and Berthoud, 2011). Bariatric surgery and following weight loss also improves the glucose metabolism in the brain (Tuulari et al.,

2013), which may partly affect the control of appetite. Recent animal work suggests that both bariatric surgery and caloric restriction increase the amount of dopamine but decrease the amount of noradrenaline in dorsal striatum compared to fed animals (Reddy et al., 2014).

Cross-sectional studies do not reveal whether the changes in neuroreceptor systems and brain densities have existed before weight gain or have developed after weight gain. Bariatric surgery provides a method for investigating whether molecular and structural changes in the brain are causes or consequences of obesity. Two small-scale human PET studies have investigated the effects of bariatric surgery and following weight loss to D_2Rs. Steele and colleagues observed increased availability after the binding after the bariatric surgery (Steele et al., 2010). On the other hand, Dunn and colleagues measured decreased D_2R availability in many parts of the reward circuit (Dunn et al., 2010). Moreover, one SPECT study with 19 female obese subjects observed no change in striatal D_2R availability 6 weeks after RYGB surgery (de Weijer et al., 2014). It remains completely unknown how weight loss influences the MOR availability. It is also previously unsolved how receptor availabilities affect mental states and behaviour in obesity, assessed by different questionnaires measuring reward functioning and eating behaviour. Moreover, there are no studies addressing the changes in brain GM or WM densities after weight loss.

2.5 Summary of the literature

Altogether, existing evidence suggests that obesity affects both brain structure and function of specific neurotransmitter systems. To date, reductions in global and focal GM densities have been shown in many studies, but research concentrating to WM integrity and obesity is still fractional. The actual mechanisms causing GM and WM density alterations remain unresolved. Furthermore, molecular function of reward circuit and its interplay with homeostatic mechanisms of food intake in obesity is not fully understood. Critically, the role of endogenous opioid system in human obesity remains elusive, albeit animal research support its contribution to human obesity as well. Finally, only a few longitudinal studies examining the brain structure and function over time exists. The link between obesity and brain-level changes remains thus unclear.

3 OBJECTIVES OF THE STUDY

This doctoral thesis presents a multimodal neuroimaging approach on the neural basis of human obesity using positron emission tomography (PET) and magnetic resonance imaging (MRI), including both voxel-based morphometry (VBM) and diffusion tensor imaging (DTI). Brain neurotransmitter systems and anatomy was investigated both in morbidly obese and non-obese subjects using aforementioned brain imaging methods. Obese subjects underwent bariatric surgery during the project. They were scanned before and six months after the operation. Thus, it was possible to evaluate whether possible alterations in obese subjects' brains change after the surgical operation and concomitant weight loss, or whether the alterations represent stable individual characteristics.

The specific objectives of this thesis are:

1. To characterize the differences in two specific neurotransmitter systems (MOR and D_2R) between obese and non-obese subjects (**Study I**).
2. To assess the role of interactions between MOR and D_2R in obesity (**Study II**).
3. To investigate the effect of bariatric surgery and following weight loss to MOR and D_2R (**Study III**).
4. To characterize the differences in brain white and gray matter densities as well as white matter integrity between obese and non-obese subjects (**Study IV**).
5. To determine the effect of bariatric surgery and following weight loss brain white and gray matter densities in obesity (**Study V**).

4 GENERAL METHODOLOGY

4.1 Subjects

Subjects were collected from two larger studies, Sleevepass (NCT00793143) and SleevePET2 (NCT01373892) (http://www.clinicaltrials.gov), both of which investigated the effects of bariatric surgery to various organ systems including the brain (Table 1). Altogether, 49 morbidly obese subjects and 42 non-obese control subjects participated in this project.

Morbidly obese study subjects were recruited from a patient population selected to undergo bariatric surgery as their standard clinical treatment for morbid obesity. Non-obese control subjects were volunteers recruited using newspaper advertisements. Morbidly obese subjects and controls were matched for age, height, and sex.

Studies were conducted in accordance with the Declaration of Helsinki. Study protocol, patient information, and informed consent form were submitted to the Ethical Committee of the Hospital District of the South-Western Finland and the permission was granted before the recruitment of the subjects. All the subjects signed the ethical committee approved informed consent form prior to studies.

Table 1. Number of participants in each study. Participants were all female unless otherwise stated.

	Sleevepass (NCT00793143)	**SleevePET2 (NCT01373892)**
Study I		13 morbidly obese 14 controls
Study II		25 morbidly obese 20 controls
Study III		16 morbidly obese 14 controls
Study IV	23 morbidly obese (5 males) 22 controls (7 males)	
Study V	24 morbidly obese (5 males) 15 controls (6 males)	23 morbidly obese 14 controls

For the morbidly obese subjects, inclusion criteria were 1) BMI ≥ 40 kg/m^2, or BMI ≥ 35 kg/m^2 if there was an additional risk factor such as type 2 diabetes or hypertension, and 2) age 18-60 years. Exclusion criteria were 1) diabetes requiring insulin treatment, 2) BMI over 60 kg/m^2 or weight over 170 kg, and 3) eating disorders or severe mental disorders.

For the control subjects, inclusion criteria were 1) BMI 18-27 kg/m^2, 2) age 18-60 years 3), and normal oral glucose tolerance test (OGTT), and exclusion criteria were 1) regular use of any medication, and 2) any chronic disease.

For both groups, exclusion criteria included 1) excessive use of alcohol or any use of other drug of abuse, 2) poor compliance, 3) pregnancy, 4) past dose of radiation from nuclear imaging studies, 5) presence of any ferromagnetic objects that would contraindicate MR imaging, and 6) notable claustrophobia that would possibly contraindicate brain imaging studies.

4.2 Overall study design

The main research site of this project was Turku PET Centre (http://www.turkupetcentre.fi). Clinical screening consisted of medical history, physical examination, anthropometric measurements as well as laboratory tests including an OGTT. Depression was assessed using Beck Depression Inventory II (BDI-II) questionnaire (Beck et al., 1996), anxiety levels were measured using State-Trait Anxiety Inventory (STAI) questionnaire (Spielberger et al., 1983), food craving and eating behaviour were assessed with Food Craving Questionnaire (FCQ) (Cepeda-Benito et al., 2000), Dutch Eating Behaviour Questionnaire (DEBQ) (van Strien et al., 1996), and Yale Food Addiction Scale (YFAS) (Gearhardt et al., 2009).

After the clinical screening, baseline MRI and PET imaging studies were performed. These studies were performed before the standard very low calorie diet, after which the morbidly obese proceeded to bariatric surgery. Non-obese controls were studied only once. For the morbidly obese subjects, imaging procedures were repeated 6 months after the bariatric surgery (Figure 1). They were also clinically examined both pre- and postoperatively.

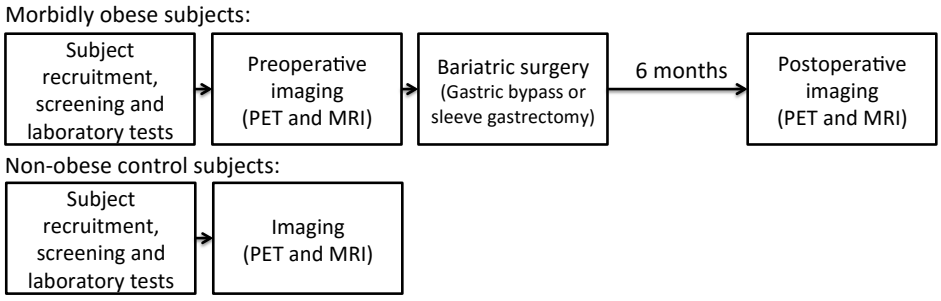

Figure 1. Overall design of the studies.

4.3 Positron Emission Tomography (PET)

Positron emission tomography (PET) is a quantitative and non-invasive *in vivo* imaging method, which utilizes positron-emitting isotopes to study various physiologic, metabolic, and functional processes in the human body. A wide variety of biological molecules can be labelled with radioactive isotopes (most commonly ^{11}C, ^{18}F, and ^{15}O). The labelled molecules are called radioligands, which can be used to investigate, for example, glucose metabolism and receptor-ligand interactions. Almost every biological system can be imaged with PET as long as molecules binding specifically to the target system can be labelled with radioactive isotopes.

A cyclotron is used to produce the unstable isotopes that have one excess proton in their nucleus. When a radioligand injected in the human body, the unstable nucleus undergoes positron decay, causing the nucleus to emit a positron (e^+) and a neutrino (v) (Turkington, 2001). The positron can travel only a short distance in the tissue after which a collision with an electron will occur. This collision is called annihilation, in which the positron and the electron will be transformed into two 511 keV photons travelling to opposite directions (Figure 2). (Turkington, 2001; Bailey, 2005).

The detectors in the PET scanner identify these two photons. This simultaneous detection is called a coincidence event, which is detected by the scanner (Figure 2). Because the photons are thought to travel to opposite directions, an imaginary line known as line of response (LOR) can be drawn between the detectors. Each LOR is registered by its location and the angle of orientation, and coincidence events are often histogrammed according to the LORs in a table called sinogram. Finally, the data in sinograms are reconstructed into PET images. These PET images are degraded by many physical factors, such as scatter (false localization of the event due to aberration from the original LOR), attenuation (reduced signal due to photon absorption by tissue), random events (simultaneous false counts interpreted as true counts by the scanner), and dead-time (inability to record true counts at high event rates), but at least some of these can be corrected by specific techniques. (Turkington, 2001; Bailey, 2005).

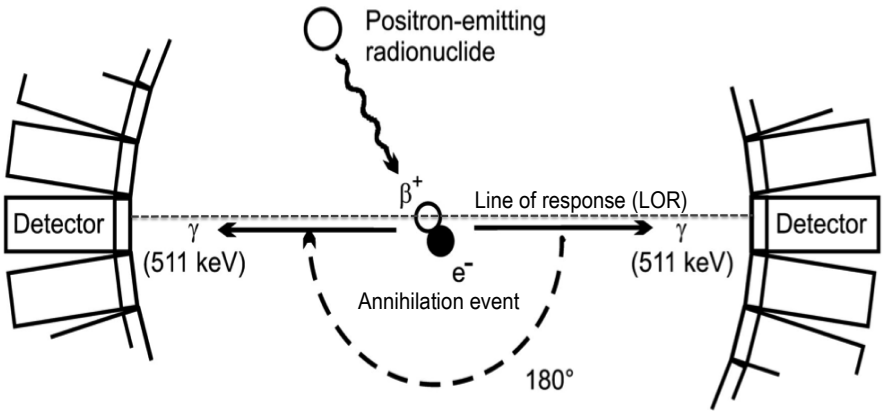

Figure 2. A schematic illustration of annihilation. Modified from (Verel et al., 2005).

4.3.1 PET image acquisition (Studies I-III)

In the PET imaging studies of this project, two different neurotransmitter systems were focused on (Figure 3). Dopaminergic system was studied with [^{11}C]raclopride, which is a dopamine D_2 receptor antagonist binding mainly to striatal and thalamic areas (Farde et al., 1986). Opioidergic system was measured with [^{11}C]carfentanil, which is a high-affinity agonist to μ-opioid receptors (Frost et al., 1985). These radioligands are well validated for human studies, having high test-retest reliability (Hirvonen et al., 2003; Hirvonen et al., 2009). In addition, these radioligands have been used is numerous clinical settings during the past years.

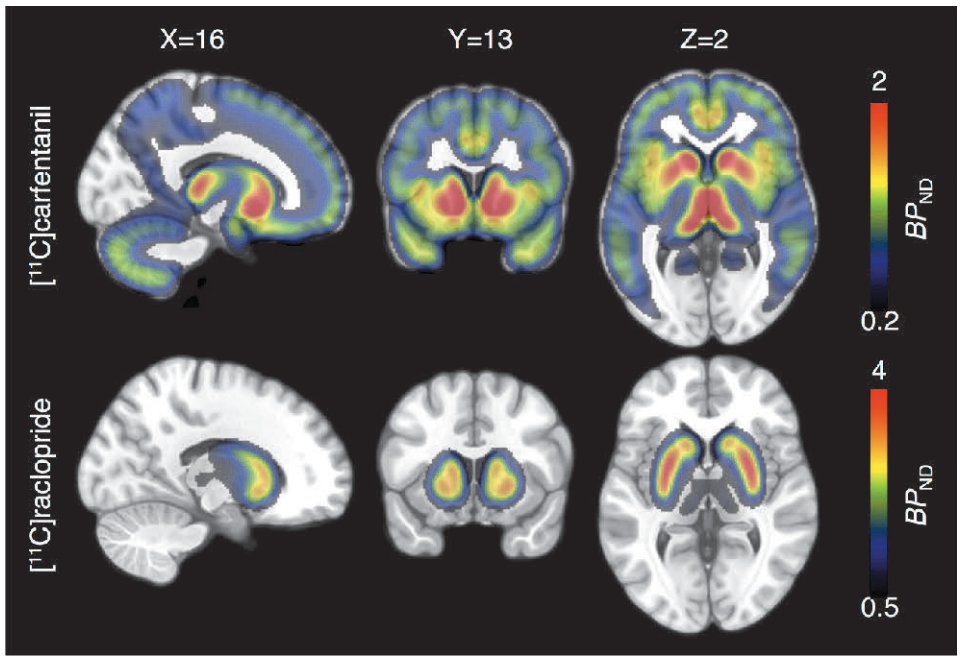

Figure 3. Distribution of μ-opioid receptors measured with [^{11}C]carfentanil (as indexed by BP_{ND} levels; upper panel) and dopamine D_2 receptors measured with [^{11}C]raclopride (lower panel) in the human brain (Original publication II).

Synthesis of the both radioligands took place in the Accelerator Laboratory of Turku PET Centre at Åbo Akademi University. CC18/9 cyclotron (Efremov Institute, Saint-Petersburg, Russia) was used to produce [^{11}C]methane, which was later on converted into [^{11}C]methyl triflate and linked to precursor molecules. The identity, radiochemical purity and the specific radioactivity of the products were determined using high performance liquid chromatography and UV detection. Both radioligands had high radiochemical purity (>99 percent). For detailed information of the radioligand production, see the original publication (Karlsson et al., 2015).

Subjects were scanned during satiated state. Before the PET scans, CT scans were taken to obtain attenuation maps and reference anatomical images of the brain. Radioligand was administered intravenously as bolus injections via a catheter placed in the antecubital vein. Radioactivity in brain was measured with the GE Healthcare Discovery™ 690 PET/CT scanner (General Electric Medical Systems, Milwaukee, WI, USA; Figure 4). Effective resolution of the PET scanner was 4.7 mm full width at half maximum (FWHM). Total scanning time was 51 minutes during which 13 time frames were collected (3 x 1 min, 4 x 3 min, 6 x 6 min). Data were corrected for dead-time, decay, and CT-measured photon attenuation. Brain scans covered the whole brain in 3D mode, and dynamic PET-scans were reconstructed using time-of-flight information.

To exclude structural abnormalities and to provide anatomical reference images for the PET scans, MR imaging was performed with Philips Gyroscan Intera 1.5 T CV Nova Dual scanner (Philips Medical Systems, Best, Netherlands). Whole-brain T1-weighted images were obtained using Repetition time (TR) = 25 ms, Echo time (TE) = 4.6 ms, flip angle = 30°, scan time = 376 s, voxel size = 1 mm^3.

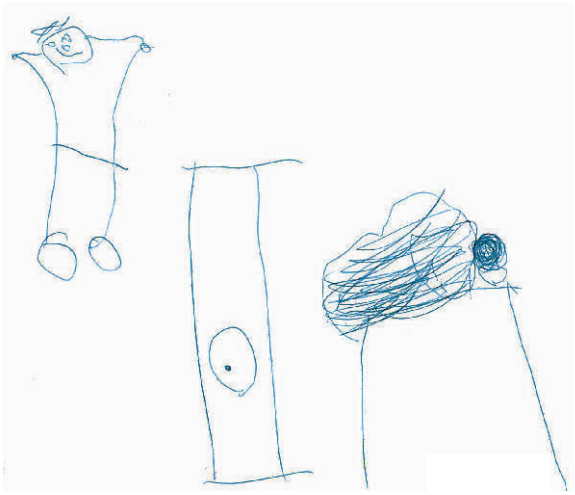

Figure 4. Schematic illustration of GE Healthcare Discovery™ 690 PET/CT scanner located at Turku PET Centre (drawing by 3-year-old Kerttu Ranki)

4.3.2 PET data analysis (Studies I-III)

Reconstructed PET images need to be modelled in order to convert radioactivity concentration into meaningful biological data, such as receptor binding in the brain. Kinetic compartmental models are used to estimate the radiotracer pharmacokinetics (Gunn et al., 1997). Models also provide assumptions about rate constants between the distinct compartments. In all the PET studies to be included in this thesis, a two-tissue

compartment model known as the simplified reference tissue model (SRTM) was utilized (Figure 5). It is based on the comparison of radioligand concentration in receptor-rich to receptor-free regions. Unlike in dynamic PET studies, where arterial plasma concentration of the radiotracer is needed as input data, SRTM allows quantification of receptor availability without any arterial cannulation or blood samples (Lammertsma and Hume, 1996). In this model, time activity curves (TAC) from specific reference regions serve as input data (Gunn et al., 1997).

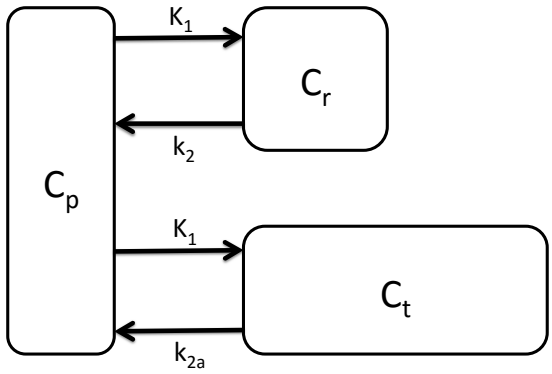

Figure 5. Schematic illustration of the simplified reference tissue model. Adopted from (Lammertsma and Hume, 1996; Lammertsma, 2014). C_p = tracer concentration in arterial plasma; C_r = tracer concentration in the reference tissue; C_t = tracer concentration in the target region; K_1 = rate constant for transfer from arterial plasma to tissue; k_2 = rate constant for transfer from tissue to arterial plasma; k_{2a} = apparent rate constant for transfer from tissue to plasma.

Using SRTM, receptor availability can be measured for each voxel as the binding potential (BP_{ND}), which is the ratio of specific to non-displaceable binding in brain (Innis et al., 2007). In this model BP_{ND} is calculated using the following equation:

$$BP_{ND} = f_{ND} \times 1/K_d \times B_{max}$$

In the equation, f_{ND} stands for the free fraction of the ligand in the non-displaceable tissue, $1/K_d$ is the receptor affinity and B_{max} is the receptor availability. Consequently, BP_{ND} is the product of all these three factors. SRTM rests on numerous assumptions. First, a reference tissue is assumed to have fully non-specific binding. Second, non-specific binding is assumed to be same in both reference and target regions. Third, free ligand and non-specifically bound ligand cannot be separated, and the model assumes that the exchange between these compartments has to be fast to be considered as one compartment.

Before statistical analysis, head motion during scanning as well as individual differences

in brain anatomy need to be accounted for. In the present PET studies, Statistical parametric mapping 8 (SPM8) software (Wellcome Trust Centre for Neuroimaging, London, UK; www.fil.ion.ucl.ac.uk/spm/), running on Matlab R2012a (The Mathworks Inc., Sherborn, Massachusetts), was used in the preprocessing of the PET images. First, head motion was corrected by realigning the dynamic PET images frame-to-frame. Second, subjects' T1-weighted MR images were coregistered to the summation images calculated from previously realigned frames. PMOD 3.4 software (PMOD Technologies Ltd., Zurich, Switzerland) was used to manually draw the reference regions. Occipital cortex was delineated as the reference region for [^{11}C]carfentanil and cerebellum for [^{11}C]raclopride. TACs needed for SRTM were acquired from the reference regions using PMOD software. Normalization of the parametric BP_{ND} images into MNI space was done by first coregistering the PET images with T1-weighted MR images. After normalization of the T1-weighted images, the obtained warping parameters were applied to the PET images. Normalized parametric BP_{ND} maps were smoothed with a Gaussian kernel of 8 mm FWHM.

In the full-volume statistical analyses, voxel-wise differences in [^{11}C]carfentanil and [^{11}C]raclopride BP_{ND}s were compared using independent samples t-tests between morbidly obese and non-obese groups (Studies I and II) and repeated measures t-test within subjects (Study III) in SPM8. The statistical threshold was set at $p < 0.05$, with the False Discovery Rate (FDR) corrected at cluster level. In addition to the full-volume SPM analyses, the AAL (Tzourio-Mazoyer et al., 2002) and Anatomy (Eickhoff et al., 2005) toolboxes were used to automatically delineate ROIs and extract ROI-wise BP_{ND}s in different brain areas: ventral striatum, dorsal caudate nucleus, putamen, insula, amygdala, thalamus, orbitofrontal cortex, anterior cingulate cortex, medial cingulate cortex, and posterior cingulate cortex.

In all the PET studies, the same amount of radioactivity was injected to both obese and non-obese subjects. Although this may lead to smaller count rates in obese subjects' brains, it is unlikely that reconstruction used by GE Healthcare Discovery^{TM} 690 PET/CT scanner would cause an underestimation of SRTM derived BP_{ND}. To further ensure that BP_{ND}s were accurate, the injected amounts of radioactivity per weight (kg) were correlated with MOR and D_2R availabilities in all ROIs, but no correlation was found.

Associations between ROI-wise BP_{ND}s, questionnaire scores, and biological variables were calculated using Pearson's correlations (Studies I and III). Pearson's correlations were also used to estimate regional interactions between receptor systems (Study II). Between-groups differences in the ROI-wise Pearson correlations between receptor systems were calculated using Fisher's test (Study II).

4.4 Magnetic Resonance Imaging (MRI)

Magnetic resonance imaging (MRI) provides an outstanding non-invasive imaging method for different anatomical structures. It is based on the strong (typically > 1.5 Tesla) static magnetic field by the MRI scanner, which can be used to produce different magnetic resonance (MR) signals in order to form high-contrast images of the human body. MR signal originates from the hydrogen atoms (H^1), which are abundant in the human tissue in free water or fat molecules. Hydrogen nuclei consist of a single proton. Protons have

an intrinsic feature called the nuclear spin, which makes every proton to form a tiny magnetic field known as magnetic momentum. Without any external magnetic field (B_0), these magnetic moments are randomly oriented. However, the strong static magnetic field produced by the MRI scanner forces the magnetic moments to align in a direction of parallel or anti-parallel compared to B_0. This results in a net magnetic field or net magnetisation (M_0). (Hendee and Morgan, 1984; Duerk, 1999; Nitz, 1999; McRobbie et al., 2006; Ridgway, 2010).

To create MR signal from M_0, another magnetic field (B_1) is applied using scanner's transmitter / receiver coils. This field is perpendicular to B_0, causing a rotating movement to the net magnetisation. When the duration and the amplitude of B_1 are changed, different radio frequency pulses can be generated. Radio frequency pulses cause the net magnetic moment of the nuclei, to tilt away from equilibrium, bringing them into a higher energy state. When these excitation pulses stop, spin population starts to return back to its previous alignment. This process is called relaxation. The relaxation time differs depending on the proton density and the molecular structure of the tissue. Tissue contrast in MR images arises from the differences in relaxation times between different tissues. There are two different relaxation processes utilized in MRI. The longitudinal relaxation or T1 (spin-lattice) relaxation means the longitudinal realignment of the magnetisation with B_0, whereas transverse relaxation or T2 (spin-spin) is the process in which protons phase or dephase with each other. T1-weighted images offer excellent discrimination between water and fat, and thus differentiation of brain gray and white matter is possible. Weighting transverse (T2) on the contrary produces images in which the signals of water and fat are opposite than in T1-weighted images. (Hendee and Morgan, 1984; Duerk, 1999; McRobbie et al., 2006)

4.4.1 MR Image acquisition (Studies I-V)

MRI was performed using Philips Gyroscan Intera 1.5 T CV Nova Dual scanner. Anatomical images with 1 mm^3 resolution were acquired using a T1-weighted sequence (TR 25 ms, TE 4.6 ms, flip angle 30°, scan time 376 s). T1-weighted images were used in VBM analysis as well as reference images for PET scans. For DTI analysis, 32 non-colinear directions of gradients and non-diffusion weighted b0 image were acquired to obtain the whole diffusion tensor, using an echo-planar imaging (EPI) single-shot sequence (TR 5947 ms, TE 89 ms, 90° flip angle, FOV 240 112 × 112 imaging matrix [reconstructed to 256 x 256], 3 mm slice thickness, 1 mm gap between slices, 36 transverse slices).

The volume of abdominal subcutaneous (SAT) and visceral (VAT) adipose tissue was measured for all the studies (I-V). To this end, axial T1-weighted dual fast field echo images covering the abdominal area were acquired (TR 120 ms, TE 2.3 and 4.6 ms, slice thickness 10 mm without gap). SAT and VAT were assessed from top of liver until the top of femoral bone and analysed using the SliceOmatic software version 4.3 (http://www.tomovision.com/products/sliceomatic.htm).

4.4.2 VBM data analysis (Studies IV-V)

Voxel-based morphometry (VBM) is a method for assessing focal brain structure differences. It allows voxel-wise comparison of brain GM and WM tissue densities between different subjects using information from T1-weighted MR images. (Ashburner and Friston, 2000). For example, comparison between a healthy control group and a group of patients with a particular condition or disease can be made. T1 images are first automatically segmented into probabilistic GM and WM volumes. Following normalization into standard stereotactic space, the smoothed GM and WM images can be compared between groups.

VBM was performed with SPM5 (Study IV) and with SPM8 (Study V) (Wellcome Trust Centre for Neuroimaging, London, UK; www.fil.ion.ucl.ac.uk/spm/). Using SPM software, spatial normalization, tissue classification, and radio-frequency bias correction were combined into a single step with segmentation to grey (GM) and white matter (WM) and cerebro-spinal fluid. Cut-off of spatial normalization was 25 mm, medium affine regularization (0.01) was used. Smoothing with a Gaussian kernel of 10 mm FWHM was performed to the segmented, normalized, and modulated GM and WM images.

General linear model (GLM) (Friston et al., 1994) was used to compare GM and WM densities between obese and non-obese groups (Study IV and V) and within subjects to assess GM and WM changes following bariatric surgery (Study V). Effects of age and gender on brain volume were controlled by modelling them as regressors of no interest. Statistical threshold was set at $p < .05$, FDR corrected at the cluster level while controlling for non-stationarity in VBM analysis (Hayasaka et al., 2004). Moreover, clusterwise GM and WM volumes were extracted using Marsbar software (http://marsbar.sourceforge.net) and correlated with anthropometric and metabolic variables before (Studies IV and V) and after the bariatric surgery (Study V).

4.4.3 DTI data analysis (Study IV)

Diffusion-weighted (DW) MRI is based on detecting the motion of water in different dimensions, making it suitable to image biological tissues with restricted diffusion (Le Bihan et al., 1986). Using DTI, it is possible to extract different measures of water diffusion from DW images to investigate neural microstructure. In the human brain, diffusion is quite restricted in the white matter, where myelinated axons allow the motion of water mainly along the axis of the axons. Most widely used scalars are fractional anisotrophy (FA) and mean diffusivity (MD). FA shows the directionality (FA of 0 indicates free water motion to all directions i.e. isotropic diffusion, but FA of 1 refers to restricted movement to only one direction) (Basser and Pierpaoli, 1996; Alexander et al., 2007). FA is sensitive to microstructural changes, but it cannot differentiate between the types of damage. Therefore other scalars are needed. For example, MD shows the mean squared displacement of water molecules, and it cannot tell anything about the direction of the diffusion. High MD values however reflect less restricted diffusion. MD is basically the inverse measure of the membrane density and fluid viscosity, and can reveal oedema or necrosis (Alexander et al., 2011).

In the first part of the DTI analysis, DW images were corrected for eddy currents and head motion. Subsequently, fractional anisotrophy (FA) and mean diffusivity (MD) diffusion tensors were computed using FDT analysis package in FSL software (www.fmrib.ox.ac.uk/fsl). MD and FA images were normalized to the MNI space using linear and nonlinear transformations and smoothing using a Gaussian kernel of 10 mm FWHM. Next, smoothed FA and MD maps were analysed using SPM5. Comparisons between obese and non-obese groups were calculated using GLM. Effects of age and gender on diffusion tensors were set as regressors of no interest. Statistical threshold was set at $T > 3.30$ and $p < .05$, FWE corrected at the cluster level.

5 RESULTS

5.1 Obesity is associated with decreased cerebral MOR availability (Study I)

The differences in MOR and D_2R availabilities between morbidly obese and non-obese subjects were characterized in a cross-sectional design. MOR and D_2R availabilities were measured with PET using [^{11}C]carfentanil and [^{11}C]raclopride, respectively. Significantly lower [^{11}C]carfentanil BP_{ND} ($p < 0.05$, FDR corrected) was found globally in the obese group compared with the non-obese group. These areas include many parts of the brain that are relevant for reward processing, such as ventral striatum, thalamus, and insula (Figure 6). BMI also correlated negatively with [^{11}C]carfentanil BP_{ND} in most of these areas. Neither full-volume SPM analysis nor ROI analysis revealed any significant differences in [^{11}C]raclopride BP_{ND} in any brain region.

MOR availability and anxiety (STAI) scores were negatively correlated in anterior cingulate cortex, medial cingulate cortex, and posterior cingulate cortex (rs < -0.38, ps < 0.05). Moreover, DEBQ restrained eating score negatively associated with MOR availability in ventral striatum, amygdala, and thalamus (rs < -0.38, ps < 0.05).

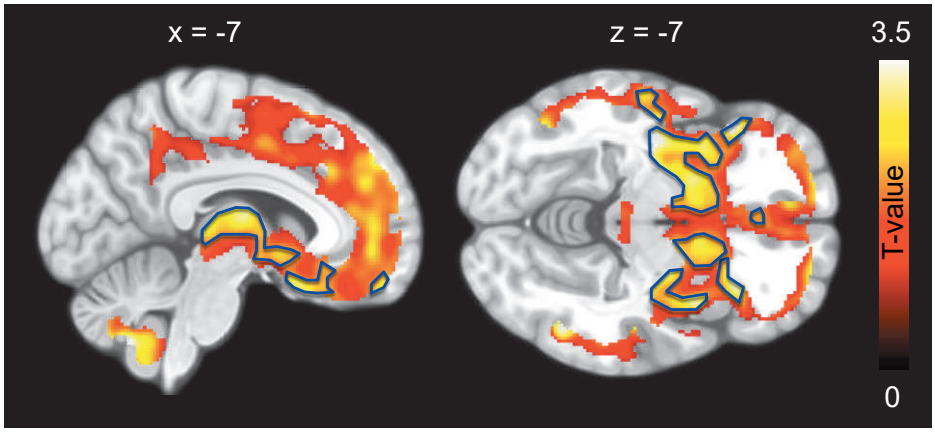

Figure 6. Brain regions where [^{11}C]carfentanil BP_{ND} is lowered in morbidly obese subjects versus non-obese subjects ($p < 0.05$, FDR corrected). The blue outline shows regions where the effect is observed with more stringent statistical threshold ($p < 0.01$, FDR corrected) (Original publication I).

5.2 Interaction between dopamine and opioid systems is disrupted in morbid obesity (Study II)

The interaction between MOR and D_2R systems was investigated in morbidly obese and non-obese subjects. MOR and D_2R availabilities were measured with PET using [^{11}C]carfentanil and [^{11}C]raclopride, respectively. Subsequently, voxel-by-voxel correlations were computed between [^{11}C]carfentanil and [^{11}C]raclopride BP_{ND}s in the obese and lean subjects.

Both voxel-level analysis and ROI analysis[1] showed that in the non-obese subjects, regional MOR and D_2R availability were positively correlated in ventral striatum and dorsal caudate, but not in putamen (Figure 7). In the morbidly obese subjects, the significant association between MOR and D_2R availability was found in dorsal caudate, but the association was disrupted in ventral striatum (Fisher's $z = 1.872$, one-tailed $p = .031$; Figure 8). The association was not driven by group differences in GM density.

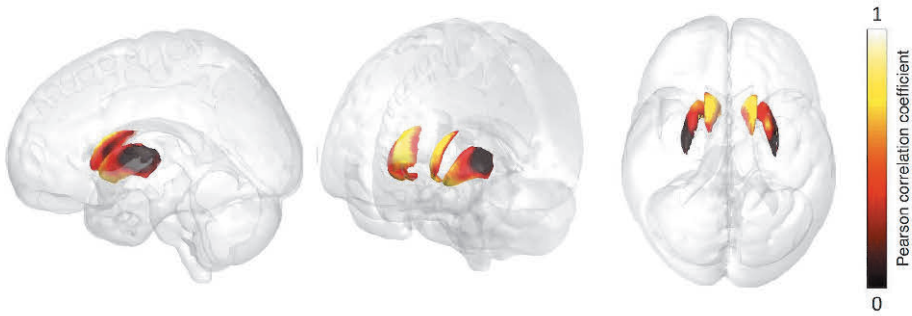

Figure 7. In non-obese subjects MOR and D_2R availability were associated in ventral striatum and dorsal caudate. Colorbar shows the voxelwise Pearson correlation coefficient (Original publication II).

[1] Because [^{11}C]raclopride binding outside the striatal areas is relatively unspecific, the analysis was restricted to this region.

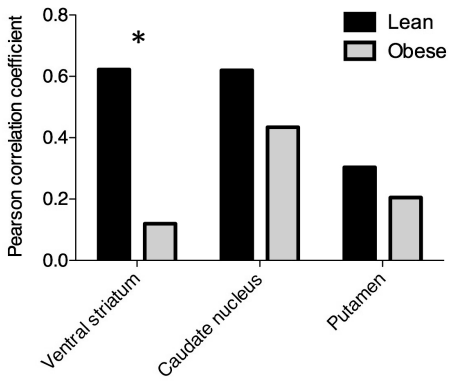

Figure 8. Association between MOR and D_2R availability was significantly decreased in ventral striatum among obese subjects (p = .031), but not in caudate nucleus or putamen (Original publication II).

5.3 Bariatric surgery and weight loss normalizes MOR availability in morbid obesity (Study III)

The effect of bariatric surgery induced weight loss on MOR and D_2R availability was assessed in this study. MOR and D_2R availabilities were measured in the morbidly obese subjects before and six months after the bariatric surgery using PET with [^{11}C]carfentanil and [^{11}C]raclopride. Control subjects were scanned once. Preoperatively, obese had significantly lower MOR availability globally but there were no differences in D_2R availability. Bariatric surgery resulted in mean weight loss of 26 kg. Significant increase in MOR availability was observed in many brain regions relevant for reward functions, such as ventral striatum, thalamus, amygdala, and insula ($p < 0.05$, FDR corrected; Figure 9). This effect was observed in all but one subject (15 out of 16). However, bariatric surgery and weight loss did not cause any changes in D_2R availability (Figure 9).

Before the operation, BMI correlated negatively with [^{11}C]carfentanil BP_{ND} in every ROI except for anterior cinculate cortex (rs < -0.36, ps < 0.05), and after the surgery, [^{11}C]carfentanil BP_{ND} was found to negatively correlate with BMI in ventral striatum and dorsal caudate (rs < -0.45, ps < 0.05). Glycaemic status or insulin sensitivity did not affect MOR availability before or after the operation.

Questionnaire scores revealed, that obese subjects improved postoperatively in food addiction (YFAS), food craving and pathological eating patterns (DEBQ emotional and external eating) (ps < 0.05). No correlations between receptor availabilities and questionnaire scores were found except with STAI scores, which were negatively associated with preoperative [^{11}C]carfentanil BP_{ND}s in all ROIs (rs < -0.31, ps < 0.04).

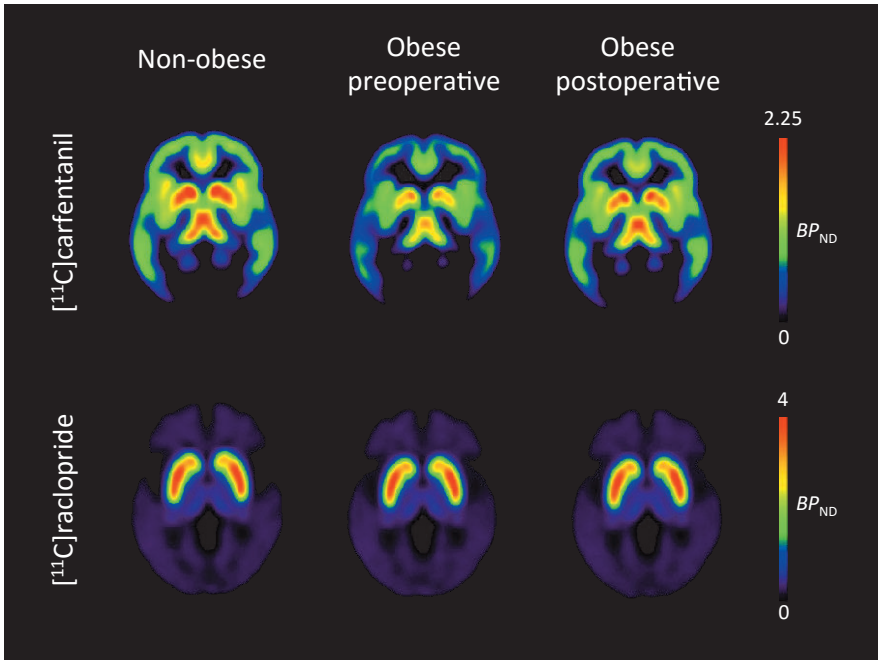

Figure 9. Preoperatively MOR availability was lower in the morbidly obese versus non-obese subjects. Bariatric surgery and weight loss however normalized the MOR availability (upper row) whereas D_2R availability was unaffected (lower row) (Original publication III).

5.4 Obesity is linked to cerebral atrophy and decreased white matter integrity (Study IV)

In this study, the differences in brain white and gray matter densities as well as white matter integrity between obese and non-obese subjects were investigated using VBM and DTI methods. In the VBM analysis, morbidly obese subjects had lower GM densities in right inferior frontal gyrus, left postcentral gyrus, inferior temporal gyri, right middle temporal gyri, temporal lobes, and occipital gyri (Figure 10). WM densities were decreased in the limbic regions and under superior and middle temporal lobes (Figure 10). Notably, GM and WM densities were not larger in morbidly obese versus non-obese controls in any brain region.

DTI analysis showed that morbidly obese versus non-obese subjects had lower FA values in corticospinal tracts, mamillary bodies, optic radiations, corpus callosum, and right inferior occipito-frontal fascicle, while lower MD values were found in uncinate fascicles and inferior occipito-frontal fascicles (Figure 11).

Anthropometric and metabolic variables were also correlated with the regional estimates of brain atrophy. BMI was associated with all the measures of brain atrophy (GM, WM, FA and MD) across the whole BMI range. In addition, percentage of fat correlated with the brain atrophy in most parts of the brain, while subcutaneous adipose tissue volume

associated negatively with most regional GM densities. Glycaemic status or blood pressure levels were not associated with any measures of cerebral atrophy.

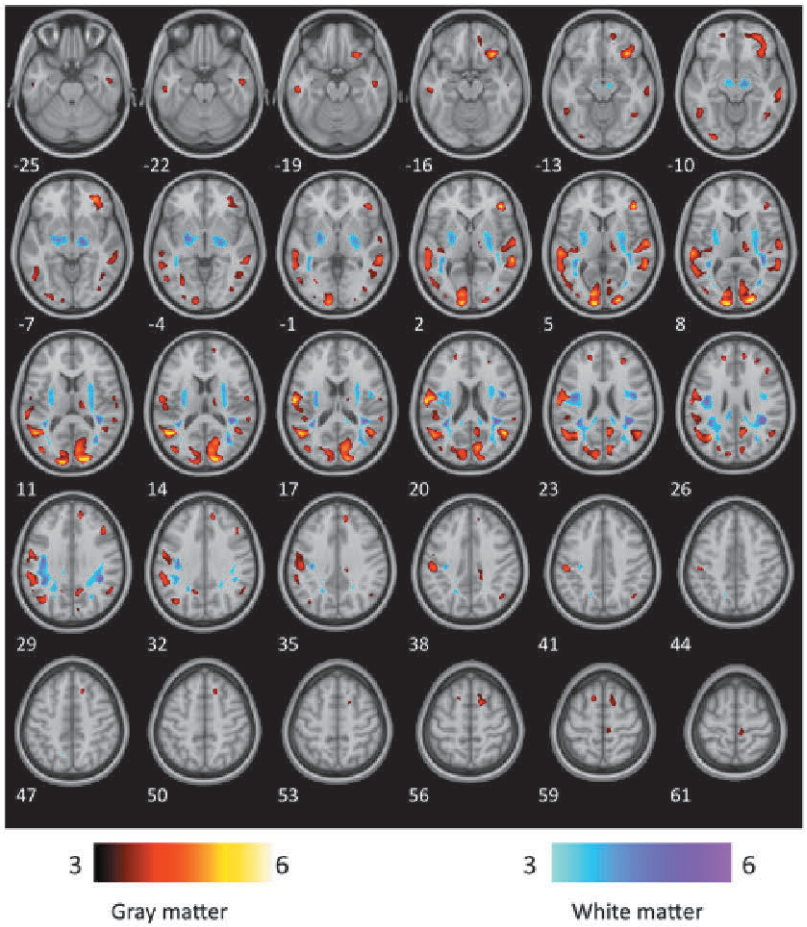

Figure 10. Brain regions that have decreased GM and WM densities in morbidly obese versus non-obese subjects (Original publication IV).

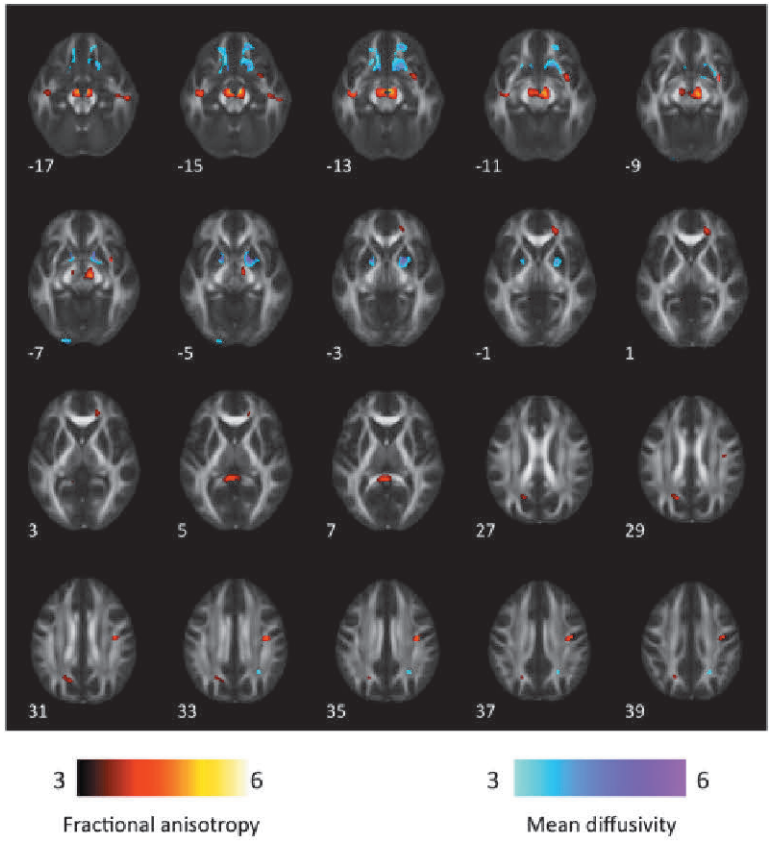

Figure 11. Brain regions that have decreased FA and MD densities in morbidly obese versus non-obese subjects (Original publication IV).

5.5 Bariatric surgery and weight loss recovers GM and WM atrophy in morbid obesity (Study V)

This study was performed in order to test whether bariatric surgery and concomitant weight loss would recover brain white and gray matter densities. VBM analysis was used to investigate i) GM and WM densities between non-obese subjects in the preoperative state and ii) the effect of surgery-induced weight loss on cerebral atrophy in the obese subjects.

Before the surgery, morbidly obese subjects had lower GM density in inferior orbitofrontal regions, frontal regions, and bilateral insula as well as temporal, occipital, and cerebellar regions (Figure 12). WM density was lower under the orbitofrontal gyri and in the midbrain/medulla (Figure 13).

Significant negative correlations were found between preoperative brain densities and

many metabolic variables such as fat percent, fat volumes, plasma lipids, and systolic blood pressure were found. Moreover, HDL cholesterol levels and physical activity associated positively with GM and WM densities in most areas.

Obese subjects' WM densities recovered significantly following the surgery-induced weight loss. The effect was observed globally in WM (Figure 13). GM recovery was found in smaller areas, mainly in occipital and inferior temporal regions (Figure 12).

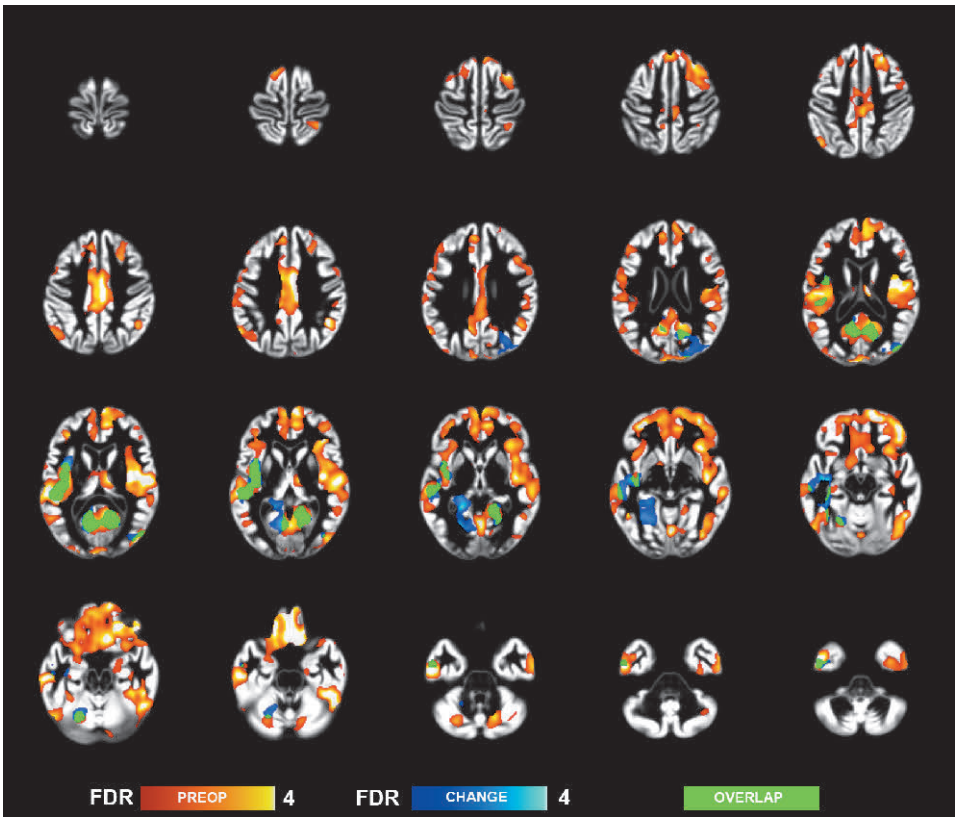

Figure 12. Brain regions with decreased GM density among obese subjects before the surgery (warm) and regions with increased GM density postoperatively (cool) (Original publication V).

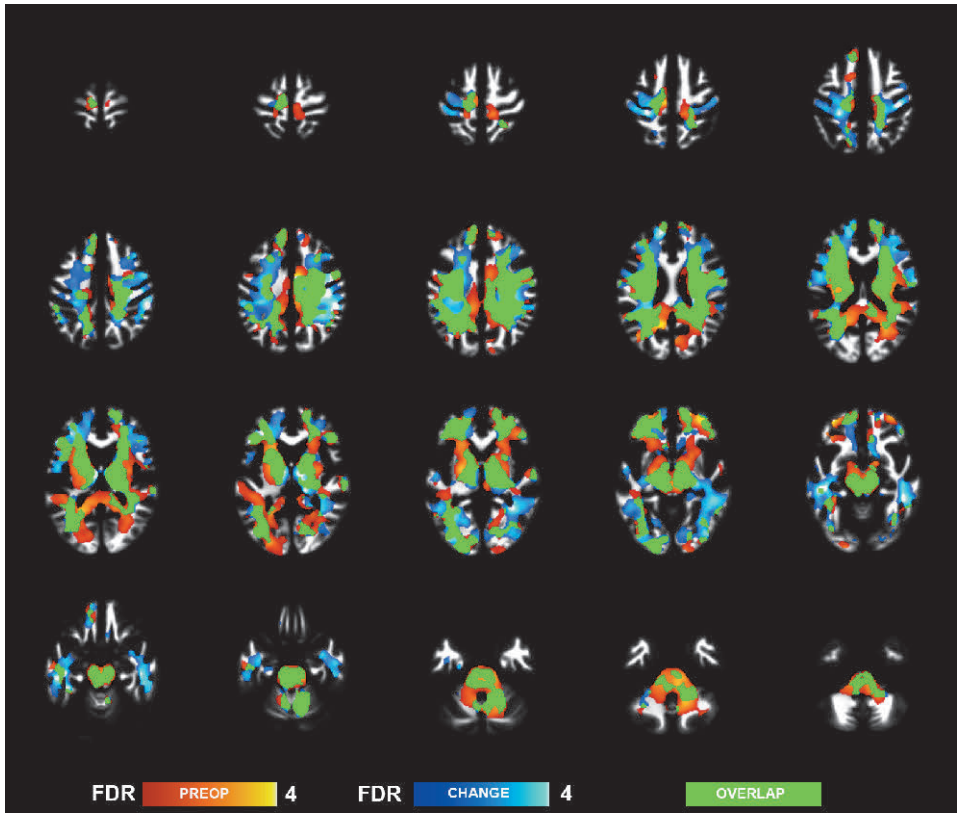

Figure 13. Brain regions with decreased WM density among obese subjects before the surgery (warm) and regions with increased WM density postoperatively (cool) (Original publication V).

6 DISCUSSION

6.1 Obesity is associated with opioidergic dysfunction

Main finding of this study was that morbidly obese subjects had lowered MOR but unaltered D_2R availability in many areas associated with the control of appetite. Decreased MOR availability was observed in the key region of the reward circuit (ventral striatum), regions implicated in emotional functioning (amygdala and prefrontal cortex), as well those subserving homeostatic control (thalamus). Animal studies have demonstrated that endogenous opiod system is important in hedonic aspects of feeding (Pecina and Smith, 2010; Fields and Margolis, 2015), and pharmacological challenge studies have shown that both MOR antagonists and inverse agonists reduce human eating behaviour (Nathan et al., 2012; Cambridge et al., 2013). Opioidergic system also contributes to pleasurable sensations triggered by eating regardless of the type of food: even bland or aversive food can be rendered rewarding (Olszewski et al., 2011). The present study is however the first one to clearly point out that abnormalities of opioidergic neurotransmission are associated with human morbid obesity.

Early animal studies suggested that consuming palatable food elevates the level of β-endorphins in hypothalamus (Dum et al., 1983). More recent work in rodents has shown that palatable food consumption decreases MOR expression in ventral striatum, and withdrawal from palatable food increases it (Pitman and Borgland, 2015). In a similar vein, continuous intake of palatable food in obese individuals may increase the levels of cerebral β-endorphins and cause downregulation in MOR proteins to compensate for the perpetual overstimulation. This may lead to a vicious circle, where decreased amount of receptors or lower affinity of receptors lead to decreased net stimulation of opioidergic system, and in turn, force these individuals to eat more palatable food to get the normal hedonic response.

Human PET studies have shown that subjects with alcohol dependency have increased MOR availability in ventral striatum (Heinz et al., 2005), and also subjects with cocaine addiction have increased MOR availability even more widespread areas (Gorelick et al., 2005). Even though MOR system plays a significant role in both obesity and substance addiction, the underlying mechanisms seem to be different. Smoking did not have any significant affect to MOR availability in this study, which is in line with previous evidence employing PET with [^{11}C]carfentanil (Kuwabara et al., 2014).

Interestingly, a link between anxiety and MOR availability was also found. Endogenous opioid system is involved in neuromodulation of anxiety (Bodnar, 2008; Bowers et al., 2012), and it is known that affective states and stress can cause dynamic changes in the MOR system (Drolet et al., 2001; Zubieta et al., 2003; Ribeiro et al., 2005; Barfield et al., 2013). It is possible that overeating is partly maintained by inability to control emotional arousal states such as anxiety. Thus, obese subjects may use food to alleviate the sensation of anxiety. It has previously been shown that subjects with mood disorders have often

diets high in sugar and fat (Davison and Kaplan, 2012). Reduction of negative affective states may partly arise from overeating, which can also affect to MOR system.

Somewhat surprisingly, no differences between D_2R availabilities in morbidly obese versus non-obese subjects were observed. This contradicts some previous imaging studies (Wang et al., 2001; de Weijer et al., 2011), yet present data are in line with majority of reports (Haltia et al., 2007b; Haltia et al., 2008; Steele et al., 2010; Eisenstein et al., 2013; Guo et al., 2014). Different results in PET studies might be related to, for example, the severity of obesity in the subjects, but also different feeding conditions. These PET scans were performed two hours after last meal. D_2R availability is likely very sensitive to feeding status (Small et al., 2003), although intravenous glucose administration does not affect on D_2R availability, possibly because of overtaken gut hormone activation. Sensitivity of D_2R availability to eating was also speculated in a recent work, where increased D_2R availability was found after 8,5 hours fasting period (Dunn et al., 2012). Authors hypothesized that this might be due reduced dopamine levels of fasting state. In the study showing the greatest effect between BMI and D_2R availability, feeding status of subjects remains indefinite (Wang et al., 2001). Moreover, in the same study, subjects had very high BMI (mean 51) and were completely unmedicated and free of chronic diseases.

In Study II, striatal opioid and dopamine systems were found to be tightly coupled in non-obese but not in obese subjects. In other words, the more MOR the subjects had, the higher were their D_2R levels. Interaction was strongest in ventral striatum, but significant also in dorsal caudate. Interrelated expression between these receptors has not been previously characterized in human reward circuit. Some evidence from rodent studies exists that MOR and D_2R are expressed in same striatal neurons (Ambrose et al., 2004; Margolis and Hjelmstad, 2014). The interaction between these receptor systems is likely crucial in regulating appetite because it breaks down in ventral striatum in the obese subjects, while association in dorsal caudate remains intact. This might explain unaltered D_2R levels in obesity: although obesity-dependent dysfunction in dopaminergic system is shown in numerous animal studies, it may be mediated through MOR-dependent mechanisms without having any effect on the actual amount of D_2R protein. Even if the amount of D_2R protein is not altered in obesity, decoupling of MOR and D_2R in ventral striatum may cause altered dopaminergic functions.

6.2 Bariatric surgery and weight loss recovers the opioidergic dysfunction in the brain reward circuit

Bariatric surgery normalized MOR expression in the morbidly obese subjects, confirming that there exists a link between weight changes and MOR system. This is the first-ever demonstration of the weight loss dependent changes in MOR signalling in humans. This fits with animal studies showing that MOR expression increases after withdrawal from palatable and high-fat diet has been observed (Pitman and Borgland, 2015). Normalization of MOR in the reward circuit after weight loss suggests that excessive eating may lead to perpetual overstimulation of the MOR system, which subsequently causes MOR downregulation. This may lead to overeating in order to get compensate for lowered hedonic impact of food intake. However, downregulated MOR can be brought back to the normal levels, which may also impact eating behaviour. In line with this, increase in MOR

availability was paralleled with lowered self-reported food craving as well as emotional and external eating. Behaviourally this is in line with previous studies, that show improved satiety and lowered appetite after bariatric surgery (Morinigo et al., 2006; Karamanakos et al., 2008), which might be due to change in gut hormones such as ghrelin (Munzberg et al., 2015). Interestingly, both natural food reward and systemic ghrelin administration activates MORs. Ghrelin also influences on dopamine release in ventral striatum (Kawahara et al., 2013). This shows a possible link between gut hormone alterations and MOR changes after bariatric surgery.

It is however possible that healthier eating habits do not arise only from the recovery of MOR availability. Reduced appetite after bariatric surgery may partly be due the change in gastrointestinal hormone secretion (Schmidt et al., 2013). Based on this study, it is not possible to fully differentiate the effect of bariatric surgery *per se* versus weight loss on MOR system. However, it was recently shown, that also weight loss caused by low-calorie diet can cause similar increase of MOR availability as observed here. Obese men without binge-eating habits have decreased MOR availability but this is partly recovered after 15 % weight loss (Burghardt et al., 2015). This study had only a few subjects (7 obese and 7 controls), but they were scanned both in fasting and fed states. Normal-weight men had increased MOR availability after a standard meal, but within the obese subjects the activation of MOR system was lower. Based on this study, obesity seems to affect both receptor density and endogeneous opioid release. This suggests that the surgical operation itself is unlikely to underlie the normalization of the MOR system, and that weight loss and altered eating habits play a major role here.

It should be noted that also other neuroreceptor systems might be involved in the normalization of appetite after bariatric surgery. Recently, changes in the serotonin receptor binding were observed after Roux-en-Y gastric bypass (RYGB) operation in obese subjects (Haahr et al., 2015), which suggest that the control of appetite involves the serotonergic system as well. To make the picture even more complicated, MOR system is interacting with endocannabinoid system in food intake (Lockie et al., 2011). All in all, many homeostatic and neuroreceptor systems play role in appetite control, and subtle changes in these may have intense affects on weight balance.

Increased substance abuse has been reported among patients who have undergone bariatric surgery (Suzuki et al., 2012), and a recent study with rats showed significantly increased morphine self-administration after Roux-en-Y gastric bypass (RYGB) operation (Biegler et al., 2015). Furthermore, rodent studies have shown increase in alcohol reward (Hajnal et al., 2012) and increase in alcohol self-administration (Polston et al., 2013) after RYGB. It is possible that this is at least partly due to changes in opioidergic signalling, which was also observed in this study. Individuals undergoing RYGB may compensate lacking food-driven hedonia (restricted by RYGB) by stimulating the MOR system with drugs of abuse (Volkow and Wise, 2005). However, in this study none of the obese subjects reported any increase in substance abuse.

No postoperative change in the D_2R availability was observed. This is line with an earlier SPECT study that did not find any change in D_2R availability after weight loss (de Weijer et al., 2014), although two other PET studies with significantly smaller samples have shown contradictory results, one increased (Steele et al., 2010) and other decreased (Dunn et al., 2010) D_2R availability. The follow-up period in all these studies was 6 weeks, which does not make these studies fully comparable to our study. However, a recent SPECT study has observed significant increase in D_2R availability after two years from

bariatric surgery (van der Zwaal et al., 2016), which implies that changes in D_2R availability may occur slowly over time. Although it was not possible to detect any change in D_2R availability in Study III, dopaminergic system is likely extremely important in the function of the reward circuit, especially in wanting and craving, but this cannot be seen with PET imaging. Changes in the interaction between D_2R and MOR might explain some of the changes in the eating pattern, but this remains to be investigated.

6.3 Obesity has a profound impact on brain anatomy and integrity but this is reversed after bariatric surgery

Obesity was associated with lowered GM densities in wide regions of the brain. This accords with previous studies, which have showed a clear inverse association between BMI and GM densities. Studies IV and V revealed lowered focal GM densities basically in the same areas than in most previous studies. (Pannacciulli et al., 2006; Gunstad et al., 2008; Taki et al., 2008; Ho et al., 2010; Raji et al., 2010; Cazettes et al., 2011; Horstmann et al., 2011; Bobb et al., 2014; Debette et al., 2014; Janowitz et al., 2015). No difference between subject sex and atrophy was seen, which contradicts some previous studies (Taki et al., 2008). Based on Studies IV and V, both sexes seem to be at risk to obesity-related brain atrophy.

WM was also preoperatively decreased in obese subjects, but in more limited regions. WM reduction has also been showed earlier (Raji et al., 2010), although contradictory studies exist too (Haltia et al., 2007a). This finding was corroborated with the DTI analysis, which revealed loss of integrity in more specific parts of the brain. Decreased FA and MD values were found in cortical and limbic areas of the brain. Lower FA refers to axonal degeneration and demyelination, which is in line with VBM results. Lower FA values are observed, for example, in multiple sclerosis, which is a demyelinating disease (Roosendaal et al., 2009). Lower MD values found in uncinate fascicles and inferior occipito-frontal fascicles are more difficult to interpret. High MD is a measure of membrane dysfunction; lower values suggest higher myelination and denser axonal packing. However, low MD values can be interpreted as dysfunctional energy metabolism, which can explain the observed changes, given that obesity is associated with altered glucose metabolism (Tuulari et al., 2013). Low MD values are seen, for example, in ischemic stroke (Bhagat et al., 2008).

Obese subjects' grey matter density was significantly lower in cortical areas that govern executive function, long-term memory, vision processing and somatosensory functions. It is possible that these structural changes could partly underlie altered reward processing and overeating in obesity. Also previous studies have established that obese individuals have structural changes in the regions that control feeding behaviour (Pannacciulli et al., 2006), but the importance of these structural findings is elusive.

In both Studies IV and V, preoperatively observed brain abnormalities in the obese subjects correlated with body fat percentage, and lowered GM densities also correlated with the volume of subcutaneous fat. It is thus likely that some of the changes in brain structure are due to low-grade systemic inflammation caused by adipose tissue (Vachharajani and Granger, 2009; Gregor and Hotamisligil, 2011; Lumeng and Saltiel, 2011). Association

between lowered brain integrity and inflammation has been observed before in overweight and obese subjects (Cazettes et al., 2011). White adipose tissue is know to produce many different inflammatory molecules that can cause damage to brain tissue (Debette et al., 2010; Aguilar-Valles et al., 2015). These inflammatory markers such as C-reactive protein and interleukin-6 are significantly reduced after bariatric surgery (Rao, 2012), which is beneficial to brain health.

Using a larger sample in Study V, clear associations were established between cerebral atrophy and previously suggested risk factors for atrophy, such as hypertension (Taki et al., 2004; Enzinger et al., 2005), diabetes (Korf et al., 2007) and abnormal cholesterol profile (Cohen et al., 2011). HDL and exercise might have a protective role in brain atrophy while high levels of triglycerides are linked to brain density reductions. Risk factors for brain atrophy are thus quite similar than those for atherosclerosis. This emphasizes the need for strict glycaemic control as well as anti-hypertensive and anti-cholesterol therapies also for those obese patients who do not seem to achieve significant results in weight loss. Additionally, physical activity can be recommended to obese patients to protect brain from deterioration.

Significant recovery in WM densities after bariatric surgery was observed, which is probably due to reduction in low-grade inflammation. This is in line with one prospective study, which showed that lowered WM densities – rather than lowered GM densities – are secondary to weight gain (Yokum et al., 2012). GM density increase was also observed, but its extent was more limited. It is possible that six months is a too short interval to observe changes in GM. Thus, it is too hasty to predict whether some of the alterations in GM have preceded weight gain. It has to be pointed out, that some of the decreased focal GM densities in the cross-sectional analyses were found in areas implicated in reward processing. For example, there was reduction in the density of orbito-frontal areas, which have previously been linked to hedonic processing (Kringelbach, 2005). Some researchers have indeed proposed that structural alterations in hedonic and homeostatic systems contribute significantly to weight gain and eating behaviour (Horstmann et al., 2011). Longer follow-up studies with more intermediate scans are thus needed to reveal whether more widespread GM recovery occurs following weight gain, and also to establish the trajectories of the grey and white matter density recovery.

6.4 Clinical implications

The dysfunction of endogenous opioid system in the reward circuit is a key component underlying overeating, and thus a feasible target for pharmacological and behavioural interventions. This has already been noted in pharmacological studies. For example, MOR antagonist naltrexone reduces pleasantness of food in human subjects (Murray et al., 2014). Even though naltrexone therapy alone does not lead to significant weight loss, more promising results are obtained when it is coupled with bupropion (a dopamine and norepinephrine re-uptake inhibitor) (Lee and Fujioka, 2009; Greenway et al., 2010; Smith et al., 2013; Billes et al., 2014). This might be due to tight coupling of MOR and D_2R, as shown in Study II. Moreover, the success of the combination therapy underlines the complex pattern of neurotransmitter networks underlying overeating, and suggests that both aspects of reward functions – *wanting* and *liking*, processes mediated by dopaminergic

and opioidergic systems, respectively (Berridge and Kringelbach, 2015) – have to be taken care of in order to treat obese patients. Although no obesity-dependent changes was observed in dopaminergic system *per se* using PET with [^{11}C]raclopride, previous animal studies strongly support the relevance of dopaminergic pathways in obesity (Baik, 2013). However, the dopamine-centered reward deficiency theory of food addiction is likely obsolete, which is also supported by a recent meta-analysis (Benton and Young, 2016). Obesity and overeating can also affect dopaminergic signalling via the function of DATs, which were not investigated in these studies. Of note, some of the effects driven by dopaminergic system can be mediated through opioid system, as close interaction with these systems was shown in Study II.

It should be noted that not all the patients benefit from the anti-obesity drugs. Furthermore, behavioural interventions are often inefficient. Bariatric surgery needs to be used with certain obese patients who have failed in non-invasive weight loss strategies. Surgical procedures cause long-term weight loss and also reduce mortality (Sjostrom et al., 2007). However, bariatric surgery involves significant risks and is quite expensive (total cost around 10 000 € per operation in Finland during the year 2015). Thus, the price per one kilo's weight loss is significantly higher than successful weight loss using behavioural or pharmacological therapies. This is why the selection of subjects to undergo a bariatric surgery must be strict. Nonetheless, due to the prevalence of obesity, cheaper and more effective treatments are urgently needed.

Obesity has a unique neurochemical profile in the brain reward system, which is not entirely shared by addictive disorders and substance abuse. Characterizing obesity merely as an addiction would thus be oversimplification. Abuse of drugs causes direct neurochemical effects to reward circuit, which in turn leads to addiction, without any natural homeostatic mechanisms involved. Thus, obese subjects should not be treated just like patients with addicted disorders. Although there were similar changes in the opioid system than some opioid users have (Koch and Hollt, 2008), physiological regulation of feeding as well as the necessity of regular food intake makes obesity a much more complex problem.

Many metabolic variables were associated with brain atrophy among obese. For example, cholesterol profile has a profound impact on brain atrophy in obese. This alludes to more intensive anti-cholesterol therapies to protect brain. Weight loss due to bariatric surgery was found to have beneficial effects to brain structure even in relatively short follow-up period. For example, obesity increases risks for neurodegenerative disorders such as Alzheimer's and Parkinson's diseases (Kivipelto et al., 2005), and weight loss protects against these diseases (Ashrafian et al., 2013). In a large prospective cohort study, BMI had a strong negative correlation with cognitive performance, and higher baseline BMI also associated with more prominent cognitive decline in the future (Cournot et al., 2006). Especially male subjects seem to be in risk for obesity-related cognitive impairment (Elias et al., 2005). Bariatric surgery might thus be very beneficial for countering the obesity-induced cognitive decline. There is clear evidence that weight loss after surgery improves cognitive functions and memory significantly (Miller et al., 2013; Stanek and Gunstad, 2013; Alosco et al., 2014b). All in all, weight loss is very advantageous in protecting the brain from cognitive decline. Moreover, also moderate physical exercise such as walking can significantly protect from brain volume reductions and cognitive impairment (Erickson et al., 2010; Ho et al., 2011), which was also seen in Study V.

When treating patients with obesity, the diversity of neural mechanisms behind the control of appetite should be kept in mind. Individual differences might also have an important role in eating; there might even different neural or psychological phenotypes causing obesity, which we are not yet aware of. For example, studies utilizing fMRI have showed difference between obese individuals with and without binge eating disorder (Balodis et al., 2013b; Balodis et al., 2013a). In the future, more personalized therapies may come into picture when treating different patients. Cognitive reappraisal strategies may be effective to some obese subjects (Yokum and Stice, 2013), some may have benefit from behavioural therapies while others must be operated using surgical procedures. Even video games may turn helpful in this task, knowing that they can directly affect at least dopaminergic system (Koepp et al., 1998).

6.5 Limitations of the studies

PET studies have certain limitations. In data analysis, using BP_{ND} as a measure of receptor binding, changes in receptor availability may be due to changes in the amount of actual receptor protein, receptor affinity, or the amount of endogenous neurotransmitter occupancy in the receptor proteins. Moreover, in the PET studies only a D_2R antagonist [^{11}C]raclopride was used, while a D_2R agonist ligand could be more sensitive for detecting changes in receptor availability among subjects with moderate BMI (Caravaggio et al., 2015). Moreover, we measured only baseline receptor availabilities, and did not measure the effects of eating on receptor system activity, which may effect both MOR (Burghardt et al., 2015) and D_2R availabilities (Small et al., 2003).

In the study III, it was not possible to differentiate the combined effects of postoperative weight loss and altered gut anatomy and function. Altered neuroreceptor availability may be due to the changes in gut hormones but also due to reduced intake of palatable foods. Further studies are needed to elucidate the sole effect of weight loss due to altered energy intake on MOR availability by comparing the effects of weight loss by surgery versus dieting. However, a recent study using conventional weight loss manipulation by dieting suggests that the changes in MOR availability are due to weight loss, not due to gastrointestinal changes (Burghardt et al., 2015). Some patients may start gaining weight later than six months from the bariatric surgery (Cooper et al., 2015) suggesting that longer follow-up studies are needed. Based on preoperative receptor availabilities, future weight gain might be predicted, which opens up new possibilities for research.

Optimally, to get a more complete picture of causal relationships between obesity and brain changes, prospective studies concentrating on developmental trajectories should be performed. Weight loss seems to normalize MOR availability but we do not know whether weight gain decreases it. However, using PET with follow-up studies is problematic due to high radiation doses. MRI follow-up studies are less problematic, and these could be combined with extensive metabolic data to understand the factors behind changes appeared in VBM and DTI. Furthermore, the actual molecular mechanisms behind the increased MOR availability following weight loss still remain unknown, and may be achievable only using translational studies with rodents and humans.

To avoid high radiation doses caused by a longitudinal PET study such as this, the same amount of radioactivity was injected to both obese and non-obese subjects. Due to smaller count rates in the obese subjects' brains, a theoretical risk of underestimation of the SRTM derived BP_{ND} remains. The risk may be more prominent with high-resolution research PET scanners using iterative reconstruction, but less crucial with more conventional whole body PET scanners such as GE Healthcare Discovery 690 PET/CT, which was used in these studies. However, to further ensure that the SRTM estimated BP_{ND}s were accurate, the injected amounts of radioactivity per weight (kg) were correlated with MOR and D_2R availabilities in all ROIs, but no correlation was found. Moreover, normalization of MOR availability postoperatively did not depend on the amount of lost weights, indicating that underestimation of BP_{ND}s is unlikely. Our results also resemble the findings of a recent study (Burghardt et al., 2015), which used [^{11}C]carfentanil with bolus and infusion method as well as iterative reconstruction.

The study groups also limit the generalizability of the findings. In the PET studies, only female subjects were studied, and the results may not be generalizable to male subjects. In the MRI studies, majority of the subjects were also females. Although no gender differences were observed, the groups could have been more balanced. Moreover, morbidly obese subjects were compared with non-obese controls. Studying also subjects with moderate levels of obesity (e.g. BMI between 25 and 35 kg/m^2) could have revealed the critical 'tipping point' in BMI after which brain anatomy and function begins to alter.

6.6 Future directions

The prevalence of obesity is dramatically increasing and there is urgent need for efficient therapies. Obesity is highly expensive for the society, especially due to the secondary chronic diseases. The obesity-linked sequelae such as type 2 diabetes also reduce person's life expectancy and quality of life. It is plausible that in order to tackle the obesity epidemic, many different therapies must be combined.

Pharmacological therapies against obesity have emerged over the years. However, most anti-obesity drugs have adverse side effects, and this has forced the medical companies to withdraw them from markets. Together with naltrexone and bupropion combination therapy, homeostatic mechanisms of food intake may however provide a feasible target for obesity medication. The newest and the most promising anti-obesity drug is liraglutide, a GLP-1 receptor agonist, which is already sold also in the European markets. It is clearly more efficient for weight loss than orlistat, and improves many cardiovascular risk factors (Astrup et al., 2012; Vilsboll et al., 2012; Pi-Sunyer, 2015). GLP-1 binds to GLP-1 receptors located in the areas related to appetite and reward, but it has also direct effect on mesolimbic neurons in ventral tegmental area (Skibicka, 2013; Baggio and Drucker, 2014). However, to achieve significant weight-loss, efficient life-style interventions should be combined with GLP-1 receptor agonists. Recently, an animal study showed that a drug affecting three gut hormone receptors (GLP-1, glucose-dependent insulinotropic polypeptide and glucagon) causes notable reduction of weight (Finan et al., 2015). In the future, we need more data how these gut hormones affect brain's appetite control.

Opioidergic and dopaminergic systems play a role in governing the motivational and rewarding aspects of feeding and the choice of food. This project shed light on the opioidergic dysfunction in obesity, but also the close interaction between these two neurotransmitter systems was shown in Study II. Molecular basis of this interaction – and the disruption of the interaction among obese - needs further verification using animal work. In addition, more studies about the associations between homeostatic regulation of appetite and reward circuit should be performed to get the comprehensive picture of the human appetite control. Gut hormones are known to have direct effect to dopaminergic system; for example, leptin and insulin inhibit dopaminergic system while ghrelin activates it (Palmiter, 2007; Kenny, 2011). Furthermore, the interaction between ghrelin and MOR system in weight loss should be further investigated, because promising new data has been observed (Kawahara et al., 2013). Combining different modalities of brain research, such as PET and fMRI, might be proven successful in this task, although few studies exist yet (Dougherty et al., 2008) Finally, volitional appetite control (Tuulari et al., 2015) and insulin resistance (Heni et al., 2015) also play a role in weight regulation. For example, nasal insulin influences hunger in male subjects (Kullmann et al., 2015). The interaction between gut hormones and brain reward systems needs further research to find implications for management of obesity.

It is unlikely that we can achieve this goal by studying human subjects only. Intensive translational research combining animal studies to human neuroscience studies will lead us closer to the understanding of appetite control. Within this project, it has been shown that obesity has adverse effects on brain function and structure. The actual mechanisms behind these alterations as well as the mechanisms behind the positive change in structure and function after bariatric surgery still remain unclear and should be addressed in future research.

7 CONCLUSIONS

This multimodal brain imaging project revealed that obesity and weight loss influence brain structure and the neural circuits responsible for reward processing in obesity. Although obesity is fundamentally caused by an imbalance between energy gain and energy expenditure, it was shown for the first time that the dysfunction of the endogenous opioid system inside the reward circuit is intimately linked to human obesity.

It is critical to understand that the adverse changes in the opioidergic system may lead to continuance of pathological eating habits. It is now clear that obesity has adverse effects on brain structure as well, but this can be reversed by bariatric surgery and following weight loss. Results showed in this thesis suggest that the cerebral changes associated with obesity are related to the obese phenotype and have developed after weight gain, rather than representing stable neural characteristics of increased propensity to gaining weight. Consequently, obese individuals can affect to their brain health in a positive way by losing weight.

The reward system in mammals has developed by evolutional processes in such conditions, where food has been scarce. Thus, it has been crucial that an animal is willing to find food and willing to eat it. Nowadays, when especially palatable energy-dense food is abundant and easily obtainable, it is even surprising that many people remain normal-weight. Findings presented in this thesis narrow the gap between previous human and animal studies on the neural architecture of the reward circuit and its dysfunction in obesity. Based on these findings, it is too simplistic to consider common metabolic obesity as ”food addiction”. Although the motivation to eat is likely processed via same routes in the reward circuit than the motivation to use drugs of abuse, overeating comprises a much more complex problem. Moreover, its neurobiological profile is unique and distinct from that observed in other addictive disorders. In obesity, hedonic aspects of energy-dense food distort the basic need for energy to survive.

Finally, regulation of appetite occurs via multiple mechanisms. It is likely that overeating is caused by dysfunction of all these systems, and in the future, with the help of the studies presented in this thesis, we are hopefully a little bit closer to assembling the complex picture of appetite control and the mystery of obesity. The findings described in this thesis may help to develop new clinical treatments for obesity. Understanding the changes in the reward-controlling systems among obese subjects will provide information for future pharmacological interventions as well as effective behavioural and psychological interventions for obesity, and thus have a major effect on the national health.

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9 REFERENCES

Aguilar-Valles A, Inoue W, Rummel C, Luheshi GN (2015) Obesity, adipokines and neuroinflammation. Neuropharmacology 96:124-134.

Ahima RS (2008) Revisiting leptin's role in obesity and weight loss. The Journal of clinical investigation 118:2380-2383.

Albuquerque D, Stice E, Rodriguez-Lopez R, Manco L, Nobrega C (2015) Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective. Molecular genetics and genomics : MGG 290:1191-1221.

Alexander AL, Lee JE, Lazar M, Field AS (2007) Diffusion tensor imaging of the brain. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics 4:316-329.

Alexander AL, Hurley SA, Samsonov AA, Adluru N, Hosseinbor AP, Mossahebi P, Tromp do PM, Zakszewski E, Field AS (2011) Characterization of cerebral white matter properties using quantitative magnetic resonance imaging stains. Brain connectivity 1:423-446.

Alonso-Alonso M, Woods SC, Pelchat M, Grigson PS, Stice E, Farooqi S, Khoo CS, Mattes RD, Beauchamp GK (2015) Food reward system: current perspectives and future research needs. Nutrition reviews 73:296-307.

Alosco ML, Stanek KM, Galioto R, Korgaonkar MS, Grieve SM, Brickman AM, Spitznagel MB, Gunstad J (2014a) Body mass index and brain structure in healthy children and adolescents. The International journal of neuroscience 124:49-55.

Alosco ML, Spitznagel MB, Strain G, Devlin M, Cohen R, Paul R, Crosby RD, Mitchell JE, Gunstad J (2014b) Improved memory function two years after bariatric surgery. Obesity (Silver Spring, Md) 22:32-38.

Ambrose LM, Unterwald EM, Van Bockstaele EJ (2004) Ultrastructural evidence for co-localization of dopamine D2 and micro-opioid receptors in the rat dorsolateral striatum. The anatomical record Part A, Discoveries in molecular, cellular, and evolutionary biology 279:583-591.

Ashburner J, Friston KJ (2000) Voxel-based morphometry--the methods. NeuroImage 11:805-821.

Ashrafian H, Harling L, Darzi A, Athanasiou T (2013) Neurodegenerative disease and obesity: what is the role of weight loss and bariatric interventions? Metabolic brain disease 28:341-353.

Astrup A, Carraro R, Finer N, Harper A, Kunesova M, Lean ME, Niskanen L, Rasmussen MF, Rissanen A, Rossner S, Savolainen MJ, Van Gaal L (2012) Safety, tolerability and sustained weight loss over 2 years with the once-daily human GLP-1 analog, liraglutide. International journal of obesity (2005) 36:843-854.

Baggio LL, Drucker DJ (2014) Glucagon-like peptide-1 receptors in the brain: controlling food intake and body weight. The Journal of clinical investigation 124:4223-4226.

Baik JH (2013) Dopamine signaling in reward-related behaviors. Frontiers in neural circuits 7:152.

Bailey A, Gianotti R, Ho A, Kreek MJ (2005) Persistent upregulation of mu-opioid, but not adenosine, receptors in brains of long-term withdrawn escalating dose "binge" cocaine-treated rats. Synapse (New York, NY) 57:160-166.

Bailey DT, DW; Valk, PE; Maisey, MN (2005) Positron Emission Tomography. Basic Sciences.: Springer.

Balodis IM, Molina ND, Kober H, Worhunsky PD, White MA, Rajita S, Grilo CM, Potenza MN (2013a) Divergent neural substrates of inhibitory control in binge eating disorder relative to other manifestations of obesity. Obesity (Silver Spring, Md) 21:367-377.

Balodis IM, Kober H, Worhunsky PD, White MA, Stevens MC, Pearlson GD, Sinha R, Grilo CM, Potenza MN (2013b) Monetary reward processing in obese individuals with and without binge eating disorder. Biological psychiatry 73:877-886.

Barfield ET, Moser VA, Hand A, Grisel JE (2013) beta-endorphin modulates the effect of stress on novelty-suppressed feeding. Frontiers in behavioral neuroscience 7:19.

Barnes MJ, Holmes G, Primeaux SD, York DA, Bray GA (2006) Increased expression of mu opioid receptors in animals susceptible to diet-induced obesity. Peptides 27:3292-3298.

Bassareo V, Di Chiara G (1997) Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. The Journal of neuroscience : the official journal of the Society for Neuroscience 17:851-861.

Basser PJ, Pierpaoli C (1996) Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. Journal of magnetic resonance Series B 111:209-219.

Batterink L, Yokum S, Stice E (2010) Body mass correlates inversely with inhibitory control in response to food among adolescent girls: an fMRI study. NeuroImage 52:1696-1703.

Beck A, Steer R, Brown G (1996) Manual for the Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation.

Beeler JA, Faust RP, Turkson S, Ye H, Zhuang X (2015) Low Dopamine D2 Receptor Increases Vulnerability to Obesity Via Reduced Physical Activity Not Increased Appetitive Motivation. Biological psychiatry.

Bencherif B, Guarda AS, Colantuoni C, Ravert HT, Dannals RF, Frost JJ (2005) Regional mu-opioid receptor binding in insular cortex is decreased in bulimia nervosa and correlates inversely with fasting behavior. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 46:1349-1351.

Benton D, Young HA (2016) A meta-analysis of the relationship between brain dopamine receptors and obesity: a matter of changes in behavior rather than food addiction? International journal of obesity (2005) 40 Suppl 1:S12-21.

Berridge KC (2009) 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. Physiology & behavior 97:537-550.

Berridge KC, Kringelbach ML (2015) Pleasure Systems in the Brain. Neuron 86:646-664.

Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG (2010) The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. Brain research 1350:43-64.

Berthoud HR (2012) The neurobiology of food intake in an obesogenic environment. The Proceedings of the Nutrition Society 71:478-487.

Bhagat YA, Hussain MS, Stobbe RW, Butcher KS, Emery DJ, Shuaib A, Siddiqui MM, Maheshwari P, Al-Hussain F, Beaulieu C (2008) Elevations of diffusion anisotropy are associated with hyper-acute stroke: a serial imaging study. Magnetic resonance imaging 26:683-693.

Biegler JM, Freet CS, Horvath N, Rogers AM, Hajnal A (2015) Increased intravenous morphine self-administration following Roux-en-Y gastric bypass in dietary obese rats. Brain research bulletin.

Billes SK, Sinnayah P, Cowley MA (2014) Naltrexone/bupropion for obesity: An investigational combination pharmacotherapy for weight loss. Pharmacological research : the official journal of the Italian Pharmacological Society 84c:1-11.

Bobb JF, Schwartz BS, Davatzikos C, Caffo B (2014) Cross-sectional and longitudinal association of body mass index and brain volume. Hum Brain Mapp 35:75-88.

Bodnar RJ (2008) Endogenous opiates and behavior: 2007. Peptides 29:2292-2375.

Bowers ME, Choi DC, Ressler KJ (2012) Neuropeptide regulation of fear and anxiety: Implications of cholecystokinin, endogenous opioids, and neuropeptide Y. Physiology & behavior 107:699-710.

Brooks SJ, Benedict C, Burgos J, Kempton MJ, Kullberg J, Nordenskjold R, Kilander L, Nylander R, Larsson EM, Johansson L, Ahlstrom H, Lind L, Schioth HB (2013) Late-life obesity is associated with smaller global and regional gray matter volumes: a voxel-based morphometric study. International journal of obesity (2005) 37:230-236.

Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M (2000) Body mass index and the prevalence of hypertension and dyslipidemia. Obesity Research 8:605-619.

Bruce AS, Bruce JM, Ness AR, Lepping RJ, Malley S, Hancock L, Powell J, Patrician TM, Breslin FJ, Martin LE, Donnelly JE, Brooks WM, Savage CR (2014) A comparison of functional brain changes associated with surgical versus behavioral weight loss. Obesity (Silver Spring, Md) 22:337-343.

Buchwald H, Estok R, Fahrbach K, Banel D, Jensen MD, Pories WJ, Bantle JP, Sledge I (2009) Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. The American journal of medicine 122:248-256.e245.

Burghardt PR, Rothberg AE, Dykhuis KE, Burant CF, Zubieta JK (2015) Endogenous Opioid Mechanisms Are Implicated in Obesity and Weight Loss in Humans. The Journal of clinical endocrinology and metabolism 100:3193-3201.

Cambridge VC, Ziauddeen H, Nathan PJ, Subramaniam N, Dodds C, Chamberlain SR, Koch A, Maltby K, Skeggs AL, Napolitano A, Farooqi IS, Bullmore ET, Fletcher PC (2013) Neural and behavioral effects of a novel mu opioid receptor antagonist in binge-eating obese people. Biological psychiatry 73:887-894.

Caravaggio F, Raitsin S, Gerretsen P, Nakajima S, Wilson A, Graff-Guerrero A (2015) Ventral striatum binding of a dopamine d2/3 receptor agonist but not antagonist predicts normal body mass index. Biological psychiatry 77:196-202.

Carlsson LM, Peltonen M, Ahlin S, Anveden A, Bouchard C, Carlsson B, Jacobson P, Lonroth H, Maglio C, Naslund I, Pirazzi C, Romeo S, Sjoholm K, Sjostrom E, Wedel H, Svensson PA, Sjostrom L

(2012) Bariatric surgery and prevention of type 2 diabetes in Swedish obese subjects. The New England journal of medicine 367:695-704.

Carnell S, Gibson C, Benson L, Ochner CN, Geliebter A (2012) Neuroimaging and obesity: current knowledge and future directions. Obesity reviews : an official journal of the International Association for the Study of Obesity 13:43-56.

Carpenter CL, Wong AM, Li Z, Noble EP, Heber D (2013) Association of dopamine D2 receptor and leptin receptor genes with clinically severe obesity. Obesity (Silver Spring, Md) 21:E467-473.

Castro DC, Berridge KC (2014) Advances in the neurobiological bases for food 'liking' versus 'wanting'. Physiology & behavior 136:22-30.

Cazettes F, Cohen JI, Yau PL, Talbot H, Convit A (2011) Obesity-mediated inflammation may damage the brain circuit that regulates food intake. Brain research 1373:101-109.

Cepeda-Benito A, Gleaves DH, Fernandez MC, Vila J, Williams TL, Reynoso J (2000) The development and validation of Spanish versions of the State and Trait Food Cravings Questionnaires. Behaviour research and therapy 38:1125-1138.

Chartoff EH, Connery HS (2014) It's MORe exciting than mu: crosstalk between mu opioid receptors and glutamatergic transmission in the mesolimbic dopamine system. Frontiers in pharmacology 5:116.

Chen PS, Yang YK, Yeh TL, Lee IH, Yao WJ, Chiu NT, Lu RB (2008) Correlation between body mass index and striatal dopamine transporter availability in healthy volunteers--a SPECT study. NeuroImage 40:275-279.

Climie RE, Moran C, Callisaya M, Blizzard L, Sharman JE, Venn A, Phan TG, Beare R, Forbes J, Blackburn NB, Srikanth V (2015) Abdominal Obesity and Brain Atrophy in Type 2 Diabetes Mellitus. PloS one 10:e0142589.

Cohen JI, Cazettes F, Convit A (2011) Abnormal cholesterol is associated with prefrontal white matter abnormalities among obese adults, a diffusion tensor imaging study. The neuroradiology journal 1:989-997.

Colasanti A, Searle GE, Long CJ, Hill SP, Reiley RR, Quelch D, Erritzoe D, Tziortzi AC, Reed LJ, Lingford-Hughes AR, Waldman AD, Schruers KR, Matthews PM, Gunn RN, Nutt DJ, Rabiner EA (2012) Endogenous opioid release in the human brain reward system induced by acute amphetamine administration. Biological psychiatry 72:371-377.

Cole JH, Boyle CP, Simmons A, Cohen-Woods S, Rivera M, McGuffin P, Thompson PM, Fu CH (2013) Body mass index, but not FTO genotype or major depressive disorder, influences brain structure. Neuroscience 252:109-117.

Cone JJ, Chartoff EH, Potter DN, Ebner SR, Roitman MF (2013) Prolonged high fat diet reduces dopamine reuptake without altering DAT gene expression. PloS one 8:e58251.

Cooper SJ, Al-Naser HA (2006) Dopaminergic control of food choice: contrasting effects of SKF 38393 and quinpirole on high-palatability food preference in the rat. Neuropharmacology 50:953-963.

Cooper TC, Simmons EB, Webb K, Burns JL, Kushner RF (2015) Trends in Weight Regain Following Roux-en-Y Gastric Bypass (RYGB) Bariatric Surgery. Obesity surgery.

Cournot M, Marquie JC, Ansiau D, Martinaud C, Fonds H, Ferrieres J, Ruidavets JB (2006) Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology 67:1208-1214.

Davis CA, Levitan RD, Reid C, Carter JC, Kaplan AS, Patte KA, King N, Curtis C, Kennedy JL (2009) Dopamine for "wanting" and opioids for "liking": a comparison of obese adults with and without binge eating. Obesity (Silver Spring, Md) 17:1220-1225.

Davison KM, Kaplan BJ (2012) Food intake and blood cholesterol levels of community-based adults with mood disorders. BMC psychiatry 12:10.

de Weijer BA, van de Giessen E, van Amelsvoort TA, Boot E, Braak B, Janssen IM, van de Laar A, Fliers E, Serlie MJ, Booij J (2011) Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. EJNMMI research 1:37.

de Weijer BA, van de Giessen E, Janssen I, Berends FJ, van de Laar A, Ackermans MT, Fliers E, la Fleur SE, Booij J, Serlie MJ (2014) Striatal dopamine receptor binding in morbidly obese women before and after gastric bypass surgery and its relationship with insulin sensitivity. Diabetologia 57:1078-1080.

Debette S, Seshadri S, Beiser A, Au R, Himali JJ, Palumbo C, Wolf PA, DeCarli C (2011) Midlife vascular risk factor exposure accelerates structural brain aging and cognitive decline. Neurology 77:461-468.

Debette S, Beiser A, Hoffmann U, Decarli C, O'Donnell CJ, Massaro JM, Au R, Himali JJ, Wolf PA, Fox CS, Seshadri S (2010) Visceral fat is associated with lower brain volume in healthy middle-aged adults. Annals of neurology 68:136-144.

Debette S, Wolf C, Lambert JC, Crivello F, Soumare A, Zhu YC, Schilling S, Dufouil C, Mazoyer B, Amouyel P, Tzourio C, Elbaz A (2014) Abdominal obesity and lower gray matter volume: a Mendelian randomization study. Neurobiology of aging 35:378-386.

DiLeone RJ, Taylor JR, Picciotto MR (2012) The drive to eat: comparisons and distinctions between mechanisms of food reward and drug addiction. Nature neuroscience 15:1330-1335.

Dougherty DD, Kong J, Webb M, Bonab AA, Fischman AJ, Gollub RL (2008) A combined [11C]diprenorphine PET study and fMRI study of acupuncture analgesia. Behavioural brain research 193:63-68.

Drolet G, Dumont EC, Gosselin I, Kinkead R, Laforest S, Trottier JF (2001) Role of endogenous opioid system in the regulation of the stress response. Progress in neuro-psychopharmacology & biological psychiatry 25:729-741.

Duerk JL (1999) Principles of MR image formation and reconstruction. Magnetic resonance imaging clinics of North America 7:629-659.

Dum J, Gramsch C, Herz A (1983) Activation of hypothalamic beta-endorphin pools by reward induced by highly palatable food. Pharmacology, biochemistry, and behavior 18:443-447.

Dunn JP, Cowan RL, Volkow ND, Feurer ID, Li R, Williams DB, Kessler RM, Abumrad NN (2010) Decreased dopamine type 2 receptor availability after bariatric surgery: preliminary findings. Brain research 1350:123-130.

Dunn JP, Kessler RM, Feurer ID, Volkow ND, Patterson BW, Ansari MS, Li R, Marks-Shulman P, Abumrad NN (2012) Relationship of dopamine type 2 receptor binding potential with fasting neuroendocrine hormones and insulin sensitivity in human obesity. Diabetes care 35:1105-1111.

Eickhoff SB, Stephan KE, Mohlberg H, Grefkes C, Fink GR, Amunts K, Zilles K (2005) A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. NeuroImage 25:1325-1335.

Eisenstein SA, Antenor-Dorsey JA, Gredysa DM, Koller JM, Bihun EC, Ranck SA, Arbelaez AM, Klein S, Perlmutter JS, Moerlein SM, Black KJ, Hershey T (2013) A comparison of D2 receptor specific binding in obese and normal-weight individuals using PET with (N-[(11)C]methyl)benperidol. Synapse (New York, NY) 67:748-756.

Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB (2005) Obesity, diabetes and cognitive deficit: The Framingham Heart Study. Neurobiology of aging 26 Suppl 1:11-16.

Enzinger C, Fazekas F, Matthews PM, Ropele S, Schmidt H, Smith S, Schmidt R (2005) Risk factors for progression of brain atrophy in aging: six-year follow-up of normal subjects. Neurology 64:1704-1711.

Erickson KI, Raji CA, Lopez OL, Becker JT, Rosano C, Newman AB, Gach HM, Thompson PM, Ho AJ, Kuller LH (2010) Physical activity predicts gray matter volume in late adulthood: the Cardiovascular Health Study. Neurology 75:1415-1422.

Farde L, Hall H, Ehrin E, Sedvall G (1986) Quantitative analysis of D2 dopamine receptor binding in the living human brain by PET. Science (New York, NY) 231:258-261.

Fields HL, Margolis EB (2015) Understanding opioid reward. Trends in neurosciences.

Finan B et al. (2015) A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. Nature medicine 21:27-36.

Frank S, Wilms B, Veit R, Ernst B, Thurnheer M, Kullmann S, Fritsche A, Birbaumer N, Preissl H, Schultes B (2014) Altered brain activity in severely obese women may recover after Roux-en Y gastric bypass surgery. International journal of obesity (2005) 38:341-348.

Franz MJ, VanWormer JJ, Crain AL, Boucher JL, Histon T, Caplan W, Bowman JD, Pronk NP (2007) Weight-loss outcomes: a systematic review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-up. Journal of the American Dietetic Association 107:1755-1767.

Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RS (1994) Statistical parametric maps in functional imaging: a general linear approach. Human brain mapping 2:189-210.

Frost JJ, Wagner HN, Jr., Dannals RF, Ravert HT, Links JM, Wilson AA, Burns HD, Wong DF, McPherson RW, Rosenbaum AE, et al. (1985) Imaging opiate receptors in the human brain by positron tomography. Journal of computer assisted tomography 9:231-236.

Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS (2006) Leptin regulation of the mesoaccumbens dopamine pathway. Neuron 51:811-822.

Gearhardt AN, Corbin WR, Brownell KD (2009) Preliminary validation of the Yale Food Addiction Scale. Appetite 52:430-436.

Gearhardt AN, Yokum S, Orr PT, Stice E, Corbin WR, Brownell KD (2011) Neural correlates of food addiction. Archives of general psychiatry 68:808-816.

Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN (2009) Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. Neuroscience 159:1193-1199.

Giuliano C, Robbins TW, Nathan PJ, Bullmore ET, Everitt BJ (2012) Inhibition of opioid transmission at the mu-opioid receptor prevents both food seeking and binge-like eating. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 37:2643-2652.

Gloy VL, Briel M, Bhatt DL, Kashyap SR, Schauer PR, Mingrone G, Bucher HC, Nordmann AJ (2013) Bariatric surgery versus non-surgical treatment for obesity: a systematic review and meta-analysis of randomised controlled trials. BMJ (Clinical research ed) 347:f5934.

Gons RA, de Laat KF, van Norden AG, van Oudheusden LJ, van Uden IW, Norris DG, Zwiers MP, de Leeuw FE (2010) Hypertension and cerebral diffusion tensor imaging in small vessel disease. Stroke; a journal of cerebral circulation 41:2801-2806.

Gorelick DA, Kim YK, Bencherif B, Boyd SJ, Nelson R, Copersino M, Endres CJ, Dannals RF, Frost JJ (2005) Imaging brain mu-opioid receptors in abstinent cocaine users: time course and relation to cocaine craving. Biological psychiatry 57:1573-1582.

Gosnell BA, Levine AS (2009) Reward systems and food intake: role of opioids. International journal of obesity (2005) 33 Suppl 2:S54-58.

Greenway FL, Fujioka K, Plodkowski RA, Mudaliar S, Guttadauria M, Erickson J, Kim DD, Dunayevich E, Group C-IS (2010) Effect of naltrexone plus bupropion on weight loss in overweight and obese adults (COR-I): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 376:595-605.

Gregor MF, Hotamisligil GS (2011) Inflammatory mechanisms in obesity. Annual review of immunology 29:415-445.

Grissom NM, Lyde R, Christ L, Sasson IE, Carlin J, Vitins AP, Simmons RA, Reyes TM (2014) Obesity at conception programs the opioid system in the offspring brain. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 39:801-810.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997) Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. NeuroImage 6:279-287.

Gunstad J, Paul RH, Cohen RA, Tate DF, Spitznagel MB, Grieve S, Gordon E (2008) Relationship between body mass index and brain volume in healthy adults. The International journal of neuroscience 118:1582-1593.

Guo J, Simmons WK, Herscovitch P, Martin A, Hall KD (2014) Striatal dopamine D2-like receptor correlation patterns with human obesity and opportunistic eating behavior. Molecular psychiatry 19:1078-1084.

Gupta A, Mayer EA, Sanmiguel CP, Van Horn JD, Woodworth D, Ellingson BM, Fling C, Love A, Tillisch K, Labus JS (2015) Patterns of brain structural connectivity differentiate normal weight from overweight subjects. NeuroImage Clinical 7:506-517.

Gustafson D, Rothenberg E, Blennow K, Steen B, Skoog I (2003) An 18-Year Follow-up of Overweight and Risk of Alzheimer Disease. Arch Intern Med 163:1524-1528.

Haahr ME, Hansen DL, Fisher PM, Svarer C, Stenbaek DS, Madsen K, Madsen J, Holst JJ, Baare WF, Hojgaard L, Almdal T, Knudsen GM (2015) Central 5-HT neurotransmission modulates weight loss following gastric bypass surgery in obese individuals. The Journal of neuroscience : the official journal of the Society for Neuroscience 35:5884-5889.

Haghighi A, Melka MG, Bernard M, Abrahamowicz M, Leonard GT, Richer L, Perron M, Veillette S, Xu CJ, Greenwood CM, Dias A, El-Sohemy A, Gaudet D, Paus T, Pausova Z (2013) Opioid receptor mu 1 gene, fat intake and obesity in adolescence. Molecular psychiatry.

Hajnal A, Zharikov A, Polston JE, Fields MR, Tomasko J, Rogers AM, Volkow ND, Thanos PK (2012) Alcohol reward is increased after Roux-en-Y gastric bypass in dietary obese rats with differential effects following ghrelin antagonism. PloS one 7:e49121.

Haltia LT, Rinne JO, Helin S, Parkkola R, Nagren K, Kaasinen V (2008) Effects of intravenous placebo with glucose expectation on human basal ganglia dopaminergic function. Synapse (New York, NY) 62:682-688.

Haltia LT, Viljanen A, Parkkola R, Kemppainen N, Rinne JO, Nuutila P, Kaasinen V (2007a) Brain white matter expansion in human obesity and the recovering effect of dieting. The Journal of clinical endocrinology and metabolism 92:3278-3284.

Haltia LT, Rinne JO, Merisaari H, Maguire RP, Savontaus E, Helin S, Nagren K, Kaasinen V (2007b) Effects of intravenous glucose on dopaminergic function in the human brain in vivo. Synapse (New York, NY) 61:748-756.

Hardman CA, Rogers PJ, Timpson NJ, Munafo MR (2013) Lack of association between DRD2 and OPRM1 genotypes and adiposity. International journal of obesity (2005).

Hayasaka S, Phan KL, Liberzon I, Worsley KJ, Nichols TE (2004) Nonstationary cluster-size inference with random field and permutation methods. NeuroImage 22:676-687.

Heinz A, Reimold M, Wrase J, Hermann D, Croissant B, Mundle G, Dohmen BM, Braus DF, Schumann G, Machulla HJ, Bares R, Mann K (2005) Correlation of stable elevations in striatal mu-opioid receptor availability in detoxified alcoholic patients with alcohol craving: a positron emission tomography study using carbon 11-labeled carfentanil. Archives of general psychiatry 62:57-64.

Hendee WR, Morgan CJ (1984) Magnetic resonance imaging. Part I--physical principles. The Western journal of medicine 141:491-500.

Heni M, Kullmann S, Preissl H, Fritsche A, Haring HU (2015) Impaired insulin action in the human brain: causes and metabolic consequences. Nature reviews Endocrinology 11:701-711.

Hirvonen J, Aalto S, Lumme V, Nagren K, Kajander J, Vilkman H, Hagelberg N, Oikonen V, Hietala J (2003) Measurement of striatal and thalamic dopamine D2 receptor binding with 11C-raclopride. Nuclear medicine communications 24:1207-1214.

Hirvonen J, Aalto S, Hagelberg N, Maksimow A, Ingman K, Oikonen V, Virkkala J, Nagren K, Scheinin H (2009) Measurement of central mu-opioid receptor binding in vivo with PET and [11C]carfentanil: a test-retest study in healthy subjects. European journal of nuclear medicine and molecular imaging 36:275-286.

Ho AJ, Raji CA, Becker JT, Lopez OL, Kuller LH, Hua X, Dinov ID, Stein JL, Rosano C, Toga AW, Thompson PM (2011) The effects of physical activity, education, and body mass index on the aging brain. Hum Brain Mapp 32:1371-1382.

Ho AJ, Raji CA, Becker JT, Lopez OL, Kuller LH, Hua X, Lee S, Hibar D, Dinov ID, Stein JL, Jack CR, Jr., Weiner MW, Toga AW, Thompson PM (2010) Obesity is linked with lower brain volume in 700 AD and MCI patients. Neurobiology of aging 31:1326-1339.

Holtzman SG (1979) Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. Life sciences 24:219-226.

Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, DiLeone RJ (2006) Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 51:801-810.

Horstmann A, Busse FP, Mathar D, Muller K, Lepsien J, Schlogl H, Kabisch S, Kratzsch J, Neumann J, Stumvoll M, Villringer A, Pleger B (2011) Obesity-Related Differences between Women and Men in Brain Structure and Goal-Directed Behavior. Frontiers in human neuroscience 5:58.

Hsu JL, Chen YL, Leu JG, Jaw FS, Lee CH, Tsai YF, Hsu CY, Bai CH, Leemans A (2012) Microstructural white matter abnormalities in type 2 diabetes mellitus: a diffusion tensor imaging study. NeuroImage 59:1098-1105.

Huang XF, Zavitsanou K, Huang X, Yu Y, Wang H, Chen F, Lawrence AJ, Deng C (2006) Dopamine transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-induced obesity. Behavioural brain research 175:415-419.

Ibrahim IY, Ibrahim HM, Aziz NM, Rahman DM (2015) The protective role of the opioid antagonist LY255582 in the management of high fat diet-induced obesity in adult male albino rats. Endocrine regulations 49:198-205.

Innis RB et al. (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 27:1533-1539.

Jagust W, Harvey D, Mungas D, Haan M (2005) Central obesity and the aging brain. Archives of neurology 62:1545-1548.

Jalabert M, Bourdy R, Courtin J, Veinante P, Manzoni OJ, Barrot M, Georges F (2011) Neuronal circuits underlying acute morphine action on dopamine neurons. Proceedings of the National Academy of Sciences of the United States of America 108:16446-16450.

Janowitz D, Wittfeld K, Terock J, Freyberger HJ, Hegenscheid K, Volzke H, Habes M, Hosten N, Friedrich N, Nauck M, Domanska G, Grabe HJ (2015) Association between waist circumference and gray matter volume in 2344 individuals from two adult community-based samples. NeuroImage 122:149-157.

Johnson AW (2013) Eating beyond metabolic need: how environmental cues influence feeding behavior. Trends in neurosciences 36:101-109.

Johnson PM, Kenny PJ (2010) Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. Nature neuroscience 13:635-641.

Jousilahti P, Tuomilehto J, Vartiainen E, Pekkanen J, Puska P (1996) Body weight, cardiovascular risk factors, and coronary mortality - 15-year follow-up of middle-aged men and women in eastern Finland. Circulation 93:1372-1379.

Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain research Brain research reviews 18:75-113.

Karamanakos SN, Vagenas K, Kalfarentzos F, Alexandrides TK (2008) Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy: a prospective, double blind study. Annals of surgery 247:401-407.

Karlsson HK, Tuominen L, Tuulari JJ, Hirvonen J, Parkkola R, Helin S, Salminen P (2015) Obesity Is Associated with Decreased mu-Opioid But Unaltered Dopamine D2 Receptor Availability in the Brain. 35:3959-3965.

Kawahara Y, Kaneko F, Yamada M, Kishikawa Y, Kawahara H, Nishi A (2013) Food reward-sensitive interaction of ghrelin and opioid receptor pathways in mesolimbic dopamine system. Neuropharmacology 67:395-402.

Keen-Rhinehart E, Ondek K, Schneider JE (2013) Neuroendocrine regulation of appetitive ingestive behavior. Frontiers in neuroscience 7:213.

Kelley AE, Berridge KC (2002) The neuroscience of natural rewards: Relevance to addictive drugs. Journal of Neuroscience 22:3306-3311.

Kenny PJ (2011) Common cellular and molecular mechanisms in obesity and drug addiction. Nature Reviews Neuroscience 12:638-651.

Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kareholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H, Nissinen A (2005) Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Archives of neurology 62:1556-1560.

Koch T, Hollt V (2008) Role of receptor internalization in opioid tolerance and dependence. Pharmacology & therapeutics 117:199-206.

Koepp MJ, Gunn RN, Lawrence AD, Cunningham VJ, Dagher A, Jones T, Brooks DJ, Bench CJ, Grasby PM (1998) Evidence for striatal dopamine release during a video game. Nature 393:266-268.

Koob GF, Volkow ND (2010) Neurocircuitry of Addiction. Neuropsychopharmacology 35:217-238.

Kopelman PG (2000) Obesity as a medical problem. Nature 404:635-643.

Korf ES, van Straaten EC, de Leeuw FE, van der Flier WM, Barkhof F, Pantoni L, Basile AM, Inzitari D, Erkinjuntti T, Wahlund LO, Rostrup E, Schmidt R, Fazekas F, Scheltens P (2007) Diabetes mellitus, hypertension and medial temporal lobe atrophy: the LADIS study. Diabetic medicine : a journal of the British Diabetic Association 24:166-171.

Kringelbach ML (2004) Food for thought: hedonic experience beyond homeostasis in the human brain. Neuroscience 126:807-819.

Kringelbach ML (2005) The human orbitofrontal cortex: linking reward to hedonic experience. Nature reviews Neuroscience 6:691-702.

Kullmann S, Heni M, Veit R, Scheffler K, Machann J, Haring HU, Fritsche A, Preissl H (2015) Selective insulin resistance in homeostatic and cognitive control brain areas in overweight and obese adults. Diabetes care 38:1044-1050.

Kullmann S, Callaghan MF, Heni M, Weiskopf N, Scheffler K, Haring HU, Fritsche A, Veit R, Preissl H (2016) Specific white matter tissue microstructure changes associated with obesity. NeuroImage 125:36-44.

Kuwabara H, Heishman SJ, Brasic JR, Contoreggi C, Cascella N, Mackowick KM, Taylor R, Rousset O, Willis W, Huestis MA, Concheiro M, Wand G, Wong DF, Volkow ND (2014) Mu Opioid Receptor Binding Correlates with Nicotine Dependence and Reward in Smokers. PloS one 9:e113694.

Lammertsma AA (2014) Receptor kinetics - modelling and practical approach. In: PET Pharmacokinetic Course Manual (van den Hoff J, Maguire, R.P., ed). Dresden, Germany.

Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. NeuroImage 4:153-158.

Le Bihan D, Breton E, Lallemand D, Grenier P, Cabanis E, Laval-Jeantet M (1986) MR imaging of intravoxel incoherent motions: application to diffusion and perfusion in neurologic disorders. Radiology 161:401-407.

Le Merrer J, Becker JA, Befort K, Kieffer BL (2009) Reward processing by the opioid system in the brain. Physiological reviews 89:1379-1412.

Lee MW, Fujioka K (2009) Naltrexone for the treatment of obesity: review and update. Expert opinion on pharmacotherapy 10:1841-1845.

Lockie SH, Czyzyk TA, Chaudhary N, Perez-Tilve D, Woods SC, Oldfield BJ, Statnick MA, Tschop MH (2011) CNS opioid signaling separates cannabinoid receptor 1-mediated effects on body weight and mood-related behavior in mice. Endocrinology 152:3661-3667.

Lou B, Chen M, Luo X, Dai Y (2014) Reduced right frontal fractional anisotropy correlated with early elevated plasma LDL levels in obese young adults. PloS one 9:e108180.

Lumeng CN, Saltiel AR (2011) Inflammatory links between obesity and metabolic disease. The Journal of clinical investigation 121:2111-2117.

Maclullich AM, Ferguson KJ, Reid LM, Deary IJ, Starr JM, Seckl JR, Bastin ME, Wardlaw JM (2009) Higher systolic blood pressure is associated with increased water diffusivity in normal-appearing white matter. Stroke; a journal of cerebral circulation 40:3869-3871.

Maggard-Gibbons M, Maglione M, Livhits M, Ewing B, Maher AR, Hu J, Li Z, Shekelle PG (2013) Bariatric surgery for weight loss and glycemic control in nonmorbidly obese adults with diabetes: a systematic review. JAMA : the journal of the American Medical Association 309:2250-2261.

Manning S, Pucci A, Batterham RL (2015) Roux-en-Y gastric bypass: effects on feeding behavior and underlying mechanisms. The Journal of clinical investigation 125:939-948.

Männistö S, Laatikainen T, Vartiainen E (2012) Suomalaisten lihavuus ennen ja nyt. Tutkimuksesta tiiviisti 4.
Margolis EB, Hjelmstad GO (2014) Direct bidirectional mu-opioid control of midbrain dopamine neurons. 34:14707-14716.
Marks BL, Katz LM, Styner M, Smith JK (2011) Aerobic fitness and obesity: relationship to cerebral white matter integrity in the brain of active and sedentary older adults. British journal of sports medicine 45:1208-1215.
Marques-Iturria I, Scholtens LH, Garolera M, Pueyo R, Garcia-Garcia I, Gonzalez-Tartiere P, Segura B, Junque C, Sender-Palacios MJ, Vernet-Vernet M, Sanchez-Garre C, de Reus MA, Jurado MA, van den Heuvel MP (2015) Affected connectivity organization of the reward system structure in obesity. NeuroImage 111:100-106.
Martinez D, Saccone PA, Liu F, Slifstein M, Orlowska D, Grassetti A, Cook S, Broft A, Van Heertum R, Comer SD (2012) Deficits in dopamine D(2) receptors and presynaptic dopamine in heroin dependence: commonalities and differences with other types of addiction. Biological psychiatry 71:192-198.
Mattson MP, Duan W, Chan SL, Cheng A, Haughey N, Gary DS, Guo Z, Lee J, Furukawa K (2002) Neuroprotective and neurorestorative signal transduction mechanisms in brain aging: Modification by genes, diet and behavior. Neurobiology of Aging 23:695-705.
McRobbie D, Moore E, Graves M, Prince M (2006) MRI from picture to proton. United States of America by Cambridge University Press, New York: Cambridge University Press.
Michaelides M, Thanos PK, Kim R, Cho J, Ananth M, Wang GJ, Volkow ND (2012) PET imaging predicts future body weight and cocaine preference. NeuroImage 59:1508-1513.
Miller LA, Crosby RD, Galioto R, Strain G, Devlin MJ, Wing R, Cohen RA, Paul RH, Mitchell JE, Gunstad J (2013) Bariatric surgery patients exhibit improved memory function 12 months postoperatively. Obesity surgery 23:1527-1535.
Morinigo R, Moize V, Musri M, Lacy AM, Navarro S, Marin JL, Delgado S, Casamitjana R, Vidal J (2006) Glucagon-like peptide-1, peptide YY, hunger, and satiety after gastric bypass surgery in morbidly obese subjects. The Journal of clinical endocrinology and metabolism 91:1735-1740.
Mueller K, Horstmann A, Moller HE, Anwander A, Lepsien J, Schroeter ML, Villringer A, Pleger B (2014) Obesity Associated Cerebral Gray and White Matter Alterations Are Interrelated in the Female Brain. PloS one 9:e114206.
Mueller K, Anwander A, Moller HE, Horstmann A, Lepsien J, Busse F, Mohammadi S, Schroeter ML, Stumvoll M, Villringer A, Pleger B (2011) Sex-dependent influences of obesity on cerebral white matter investigated by diffusion-tensor imaging. PloS one 6:e18544.
Munzberg H, Laque A, Yu S, Rezai-Zadeh K, Berthoud HR (2015) Appetite and body weight regulation after bariatric surgery. Obesity reviews : an official journal of the International Association for the Study of Obesity 16 Suppl 1:77-90.
Murray E, Brouwer S, McCutcheon R, Harmer CJ, Cowen PJ, McCabe C (2014) Opposing neural effects of naltrexone on food reward and aversion: implications for the treatment of obesity. Psychopharmacology 231:4323-4335.
Nader MA, Morgan D, Gage HD, Nader SH, Calhoun TL, Buchheimer N, Ehrenkaufer R, Mach RH (2006) PET imaging of dopamine D2 receptors during chronic cocaine self-administration in monkeys. Nature neuroscience 9:1050-1056.
Nakanishi S, Hikida T, Yawata S (2014) Distinct dopaminergic control of the direct and indirect pathways in reward-based and avoidance learning behaviors. Neuroscience 282c:49-59.
Nathan PJ, O'Neill BV, Bush MA, Koch A, Tao WX, Maltby K, Napolitano A, Brooke AC, Skeggs AL, Herman CS, Larkin AL, Ignar DM, Richards DB, Williams PM, Bullmore ET (2012) Opioid receptor modulation of hedonic taste preference and food intake: a single-dose safety, pharmacokinetic, and pharmacodynamic investigation with GSK1521498, a novel mu-opioid receptor inverse agonist. Journal of clinical pharmacology 52:464-474.
Nitz WR (1999) MR imaging: acronyms and clinical applications. European radiology 9:979-997.
Nogueiras R, Romero-Pico A, Vazquez MJ, Novelle MG, Lopez M, Dieguez C (2012) The Opioid System and Food Intake: Homeostatic and Hedonic Mechanisms. Obesity facts 5:196-207.
Nummenmaa L, Hirvonen J, Hannukainen JC, Immonen H, Lindroos MM, Salminen P, Nuutila P (2012) Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity. PloS one 7:e31089.
Ochner CN, Stice E, Hutchins E, Afifi L, Geliebter A, Hirsch J, Teixeira J (2012) Relation between changes in neural responsivity and reductions in desire to eat high-calorie foods following gastric bypass surgery. Neuroscience 209:128-135.
Ogden CL, Carroll MD, Flegal KM (2014) Prevalence of obesity in the United States. JAMA : the journal of the American Medical Association 312:189-190.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of comparative and physiological psychology 47:419-427.

Olszewski PK, Alsio J, Schioth HB, Levine AS (2011) Opioids as facilitators of feeding: can any food be rewarding? Physiology & behavior 104:105-110.

Ostlund SB, Kosheleff A, Maidment NT, Murphy NP (2013) Decreased consumption of sweet fluids in mu opioid receptor knockout mice: a microstructural analysis of licking behavior. Psychopharmacology 229:105-113.

Palmiter RD (2007) Is dopamine a physiologically relevant mediator of feeding behavior? Trends in neurosciences 30:375-381.

Pannacciulli N, Le DS, Chen K, Reiman EM, Krakoff J (2007) Relationships between plasma leptin concentrations and human brain structure: a voxel-based morphometric study. Neuroscience letters 412:248-253.

Pannacciulli N, Del Parigi A, Chen K, Le DS, Reiman EM, Tataranni PA (2006) Brain abnormalities in human obesity: a voxel-based morphometric study. NeuroImage 31:1419-1425.

Papaleo F, Kieffer BL, Tabarin A, Contarino A (2007) Decreased motivation to eat in mu-opioid receptor-deficient mice. The European journal of neuroscience 25:3398-3405.

Pecina S, Smith KS (2010) Hedonic and motivational roles of opioids in food reward: implications for overeating disorders. Pharmacology, biochemistry, and behavior 97:34-46.

Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:9395-9402.

Pi-Sunyer X (2015) Liraglutide in Weight Management. The New England journal of medicine 373:1781-1782.

Pitman KA, Borgland SL (2015) Changes in mu-opioid receptor expression and function in the mesolimbic system after long-term access to a palatable diet. Pharmacology & therapeutics 154:110-119.

Polston JE, Pritchett CE, Tomasko JM, Rogers AM, Leggio L, Thanos PK, Volkow ND, Hajnal A (2013) Roux-en-Y gastric bypass increases intravenous ethanol self-administration in dietary obese rats. PloS one 8:e83741.

Puig J, Blasco G, Daunis IEJ, Molina X, Xifra G, Ricart W, Pedraza S, Fernandez-Aranda F, Fernandez-Real JM (2015) Hypothalamic damage is associated with inflammatory markers and worse cognitive performance in obese subjects. The Journal of clinical endocrinology and metabolism 100:E276-281.

Rada P, Barson JR, Leibowitz SF, Hoebel BG (2010) Opioids in the hypothalamus control dopamine and acetylcholine levels in the nucleus accumbens. Brain research 1312:1-9.

Raji CA, Ho AJ, Parikshak NN, Becker JT, Lopez OL, Kuller LH, Hua X, Leow AD, Toga AW, Thompson PM (2010) Brain structure and obesity. Hum Brain Mapp 31:353-364.

Rao SR (2012) Inflammatory markers and bariatric surgery: a meta-analysis. Inflammation research : official journal of the European Histamine Research Society [et al] 61:789-807.

Raz N, Rodrigue KM, Acker JD (2003) Hypertension and the brain: Vulnerability of the prefrontal regions and executive functions. Behavioral Neuroscience 117:1169-1180.

Reddy IA, Wasserman DH, Ayala JE, Hasty AH, Abumrad NN, Galli A (2014) Striatal dopamine homeostasis is altered in mice following Roux-en-Y gastric bypass surgery. ACS chemical neuroscience 5:943-951.

Ribeiro SC, Kennedy SE, Smith YR, Stohler CS, Zubieta JK (2005) Interface of physical and emotional stress regulation through the endogenous opioid system and mu-opioid receptors. Progress in neuro-psychopharmacology & biological psychiatry 29:1264-1280.

Ridgway JP (2010) Cardiovascular magnetic resonance physics for clinicians: part I. Journal of cardiovascular magnetic resonance : official journal of the Society for Cardiovascular Magnetic Resonance 12:71.

Roosendaal SD, Geurts JJ, Vrenken H, Hulst HE, Cover KS, Castelijns JA, Pouwels PJ, Barkhof F (2009) Regional DTI differences in multiple sclerosis patients. NeuroImage 44:1397-1403.

Sahr AE, Sindelar DK, Alexander-Chacko JT, Eastwood BJ, Mitch CH, Statnick MA (2008) Activation of mesolimbic dopamine neurons during novel and daily limited access to palatable food is blocked by the opioid antagonist LY255582. American journal of physiology Regulatory, integrative and comparative physiology 295:R463-471.

Salamone JD, Correa M (2013) Dopamine and food addiction: lexicon badly needed. Biological psychiatry 73:e15-24.

Saper CB, Chou TC, Elmquist JK (2002) The need to feed: homeostatic and hedonic control of eating. Neuron 36:199-211.

Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, Aminian A, Pothier CE, Kim ES, Nissen SE, Kashyap SR (2014) Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. The New England journal of medicine 370:2002-2013.

Schmidt JB, Pedersen SD, Gregersen NT, Vestergaard L, Nielsen MS, Ritz C, Madsbad S, Worm D, Hansen DL, Clausen TR, Rehfeld JF, Astrup A, Holst JJ, Sjodin A (2013) Effects of RYGB on energy expenditure, appetite and glycaemic control: a randomized controlled clinical trial. International journal of obesity (2005).

Shin AC, Berthoud HR (2011) Food reward functions as affected by obesity and bariatric surgery. International journal of obesity (2005) 35 Suppl 3:S40-44.

Shin AC, Pistell PJ, Phifer CB, Berthoud HR (2010) Reversible suppression of food reward behavior by chronic mu-opioid receptor antagonism in the nucleus accumbens. Neuroscience 170:580-588.

Sjostrom L et al. (2012) Bariatric surgery and long-term cardiovascular events. JAMA : the journal of the American Medical Association 307:56-65.

Sjostrom L et al. (2007) Effects of bariatric surgery on mortality in Swedish obese subjects. The New England journal of medicine 357:741-752.

Skibicka KP (2013) The central GLP-1: implications for food and drug reward. Frontiers in neuroscience 7:181.

Small DM, Jones-Gotman M, Dagher A (2003) Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. NeuroImage 19:1709-1715.

Smith SL, Harrold JA, Williams G (2002) Diet-induced obesity increases mu opioid receptor binding in specific regions of the rat brain. Brain research 953:215-222.

Smith SR, Fujioka K, Gupta AK, Billes SK, Burns C, Kim D, Dunayevich E, Greenway FL (2013) Combination therapy with naltrexone and bupropion for obesity reduces total and visceral adiposity. Diabetes, obesity & metabolism 15:863-866.

Soderman AR, Unterwald EM (2009) Cocaine-induced mu opioid receptor occupancy within the striatum is mediated by dopamine D2 receptors. Brain research 1296:63-71.

South T, Huang XF (2008) High-fat diet exposure increases dopamine D2 receptor and decreases dopamine transporter receptor binding density in the nucleus accumbens and caudate putamen of mice. Neurochemical research 33:598-605.

Spanagel R, Herz A, Shippenberg TS (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proceedings of the National Academy of Sciences of the United States of America 89:2046-2050.

Spielberger C, Gorsuch R, Lushene R, Vagg P, Jacobs G (1983) Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.

Stanek KM, Gunstad J (2013) Can bariatric surgery reduce risk of Alzheimer's disease? Progress in neuro-psychopharmacology & biological psychiatry 47:135-139.

Stanek KM, Grieve SM, Brickman AM, Korgaonkar MS, Paul RH, Cohen RA, Gunstad JJ (2011) Obesity is associated with reduced white matter integrity in otherwise healthy adults. Obesity (Silver Spring, Md) 19:500-504.

Steele KE, Prokopowicz GP, Schweitzer MA, Magunsuon TH, Lidor AO, Kuwabawa H, Kumar A, Brasic J, Wong DF (2010) Alterations of central dopamine receptors before and after gastric bypass surgery. Obesity surgery 20:369-374.

Steketee JD, Kalivas PW (2011) Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. Pharmacological reviews 63:348-365.

Stice E, Spoor S, Bohon C, Small DM (2008) Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. Science 322:449-452.

Stice E, Yokum S, Burger KS, Epstein LH, Small DM (2011) Youth at risk for obesity show greater activation of striatal and somatosensory regions to food. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:4360-4366.

Stoeckel LE, Weller RE, Cook Iii EW, Twieg DB, Knowlton RC, Cox JE (2008) Widespread reward-system activation in obese women in response to pictures of high-calorie foods. NeuroImage 41:636-647.

Suzuki J, Haimovici F, Chang G (2012) Alcohol use disorders after bariatric surgery. Obesity surgery 22:201-207.

Sweat V, Starr V, Bruehl H, Arentoft A, Tirsi A, Javier E, Convit A (2008) C-reactive protein is linked to lower cognitive performance in overweight and obese women. Inflammation 31:198-207.

Tabarin A, Diz-Chaves Y, Carmona Mdel C, Catargi B, Zorrilla EP, Roberts AJ, Coscina DV, Rousset S, Redonnet A, Parker GC, Inoue K, Ricquier D, Penicaud L, Kieffer BL, Koob GF (2005) Resistance to diet-induced obesity in mu-opioid receptor-deficient mice: evidence for a "thrifty gene". Diabetes 54:3510-3516.

Taber MT, Zernig G, Fibiger HC (1998) Opioid receptor modulation of feeding-evoked dopamine release in the rat nucleus accumbens. Brain research 785:24-30.

Taki Y, Kinomura S, Sato K, Inoue K, Goto R, Okada K, Uchida S, Kawashima R, Fukuda H (2008) Relationship between body mass index and gray matter volume in 1,428 healthy individuals. Obesity (Silver Spring, Md) 16:119-124.

Taki Y, Goto R, Evans A, Zijdenbos A, Neelin P, Lerch J, Sato K, Ono S, Kinomura S, Nakagawa M, Sugiura M, Watanabe J, Kawashima R, Fukuda H (2004) Voxel-based morphometry of human brain with age and cerebrovascular risk factors. Neurobiology of Aging 25:455-463.

Thomsen G, Ziebell M, Jensen PS, da Cuhna-Bang S, Knudsen GM, Pinborg LH (2013) No correlation between body mass index and striatal dopamine transporter availability in healthy volunteers using SPECT and [123I]PE2I. Obesity (Silver Spring, Md) 21:1803-1806.

Turkington TG (2001) Introduction to PET instrumentation. Journal of nuclear medicine technology 29:4-11.

Tuulari JJ, Karlsson HK, Hirvonen J, Salminen P, Nuutila P, Nummenmaa L (2015) Neural circuits for cognitive appetite control in healthy and obese individuals: an FMRI study. PloS one 10:e0116640.

Tuulari JJ, Karlsson HK, Hirvonen J, Hannukainen JC, Bucci M, Helmio M, Ovaska J, Soinio M, Salminen P, Savisto N, Nummenmaa L, Nuutila P (2013) Weight Loss After Bariatric Surgery Reverses Insulin-Induced Increases in Brain Glucose Metabolism of the Morbidly Obese. Diabetes 62:2747-2751.

Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, Mazoyer B, Joliot M (2002) Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. NeuroImage 15:273-289.

Unger RH (2003) Minireview: Weapons of Lean Body Mass Destruction: The Role of Ectopic Lipids in the Metabolic Syndrome. Endocrinology 144:5159-5165.

Vachharajani V, Granger DN (2009) Adipose tissue: a motor for the inflammation associated with obesity. IUBMB life 61:424-430.

van de Giessen E, Celik F, Schweitzer DH, van den Brink W, Booij J (2014) Dopamine D2/3 receptor availability and amphetamine-induced dopamine release in obesity. Journal of psychopharmacology (Oxford, England) 28:866-873.

van de Giessen E, Hesse S, Caan MW, Zientek F, Dickson JC, Tossici-Bolt L, Sera T, Asenbaum S, Guignard R, Akdemir UO, Knudsen GM, Nobili F, Pagani M, Vander Borght T, Van Laere K, Varrone A, Tatsch K, Booij J, Sabri O (2013) No association between striatal dopamine transporter binding and body mass index: a multi-center European study in healthy volunteers. NeuroImage 64:61-67.

van der Zwaal EM, de Weijer BA, van de Giessen EM, Janssen I, Berends FJ, van de Laar A, Ackermans MT, Fliers E, la Fleur SE, Booij J, Serlie MJ (2016) Striatal dopamine D receptor availability increases after long-term bariatric surgery-induced weight loss. European neuropsychopharmacology : the journal of the European College of Neuropsychopharmacology.

van Dijk EJ, Prins ND, Vermeer SE, Vrooman HA, Hofman A, Koudstaal PJ, Breteler MM (2005) C-reactive protein and cerebral small-vessel disease: the Rotterdam Scan Study. Circulation 112:900-905.

van Strien T, Frijters JER, Bergers GPA, Defares PB (1996) The Dutch Eating Behavior Questionnaire (DEBQ) for assessment of restrained, emotional, and external eating behavior. International Journal of Eating Disorders 5:295-315.

Verdejo-Garcia A, Bechara A (2009) A somatic marker theory of addiction. Neuropharmacology 56:48-62.

Verel I, Visser GW, van Dongen GA (2005) The promise of immuno-PET in radioimmunotherapy. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 46 Suppl 1:164s-171s.

Verstynen TD, Weinstein AM, Schneider WW, Jakicic JM, Rofey DL, Erickson KI (2012) Increased body mass index is associated with a global and distributed decrease in white matter microstructural integrity. Psychosomatic medicine 74:682-690.

Vilsboll T, Christensen M, Junker AE, Knop FK, Gluud LL (2012) Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. BMJ (Clinical research ed) 344:d7771.

Volkow ND (2010) Opioid-dopamine interactions: implications for substance use disorders and their treatment. Biological psychiatry 68:685-686.

Volkow ND, Wise RA (2005) How can drug addiction help us understand obesity? Nature neuroscience 8:555-560.

Volkow ND, Wang GJ, Fowler JS, Telang F (2008a) Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. Philosophical Transactions of the Royal Society B-Biological Sciences 363:3191-3200.

Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R (2012) Food and drug reward: overlapping circuits in human obesity and addiction. Current topics in behavioral neurosciences 11:1-24.

Volkow ND, Wang GJ, Fowler JS, Logan J, Hitzemann R, Ding YS, Pappas N, Shea C, Piscani K (1996) Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcoholism, clinical and experimental research 20:1594-1598.

Volkow ND, Wang GJ, Telang F, Fowler JS, Thanos PK, Logan J, Alexoff D, Ding YS, Wong C, Ma Y, Pradhan K (2008b) Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. NeuroImage 42:1537-1543.

Volkow ND, Chang L, Wang GJ, Fowler JS, Ding YS, Sedler M, Logan J, Franceschi D, Gatley J, Hitzemann R, Gifford A, Wong C, Pappas N (2001) Low level of brain dopamine D2 receptors in methamphetamine abusers: association with metabolism in the orbitofrontal cortex. The American journal of psychiatry 158:2015-2021.

Walther K, Birdsill AC, Glisky EL, Ryan L (2010) Structural brain differences and cognitive functioning related to body mass index in older females. Hum Brain Mapp 31:1052-1064.

Wang GJ, Volkow ND, Thanos PK, Fowler JS (2009) Imaging of brain dopamine pathways: implications for understanding obesity. Journal of addiction medicine 3:8-18.

Wang GJ, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, Netusil N, Fowler JS (2001) Brain dopamine and obesity. Lancet 357:354-357.

Wang GJ, Tomasi D, Convit A, Logan J, Wong CT, Shumay E, Fowler JS, Volkow ND (2014) BMI modulates calorie-dependent dopamine changes in accumbens from glucose intake. PloS one 9:e101585.

Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC (2005) The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. BMC neurology 5:23.

Weerts EM, Wand GS, Kuwabara H, Munro CA, Dannals RF, Hilton J, Frost JJ, McCaul ME (2011) Positron emission tomography imaging of mu- and delta-opioid receptor binding in alcohol-dependent and healthy control subjects. Alcoholism, clinical and experimental research 35:2162-2173.

Whistler JL (2012) Examining the role of mu opioid receptor endocytosis in the beneficial and side-effects of prolonged opioid use: from a symposium on new concepts in mu-opioid pharmacology. Drug and alcohol dependence 121:189-204.

WHO (2015) Obesity and overweight. Available from: http://wwwwhoint/mediacentre/factsheets/fs311/en/.

Wise RA, Spindler J, deWit H, Gerberg GJ (1978) Neuroleptic-induced "anhedonia" in rats: pimozide blocks reward quality of food. Science (New York, NY) 201:262-264.

Xu J, Li Y, Lin H, Sinha R, Potenza MN (2013) Body mass index correlates negatively with white matter integrity in the fornix and corpus callosum: a diffusion tensor imaging study. Hum Brain Mapp 34:1044-1052.

Yau PL, Javier D, Tsui W, Sweat V, Bruehl H, Borod JC, Convit A (2009) Emotional and neutral declarative memory impairments and associated white matter microstructural abnormalities in adults with type 2 diabetes. Psychiatry research 174:223-230.

Yau PL, Javier DC, Ryan CM, Tsui WH, Ardekani BA, Ten S, Convit A (2010) Preliminary evidence for brain complications in obese adolescents with type 2 diabetes mellitus. Diabetologia 53:2298-2306.

Yeomans MR, Gray RW (2002) Opioid peptides and the control of human ingestive behaviour. Neuroscience and biobehavioral reviews 26:713-728.

Yokum S, Stice E (2013) Cognitive regulation of food craving: effects of three cognitive reappraisal strategies on neural response to palatable foods. International journal of obesity (2005) 37:1565-1570.

Yokum S, Ng J, Stice E (2012) Relation of regional gray and white matter volumes to current BMI and future increases in BMI: a prospective MRI study. International journal of obesity (2005) 36:656-664.

Yu YH, Vasselli JR, Zhang Y, Mechanick JI, Korner J, Peterli R (2015) Metabolic vs. hedonic obesity: a conceptual distinction and its clinical implications. Obesity reviews : an official journal of the International Association for the Study of Obesity.

Zhou QY, Palmiter RD (1995) Dopamine-deficient mice are severely hypoactive, adipsic, and aphagic. Cell 83:1197-1209.

Ziauddeen H, Fletcher PC (2013) Is food addiction a valid and useful concept? Obesity reviews : an official journal of the International Association for the Study of Obesity 14:19-28.

Ziauddeen H, Farooqi IS, Fletcher PC (2012) Obesity and the brain: how convincing is the addiction model? Nature reviews Neuroscience 13:279-286.

Zuberi AR, Townsend L, Patterson L, Zheng H, Berthoud HR (2008) Increased adiposity on normal diet, but decreased susceptibility to diet-induced obesity in mu-opioid receptor-deficient mice. European journal of pharmacology 585:14-23.

Zubieta JK, Ketter TA, Bueller JA, Xu Y, Kilbourn MR, Young EA, Koeppe RA (2003) Regulation of human affective responses by anterior cingulate and limbic mu-opioid neurotransmission. Archives of general psychiatry 60:1145-1153.