Neuroendocrine stress responsiveness in human obesity and non-obesity controls

Focus on hypothalamic-pituitary-adrenal axis reactivity in relation to the vasopressin-surrogate copeptin and central noradrenaline and serotonin transporter availability

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ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AVP	Arginine-vasopressin
BMI	Body-mass-index
BP_{ND}	Binding potential
CRH	Corticotropin releasing hormone
CRH1R	Corticotropin releasing hormone receptor 1
[^{11}C]DASB	[^{11}C] 3-Amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile
[^{11}C]MRB	S,S-[^{11}C]O-methylreboxetine
5-HT	5-hydroxytryptamine
5-HTT	5-hydroxytryptamine transporter
HPA	Hypothalamic-pituitary-adrenal
MDMA	3,4-Methylendioxy-N-methylamphetamin
MRI	Magnetic resonance imaging
OB	Obesity
NA	Noradrenaline
NAT	Noradrenaline transporter
NOC	Non-obesity controls
OB	Obesity
PET	Positron emission tomography
PVN	Paraventricular nucleus
SERT	Serotonin transporter
SON	Supraoptic nucleus
SSRI	Selective serotonin reuptake inhibitor
V1aR	Vasopressin receptor 1a
V1bR	Vasopressin receptor 1b, formerly vasopressin receptor 3
V2R	Vasopressin receptor 2
WHR	Waist-hip-ratio

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I. BIBLIOGRAPHIC DESCRIPTION

Christian Schinke

Neuroendocrine stress responsiveness in human obesity and non-obesity controls

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84 pages, 8 figures, 3 publications, 261 references, appendices.

BACKGROUND: Obesity is a leading health burden of the 21st century. Alterations of the individual endocrine stress response and the monoamine system may pathophysiologically contribute to the obesity pandemic and its metabolic and mental complications.

OBJECTIVES: (i) to measure hypothalamic-pituitary-adrenal (HPA) axis responsiveness and its relation to serum concentrations of the arginine-vasopressin (AVP) surrogate copeptin in subjects with obesity (OB) compared to non-obesity controls (NOC), (ii) to test whether HPA axis responsiveness and copeptin are related to central noradrenaline (NA) transporter (NAT) availability, (iii) to assess brain serotonin transporter (SERT) binding potentials in OB compared to NOC.

METHODS: 40 subjects with obesity (BMI > 35kg/m^2) were compared to 25 non-obesity controls, matched for age and sex. (i) All individuals underwent the combined dexamethasone/corticotropin releasing hormone (dex/CRH) test. Plasma ACTH and cortisol curve parameters were derived, and copeptin was assessed in the 1500h sample. (ii) Positron emission tomography (PET) was applied in 10 OB and 10 NOC using the NAT-selective radiotracer S,S-[^{11}C]O-methylreboxetine (MRB) and associated with curve indicators derived from the dex/CRH test as well as with copeptin. (iii) PET using the SERT selective radiotracer [^{11}C] DASB was performed in 30 OB and 15 NOC for intergroup comparison.

RESULTS: (i) OB subjects showed an increased HPA axis responsiveness as measured by cortisol concentrations after CRH stimulation. Correspondingly, the AVP surrogate copeptin was higher in OB along with being significantly associated with HPA axis reactivity. OB subjects had a higher adrenal sensitivity as measured by a lower ACTH/cortisol ratio. (ii) In NOC, the HPA response was related to NAT availability of the amygdala and the orbitofrontal cortex while in OB, this association was located in the hypothalamus. (iii) There were no differences in SERT availability between OB and NOC, but a higher inter-regional SERT connectivity was observed in OB.

CONCLUSION: This work supports the notion of an increased endocrine stress response in human obesity, pointing to interacting alterations of the HPA and neurohypophyseal axes. Normally, these stress axes seem to be linked to prefrontal-limbic NA signaling, whereas a loss of this association in favor of a hypothalamic-centered relation is observed in OB. The SERT network pattern is more closely inter-related in OB, albeit central SERT concentrations per se do not differ between OB and NOC.

II. INTRODUCTION

2.1 Obesity as a global health burden

Obesity is a leading cause of preventable disease, disability and death (Ng *et al.*, 2014; Hruby and Hu, 2015). According to the World Health Organization (WHO), approximately 39% of the world population are overweight (Body Mass Index, BMI, > 25kg/m^2) and 11% of all men and 15% of all women obese (BMI > 30kg/m^2), affecting a total of half a billion people worldwide (WHO Global Health Observatory data repository, 2017). The German Health Interview and Examination Survey revealed a prevalence of overweight of 67.1% in men and 53.0% in women, and an increasing rate of obesity affecting about one fourth of the German population (Mensink *et al.*, 2013). Obesity is frequently associated with the *metabolic syndrome* as a cluster of life expectancy affecting cardiovascular risk factors which include abdominal obesity, dyslipidemia, hypertension and diabetes or prediabetes (Park *et al.*, 2003; International Diabetes Federation, 2017). Morbidity and mortality increase with a body mass index (BMI) > 25kg/m^2 in a J-shaped pattern, making the obesity pandemic to one of the most challenging health concerns of the 21st century (Berrington de Gonzalez *et al.*, 2010; Wang *et al.*, 2011; Di Angelantonio *et al.*, 2016; Aune *et al.*, 2016). These unfavorable physical health conditions are often accompanied by psychosocial distress (Hemmingsson, 2014), stigmatization (Hilbert *et al.*, 2008; Dutton *et al.*, 2014; Papadopoulos and Brennan, 2015), psychiatric illness (Pereira-Miranda *et al.*, 2017) and, hence, a remarkably decreased quality of life (Collins *et al.*, 2016). Obesity is caused by a long-term positive energy balance to which a multitude of environmental, genetic and epigenetic factors predispose (Heymsfield and Wadden, 2017). The neurobiological underpinnings of obesity along with its metabolic and mental complications, however, are incompletely understood, and the not-yet individualized treatment strategies frequently fail to achieve weight loss maintenance.

Over the last years, *stress* has become one of the most acknowledged contributors to the obesity pandemic (Chrousos, 2000; Incollingo *et al.*, 2015). This assumption is partly based on the observed coincidence of a growing level of perceived stress in modern societies and the increasing prevalence of obesity (Chandola *et al.*, 2006). It is noteworthy, however, that not all *stressed* individuals are equally prone to civilization diseases, underlining a varying vulnerability to stressors (van der Valk *et al.*, 2018). Hence, it is to question whether it is rather the individual *stress response* than stress per se which accounts for an endophenotype predisposing to stress-associated civilization diseases such as obesity and its associated physical and mental disorders.

2.2 Neurobiology of stress

Stress is any endangerment of the *milieu intérieur* (Bernard, 1879) triggered by an actual or perceived challenge. It is counteracted by an adaptive neuronal and hormonal response cascade to maintain equilibrium in situations of demanding environmental or internal changes (Cannon, 1932; Selye, 1946). The neuroendocrine stress response is evolutionarily highly conserved and crucial for survival (Sapolsky *et al.*, 2000; Gold, 2015), but may predispose to stress-related physical and mental disorders once its regulation is thrown out of joint (Chrousos, 2009; Holsboer and Ising, 2010).

2.3 Stress and obesity

The change of one's eating behavior in situations of mental stress is probably familiar to all of us. Analogously, the repeated administration of mild stressors leads to hyperphagia with preference for highly palatable food in rodents (Rowland and Antelman, 1976). This observation parallels the dose-dependent relation of chronic work stress exposure to the risk of the metabolic syndrome in humans (Chandola *et al.*, 2006). Stress hormones profoundly affect the intake, distribution and expenditure of energy (Cavagnini *et al.*, 2000; Anagnostis *et al.*, 2009), and the clinical picture of patients suffering from Cushing's syndrome sheds light on the causative role of elevated glucocorticoid concentrations in the pathogenesis of obesity and the metabolic syndrome (Loriaux, 2017). However, basal cortisol concentrations are normal in common obesity (Bjorntorp and Rosmond, 2000; Loriaux, 2017), and stress per se does not necessarily lead to hyperphagia in all humans (Epel *et al.*, 2001). Rather, it seems that in response to distress, especially *high cortisol reactors* preferably consume highly palatable rewarding food, probably to ameliorate symptoms of perceived distress (Adam and Epel, 2007). Hence, rather than stressors or basal cortisol concentrations per se, the individual stress *response* is likely to pre-dispose to an increased appetite and energy intake (Epel *et al.*, 2001; van der Valk *et al.*, 2018).
The stress response involves the activation of the *hypothalamic-pituitary-adrenal (HPA) axis*, leading to the subsequent release of CRH, ACTH and cortisol, and the *hypothalamic-posterior-pituitary-axis* – also called *neurohypophyseal axis* – which triggers AVP secretion into the peripheral circulation. They are centrally modulated by biogenic amines which in turn affect mood, behavior, appetite and metabolism (Hainer *et al.*, 2006; Nelson and Gehlert, 2006), as introduced to below.

2.4 Neuroendocrine correlates of the stress response – The hypothalamic pituitary-adrenal- and neurohypophyseal axes

The *hypothalamic-pituitary-adrenal axis* consists of the medial part of the hypothalamus, the *anterior* pituitary gland and the adrenal cortex which subsequently produce corticotropin-releasing-hormone (CRH) and arginine-vasopressin (AVP) to trigger the release of adrenocorticotropic hormone (ACTH) and the production of cortisol in response to stress (Scott and Dinan, 1998; Aguilera, 2011).

The *hypothalamic-neurohypophyseal* axis also consists of the medial hypothalamus but the *posterior* pituitary gland, the so-called neurohypophysis, to release AVP into the peripheral circulation to mediate water conservation and vascular regulation primarily in response to acute stressors such as osmotic stimuli and changes in blood pressure (Schrier and Bichet, 1981; Jochberger *et al.*, 2006; Balanescu *et al.*, 2011; Nickel *et al.*, 2012).

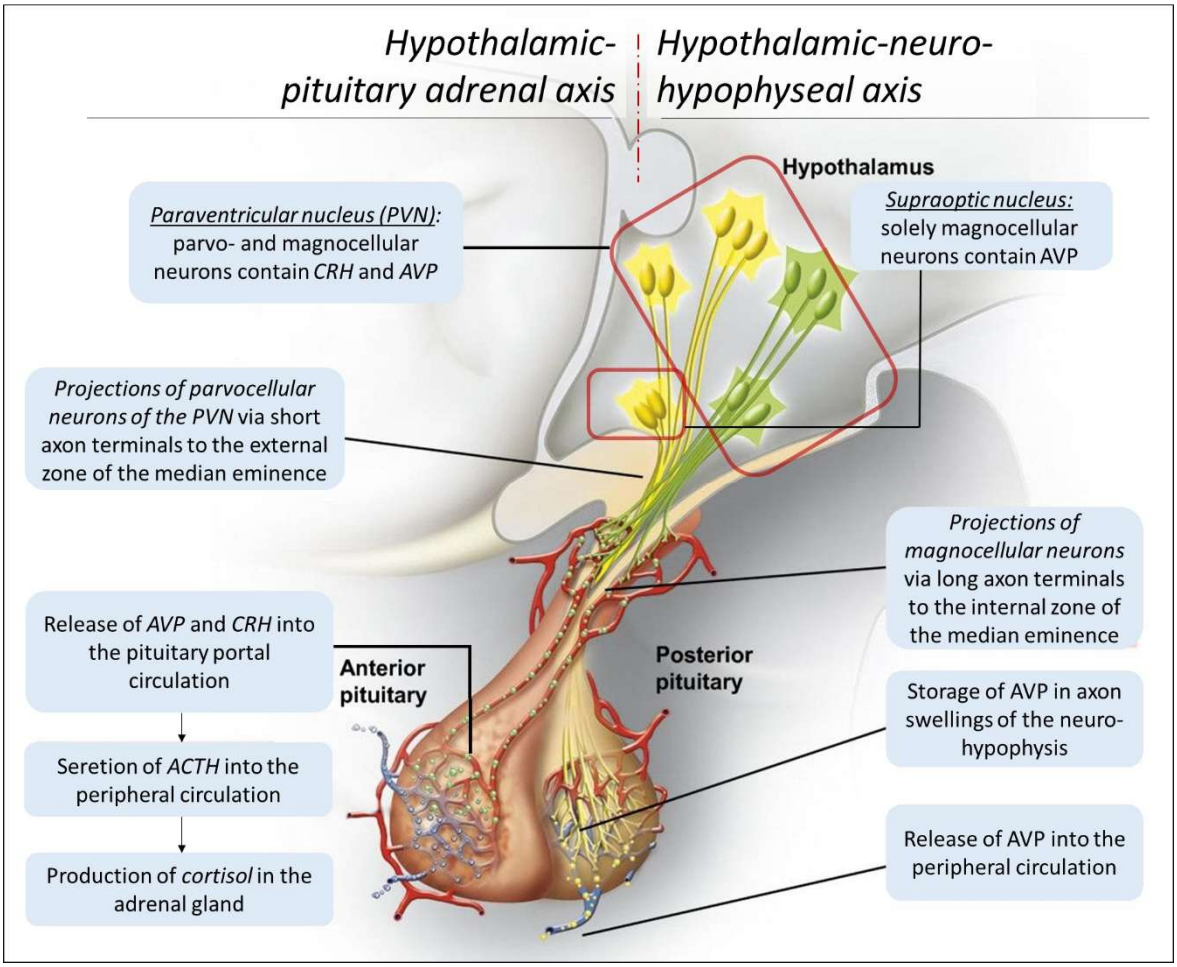


Figure 1. *The hypothalamic pituitary-adrenal- and neurohypophyseal axes.* AVP from parvocellular neurons of the hypothalamus reaches the anterior pituitary gland together with CRH to stimulate ACTH secretion. AVP from the magnocellular subdivision is released into the peripheral circulation by the posterior pituitary gland (neurohypophysis). Secretory activity of the latter can be measured by the cleaved AVP-precursor fragment

copeptin. A connection between both axes is suspected at the level of the median eminence. ACTH: adrenocorticotropic hormone; AVP: arginine-vasopressin. CRH: Corticotropin-releasing hormone. PVN: paraventricular nucleus. SON: supraoptic nucleus. Figure adapted from Nickel *et al.*, 2012.

2.4.1 Anatomy of the hypothalamic-pituitary-adrenal and neurohypophyseal axes

The medial hypothalamus contains two nuclei involved in the regulation of the neuroendocrine stress response: the *paraventricular (PVN)* and the *supraoptic nuclei (SON)*, which consist of *parvocellular* and *magnocellular* (PVN) or solely *magnocellular* (SON) neurons. It is generally accepted that the PVN is primarily involved in HPA axis regulation, and that the SON determines the neurohypophyseal stress response. However, mounting evidence challenges the former doctrine suggesting rather an interplay of the two systems than being merely parallel pathways (Holmes *et al.*, 1986; Antoni, 1993; Keck *et al.*, 2002; Tanoue *et al.*, 2004; Sivukhina and Jirikowski, 2016). The relation of serum AVP or its surrogate copeptin to HPA responsiveness has not been formally shown in humans.

The *paraventricular nucleus* lies adjacent to the third ventricle, harboring parvocellular neurons in its medial and magnocellular neurons in its lateral part. The *parvocellular* division consists of cells of 7-10 µm diameter which produce CRH and AVP (Sivukhina and Jirikowski, 2016). AVP and CRH producing neurons of the parvocellular division of the PVN project short axon terminals to the external zone of the median eminence where the neuropeptides are released into the pituitary portal circulation. The secretagogues of the *parvocellular neurons* are acknowledged to trigger ACTH release by CRH1- and V1b-receptor binding at the anterior pituitary, and hence, to be predominantly involved in the regulation of HPA axis activity (Volpi *et al.*, 2004; Aguilera, 2011).

The *supraoptic nucleus* is located dorsally of the optic tract with entirely magnocellular neurons of 20-40µm diameter, producing oxytocin and AVP. AVP derived from the *magnocellular division* of both PVN and SON is transported via long axons through the internal part of the median eminence to the posterior pituitary gland and released into the peripheral circulation by axon swellings and fenestrated capillaries, leading to vasoconstriction by V1aR binding on vessels and water conservation by V2R-triggered aquaporin-2 insertion into the renal tubules (Volpi *et al.*, 2004). Therefore, the *magnocellular division* of the PVN and SON are neurosecretory cells primarily involved in the *regulation of the hypothalamic-neurohypophyseal axis* (Burbach *et al.*, 2001; Dinan and Scott, 2005; Aguilera, 2011). A communication of the hypothalamic-pituitary and the neurohypophyseal system, however, is probably located in the median eminence, where AVP from the magnocellular system passes through, leading to physiologically relevant concentrations of the neuropeptide in the pituitary portal circulation following its stimulation (Wotjak *et al.*, 1996). This CRH/AVP synergism probably becomes

functionally more relevant under conditions of chronic stress (de Goeij, D C *et al.*, 1992; Keck *et al.*, 2002).

2.4.2 The role of CRH, ACTH and cortisol in the context of metabolism and obesity

CRH is a 41-amino acid long neuropeptide that triggers the release of ACTH from the anterior pituitary gland (Vale *et al.*, 1981; Aguilera, 2011). The nonapeptide AVP acts synergistically with CRH, potentiating ACTH secretion (Bardeleben *et al.*, 1985; Tsigos *et al.*, 2000). In situations of chronic stress, the increased synthesis of AVP facilitates HPA responsiveness to meet the enhanced demand of enduring activation (de Goeij, D C *et al.*, 1992; Antoni, 1993; Dinan and Scott, 2005; Litvin *et al.*, 2011). Acutely, the peptide hormones CRH and ACTH (Yalow *et al.*, 1964; Berson and Yalow, 1967; Schulte *et al.*, 1982) suppress appetite directly via CRH1R- or melanocortin receptor binding and indirectly via sympathetic activation during the *alarm phase* of the *fight and flight* response (Cannon, 1932; Selye, 1946; Yalow *et al.*, 1964; Glowa *et al.*, 1992; Shipp *et al.*, 2015). Then, ACTH and probably also AVP trigger the production of the steroid hormone cortisol in the zona fasciculata of the adrenal gland (Arlt and Stewart, 2005; Aguilera, 2011; Mavani *et al.*, 2015). Contrary to the afore-mentioned peptides, cortisol leads to *increased* appetite with preferential choice for rewarding high-caloric comfort food in order to restore energy resources during the *resistance* or *exhaustion phase* of the stress response (Selye, 1946; Tataranni *et al.*, 1996; Dallman *et al.*, 2005; Adam and Epel, 2007). Only slightly increasing energy expenditure, glucocorticoids lead to a markedly higher food intake with subsequent weight gain in almost all patients treated with therapeutically relevant doses of glucocorticoids as well as in patients suffering from Cushing's disease (Tataranni *et al.*, 1996; Tsigos *et al.*, 2000; Fardet and Feve, 2014). Glucocorticoids enable energy mobilization by gluconeogenesis, glycogenolysis, lipolysis and proteolysis, increasing the concentrations of circulating glucose and fatty acids acutely, and rather long-term, lead to a re-distribution of fat to its rapidly-available visceral depots with lipodystrophy (Fardet and Feve, 2014), and the higher waist-hip ratio (WHR) as a proxy of visceral fat is markedly linked with an increased mortality (Rosmond *et al.*, 1998; Despres and Lemieux, 2006; Fardet *et al.*, 2012; Katzmarzyk *et al.*, 2012; Tchernof and Després, 2013). Whereas *basal* hypercortisolism in common obesity is not supported by the majority of the literature (Abraham *et al.*, 2013; Incollingo *et al.*, 2015; Bailey, 2017), it is to question if it is rather a higher *reactivity* of the HPA axis with timely limited peaks of circulating cortisol that may account for the phenotypic similarities of patients suffering from Cushing's syndrome and subjects with common obesity. This would imply a dynamic approach to assess HPA axis responsiveness upon defined stimuli, e.g., by the use of the dex/CRH test.

2.4.3 The role of AVP in the context of metabolism and obesity

AVP activates sympathetic pathways (Grazzini *et al.*, 1999), and besides its antidiuretic and pressor functions, raises blood glucose concentrations by hepatic glycogenolysis as well as glucagon secretion (Oshikawa *et al.*, 2004; Mavani *et al.*, 2015), and affects lipid metabolism (Koshimizu *et al.*, 2012). Elevated concentrations of AVP longitudinally precede arterial hypertension, abdominal obesity, insulin resistance and diabetes which led to the assumption that an enhanced vasopressinergic tone may be causally linked with the pathogenesis of the metabolic syndrome (Enhörning *et al.*, 2011; Enhörning *et al.*, 2013; Wannamethee *et al.*, 2015). Due to the recruitment of similar metabolic effector pathways, a functional interrelation of the AVP and HPA system in the context of obesity seems likely (Saleem *et al.*, 2009).

2.4.4 Measuring HPA axis responsiveness by means of the combined dexamethasone-corticotropin-releasing hormone (dex/CRH) test

HPA axis assessment is of great interest for clinicians and researchers since disturbances of its activity have long been recognized to be pathophysiologically linked with physical and mental health disorders, such as Cushing's syndrome (Liddle, 1960), multiple sclerosis (Then Bergh *et al.*, 1999), major depression (Pariante and Lightman, 2008), schizophrenia (Lammers *et al.*, 1995), panic disorder (Erhardt *et al.*, 2006) and posttraumatic stress disorder (Kloet *et al.*, 2006).

Cortisol secretion can be divided into different temporal patterns: (i) *basal activity* with circadian fluctuations and ultradian pulses, and (ii) *stimulus-induced activity*, which constitutes HPA responsiveness upon different types of stressors (Spencer and Deak, 2017). These HPA functions can be measured by a multitude of different approaches, such as the single or repeated assessment of cortisol in patient serum, saliva, urine or hair to reflect *basal* cortisol secretion and cortisol turnover, or by the use of *dynamic* paradigms (Groot *et al.*, 2000). These *endocrine challenge tests* which dynamically assess HPA reactivity either use *non-pharmacological* stimuli, such as psychological stress induced by social evaluation in the Trier Social Stress test (Kirschbaum *et al.*, 1993), or *pharmacologically-induced* stress. In the category of the latter, the *dexamethasone suppression test* was the first provocation test and still is the clinically most relevant tool to evaluate endogenous glucocorticoid excess (Liddle, 1960; Groot *et al.*, 2000; Findling *et al.*, 2004). Later, the *CRH-stimulation test* was shown to further increase ACTH and cortisol release specifically in patients with ACTH-secreting pituitary adenomas but not in other forms of the Cushing's syndrome (Chrousos *et al.*, 1984). The combination of both HPA axis suppression by dexamethasone *and* stimulation by CRH achieved a higher diagnostic accuracy than either of the tests alone, reliably distinguishing between Cushing's

syndrome from pseudo-Cushing states, thus, subjects with mild hypercortisolism but without ectopic cortisol production (Yanovski *et al.*, 1993; Erickson *et al.*, 2007). The *combined dex/CRH test* was initially used as a laboratory test in psychiatry research, revealing a cortisol escape in patients with major depression (Bardeleben and Holsboer, 1989; Heuser *et al.*, 1994), and has later become a well-validated tool in various mental and physical health conditions (Then Bergh *et al.*, 1999; Holsboer, 2001; Ising *et al.*, 2005; Heesen *et al.*, 2007). The advantage of the dex/CRH test is its high standardization due to the use of a defined pharmacological stimulus in a defined laboratory or clinical setting in which HPA axis reactivity can be dynamically mirrored by ACTH and cortisol concentrations from patients' sera in the course of 1.5h of time (Heuser *et al.*, 1994). The main determinants of the ACTH-cortisol release pattern are a negative feedback mechanism as a function of glucocorticoid receptor integrity (Holsboer, 2000), sensitivity to circulating CRH (Nussey *et al.*, 1991), AVP co-stimulation (Bardeleben *et al.*, 1985; Keck *et al.*, 2002) and adrenal sensitivity to circulating ACTH, as measured by the ACTH/cortisol ratio (Holsboer *et al.*, 1984; Kümpfel *et al.*, 2014). The acute as well as long-term effects of monoamine reuptake inhibitors on dex/CRH test responsiveness further imply a modulatory role of the serotonergic and noradrenergic system on HPA axis functioning (Schule *et al.*, 2006; Sarubin *et al.*, 2014a). HPA axis assessment by means of the dex/CRH test has not been performed specifically in subjects with obesity. There are no studies on the relation of the ACTH and cortisol response to the AVP system or central monoaminergic signaling which are probably modulators of the HPA response.

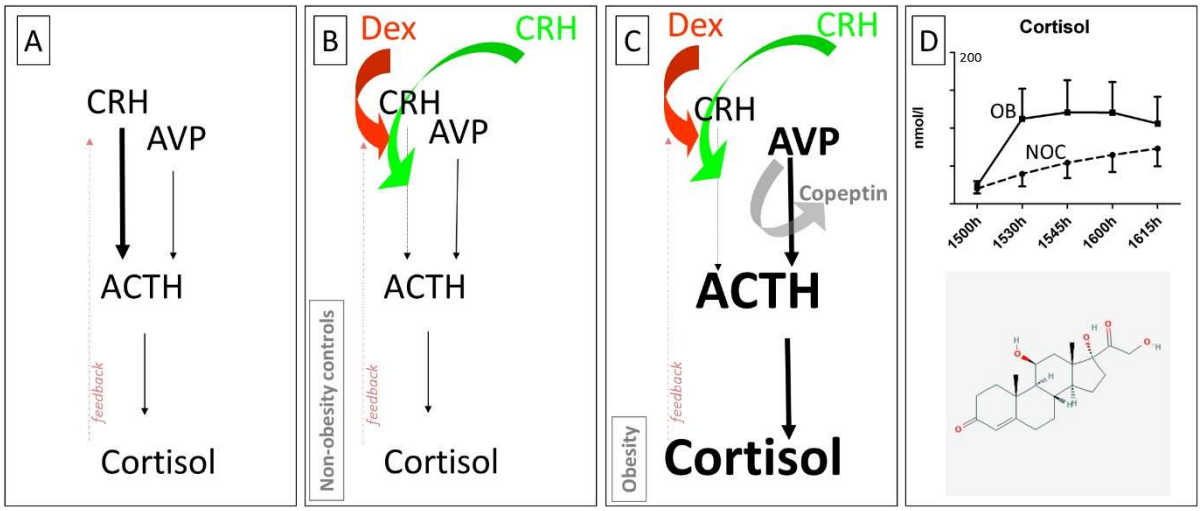


Figure 2. *Principle of the combined dexamethasone/CRH test.* (A) Under physiological conditions, CRH and its secretagogue AVP stimulate ACTH secretion. Cortisol inhibits the release of the neuropeptides by feedback control. (B) Normally, 1.5mg of dexamethasone suppress ACTH and cortisol secretion despite the stimulation by 100µg CRH at 1500h. (C) An escape of this dexamethasone suppression was hypothesized for the obesity group, as well as a co-stimulation by an enhanced AVP tone, as to be measured by copeptin. (D) Expected time curve of cortisol by means of the dex/CRH test, and its chemical structure. ACTH: adrenocorticotropic hormone; AVP: arginine-vasopressin. CRH: Corticotropin-releasing hormone; Dex: dexamethasone; NOC: non-obesity controls.

OB: obesity. Figure adapted from a presentation of Prof. Dr. F. Then Bergh (A-C) and Schinke *et al.*, 2017 (D). Chemical structure from Pubchem Open chemistry database.

2.4.5 Measuring AVP secretion by its equally-released surrogate copeptin

AVP derives from the 164-amino-acid long precursor peptide pre-pro-AVP that is proteolytically cleaved into a signal peptide, AVP, neurophsyin-II and copeptin (Land *et al.*, 1982; North, 1987; Morgenthaler *et al.*, 2006). AVP is cumbersome to measure since it binds to platelets, has a short-ex vivo half-life and cannot be assessed by sandwich-immunoassays due to the shortness of the amino-sequence (Robertson *et al.*, 1973; Szinnai *et al.*, 2007). Copeptin is the stable 39-amino-acid long c-terminal fragment of the pre-pro-AVP precursor which is probably involved in the correct folding of AVP during maturation (Acher *et al.*, 2002; Barat *et al.*, 2004). It is released into the peripheral circulation by the neurohypophysis in equimolar amounts to AVP, reflecting recent vasopressin secretion while being a more stable analyte (Robertson, 2001; Morgenthaler *et al.*, 2006; Szinnai *et al.*, 2007). It has been suggested as a new biomarker of acute illness (Katan and Christ-Crain, 2010) and is associated with an increased morbidity in patients with acute coronary syndrome (Keller *et al.*, 2010; Lattuca *et al.*, 2019) and ischemic stroke (Katan *et al.*, 2009). A subject of our study is to investigate whether the assessment of serum copeptin concentrations could be a useful tool revealing an enhanced vasopressin tone and, hence, reflect chronic humoral stress.

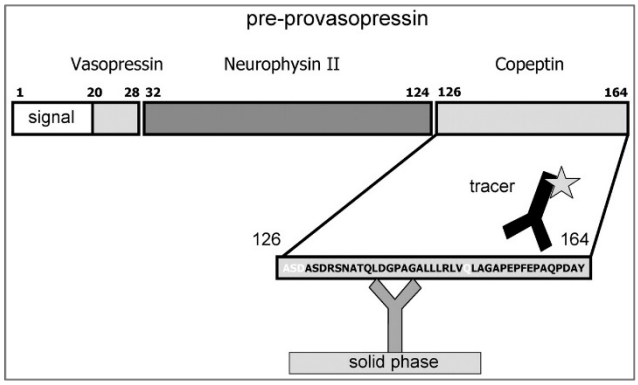


Figure 3. *Cleavage of pre-provasopressin.* Pre-provasopressin is cleaved into a signal peptide, arginine-vasopressin, neurophysin II and the c-terminal fragment copeptin. Copeptin is stable ex vivo and can be measured by sandwich-immunofluorescence assays, as used in our study (Brahms CopeptinUs®, ThermoScientific). The tracer is a labeled antibody which binds to copeptin that, in turn, binds to another antibody attached to the tube (*solid phase*). Numbers indicate amino sequence. Figure from Morgenthaler *et al.*, 2006.

2.5 The noradrenergic system in the context of obesity and stress axis modulation

Noradrenaline is deeply involved the regulation of drive, sleep, behavior and the degree of alertness and arousal (Zhou, 2004; Schou *et al.*, 2007). The association of noradrenaline to the endocrine stress

system seems intuitive given that NA chemically belongs to the group of catecholamines as the classical neuroendocrine hormones and neurotransmitters of the sympathetic nervous system (McCorry, 2007). It is biosynthesized from the amino acid tyrosine in a three-step enzymatic reaction including the hydroxylation to dopa, the decarboxylation to dopamine and finally another hydroxylation to noradrenaline (Goodall and Kirshner, 1958; Axelrod, 1970). Peripherally, NA is produced by preganglionic sympathetic nerve fibers and chromaffin cells of the adrenal medullary (Euler, 1946) which are directly derived from neural crest cells during embryonic development (Le Douarin and Teillet, 1974; Huber *et al.*, 2009). In the 1960s, the *central* noradrenaline system came out of age, proving the existence of monoamine nerve terminals in the brain (Dahlstroem and Fuxe, 1964) with highest NA-neuron densities in the locus coeruleus (LC) (Fuxe *et al.*, 1970). From there, brainstem LC neurons project long axon terminals to emotional and cognitive centers embracing limbic regions such as the amygdala, the hippocampus and prefrontal cortex and to centers responsible for endocrine function and appetite regulation of the hypothalamus (Moret and Briley, 2011). In the neuron, NA is stored in vesicles which merge with the presynaptic membrane of the nerve terminals to release the neurotransmitter into the synaptic cleft (Benarroch, 2013). There, NA exerts action primarily via postsynaptic α1-receptor binding, and, with a lower affinity, to β-adrenoceptors. Finally, its release is self-limited by feedback-inhibition through presynaptic α2-autoreceptors (Starke, 2001). All NA receptors belong to the family of heptahelical g-protein-coupled transmembrane domains (Strosberg, 1993).

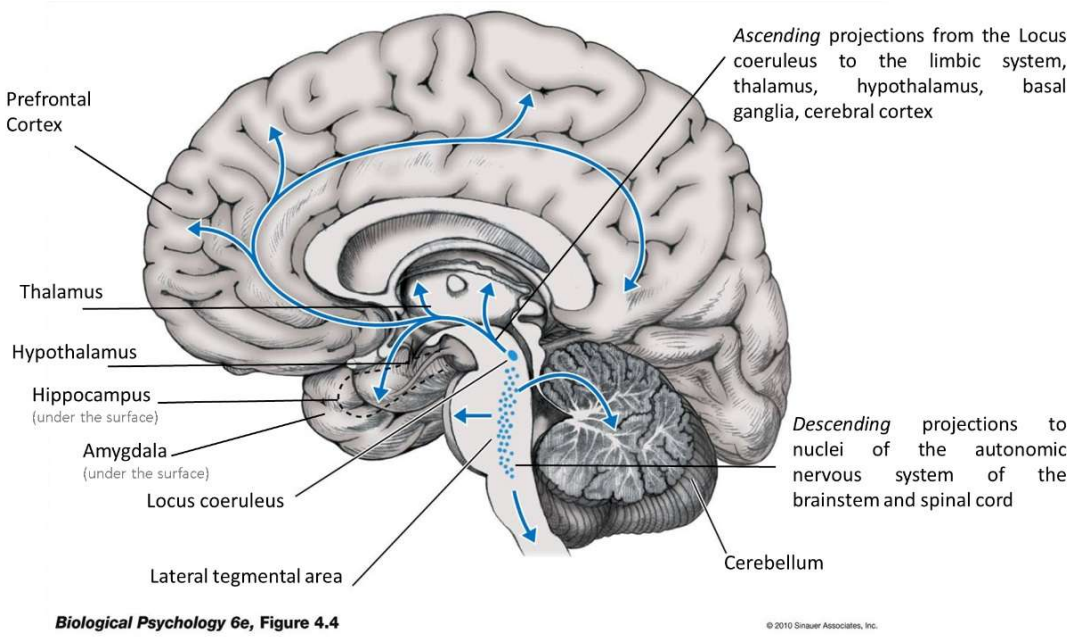


Figure 4. *The brain noradrenaline system.* The locus coeruleus (LC) is located in the median pons near the pontomesencephalic junction, harboring the largest population of NA neurons. Noradrenergic projection from the LC ascend to the limbic system, thalamus, hypothalamus and cortex to regulate arousal, emotions and endocrine function. Descending projections reach autonomic nuclei of the brainstem and spinal cord to modulate

the sympathetic and parasympathetic nervous system and motor function. Picture adapted from Kalat, 1997 and according to Samuels and Szabadi, 2008.

2.5.1 NA and its influence on feeding behavior

Clinically, the reduction of food ingestion by monoamines was observed after ephedrine-derived bronchodilators – the novel group of amphetamines – had been turned into the first antidepressants alleviating from *anhedonia*, and benzedrine was soon promoted an *advantageous* drug reducing the hedonic drive to eat (Piness G., H. Miller, and G. Alles, 1930; Lesses and Myerson, 1938). Mechanistically, the increased metabolism and diuresis, the reduced digestion and absorption of nutrients as well as the loss of appetite finally lead to significant weight loss (Nutrition Reviews, 1956; Heal *et al.*, 2013). The exploitation of these pathways paved the way to an iatrogenic epidemic of amphetamine abuse as anti-obesogenic drug in the 1960s (Rasmussen, 2008).

The amphetamine-type subjective effects of central psychostimulants are mediated by the inhibition of monoamine reuptake and release of dopamine, serotonin and, most potently, noradrenaline (Rothman *et al.*, 2001). NA exerts effects on appetite via opposing pathways, depending on the hypothalamic nucleus and receptor binding site: On the one hand, it *suppresses* food intake by binding *alpha1*-receptors in the PVN and noradrenergic trajectories to the lateral hypothalamus (Wellman, 2000) and direct central sympathetic activation. On the other hand, NA *stimulates* feeding via brainstem noradrenergic projections to the PVN of the *medial* hypothalamus, activating *alpha-2* receptors (Holtzman and Jewett, 1971; Nelson and Gehlert, 2006; Bray and Greenway, 1999; Jhanwar-Uniyal and Leibowitz, 1986) and, more downstream, fosters feeding by engaging the HPA axis (Chrousos, 2000) with the subsequent release of glucocorticoids that increase the motivation for pleasurable reward-associated food (Dallman, 2010).

2.5.2 The association of the noradrenergic system with the HPA and neurohypophyseal axes

Real or anticipated stress involves the activation of neuronal circuits of the prefrontal cortex, amygdala, hippocampus and hypothalamus which lead to the activation of the HPA and neurohypophyseal axes, resulting in a stress-adapted behavioral response (Plotsky *et al.*, 1989; Holsboer and Ising, 2010). The assumption of an NA-mediated activation of the HPA and AVP stress system is based on anatomical, experimental and clinical observations.

Anatomically, noradrenergic brainstem projections directly reach CRH- and AVP-containing neurons of the paraventricular and supraoptic nuclei which express alpha-1-receptors, integrating acute and chronic stress responses at the hypothalamic level (Alonso *et al.*, 1986; Plotsky *et al.*, 1989; Flak *et al.*, 2014). Microinjection of NA into the hypothalamic PVN and SON stimulate CRH and AVP expression and release (Armstrong *et al.*, 1986; Benetos *et al.*, 1986; Itoi *et al.*, 1994; Vacher *et al.*, 2002) whereas mechanic or immunotoxic lesions as well as alpha1-R antagonism contrarily blunt respective activities (Laakmann *et al.*, 1986; Feldman and Weidenfeld, 1998; Sawchenko, 1988; Ritter *et al.*, 2003; Flak *et al.*, 2014), hinting to an excitatory effect of alpha1-receptor binding NA on the HPA and neurohypophyseal axes (Plotsky *et al.*, 1989; Feldman and Weidenfeld, 1998).

Experimentally, the administration of the centrally acting alpha-1R-agonist methoxamine fosters the release of AVP, ACTH and cortisol into the peripheral circulation in humans (Radant *et al.*, 1992) whereas alpha-1-receptor-antagonism by prazosin reverses pharmacologically-induced *HPA axis* activation (Laakmann *et al.*, 1986). Similarly, stimulatory effects on the *neurohypophyseal axis* were reported in healthy subjects being administered the NA/5-HTT-releasing and monoamine-reuptake blocking drug MDMA, resulting in an increased AVP tone as measured by its surrogate copeptin (Simmler *et al.*, 2011). The administration of the selective NA-reuptake inhibiting antidepressant reboxetine results in HPA axis activation *acutely* but downregulating its activity *chronically* in the course of several weeks of treatment, probably due to a gradual restoration of the glucocorticoid feedback control (Schule *et al.*, 2006).

From a clinical point of view, alterations of monoaminergic signaling (Moret and Briley, 2011; Moriguchi *et al.*, 2017) and HPA dysregulation belong to the most robustly reported biological findings in major depression (Heuser *et al.*, 1994; Chrousos, 2009). Similar neurobiological mechanisms seem to apply in OB where both deficient NA signaling (Li *et al.*, 2014; Robertson *et al.*, 2010) and alterations of HPA activity were postulated to be involved in its pathogenesis (Bjorntorp and Rosmond, 2000; Pasquali *et al.*, 2006; Incollingo *et al.*, 2015; Bose *et al.*, 2009; Lee *et al.*, 2016), and may partly explain the high prevalence of comorbid psychiatric conditions such as depression, anxiety disorder, bipolar disorder and schizophrenia in people with obesity (Simon *et al.*, 2006; Kyrou and Tsigos, 2009; Jauch-Chara and Oltmanns, 2014). This indicates that alterations in the HPA-monoamine interplay may constitute an unspecific *endophenotype* predisposing to a variety of physical and mental diseases.

2.5.3 Monoamine transporters as regulators of neurotransmitter signaling

Monoamine-signaling is critically modulated by its presynaptic transporters, limiting neurotransmitter concentrations in the synaptic cleft by its reuptake into the presynaptic bouton to terminate its action

(Hertting and Axelrod, 1961; Iversen, 1971; Benarroch, 2013). About 80-90% of the released NA is re-captured and re-released (Schomig *et al.*, 1989; Ding *et al.*, 2006). Transporters of NA and 5-HTT as well as of dopamine, GABA and glycine belong to the solute carrier 6 (SLC6) superfamily which use the electrochemical gradient of sodium as driving force for neurotransmitter-recycling (Benarroch, 2013). They consist of about 600 amino-acids with twelve transmembrane spanning domains (Lesch *et al.*, 1993), and are primarily located close to the dendrites and along the axons (Zhou, 2004; Murphy and Lesch, 2008). Albeit NA transporters have been pharmacologically targeted in stress-associated diseases for decades (Zhou, 2004; Eyding *et al.*, 2010), there is no study which investigated the *status quo* of NAT availability in the living human brain together with stress axes responsiveness.

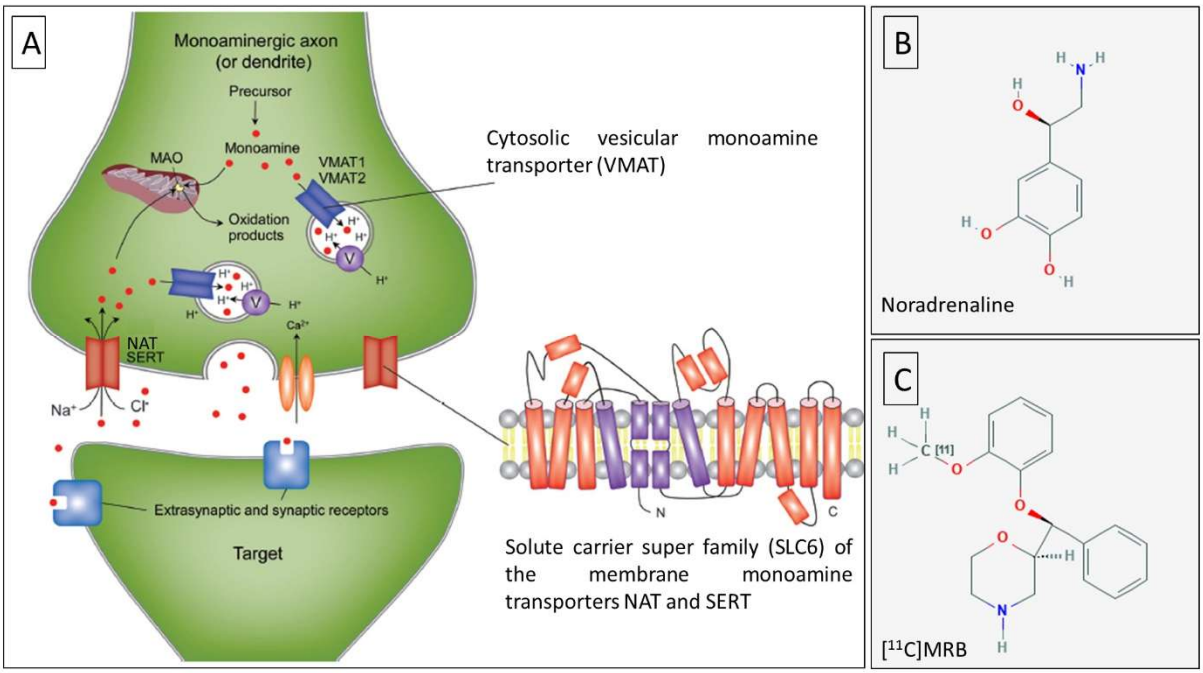


Figure 5. *The monoamine transporter and its ligands.* (A) Structure of the membrane monoamine transporter. SERT and NAT as members of the solute carrier superfamily (SLC) use the Na+ gradient for neurotransmitter recycling. (B) Chemical structure of noradrenaline and (C) the NAT-selective radiotracer S,S-[^{11}C]O-methylreboxetine. Picture from Benarroch, 2013; chemical structures from Pubchem Open chemistry database.

2.5.4 Noradrenaline transporter imaging

The noradrenaline transporter is involved in the pathophysiology and treatment of major depression, attention deficit hyperactivity disorder, substance abuse and neurodegenerative disorders (Ding *et al.*, 2006). Albeit these findings could initially only be derived from post-mortem tissue, it is since the early 2000s that *in vivo* quantification of the noradrenaline transporter in the living human brain has become possible by means of NAT brain PET with suitable radiotracers (Laruelle *et al.*, 2002; Ding *et al.*, 2006). The latter were generated by labeling noradrenaline reuptake inhibitors such as desipramine,

nisoxetine, atomoxetine or reboxetine with the beta-emitters [^{11}C], [^{3}H] or [^{18}F] (Ding *et al.*, 2006). The (S, S)-enantiomer *[^{11}C]Methylreboxetine* was the most promising ligand candidate for NAT imaging, combining the properties of a high NAT affinity, specificity, selectivity and good lipophilicity. It shows the highest binding in the locus coeruleus, thalamus and hypothalamus and the lowest in the striatum and occipital cortex which serve as reference regions (Ding *et al.*, 2003; Wilson *et al.*, 2003; Ding *et al.*, 2006). By means of this novel radiotracer, the previously mentioned alterations in NAT signaling could be verified in the living human brain, e.g. with an enhanced thalamic NAT availability in patients with major depression (Moriguchi *et al.*, 2017) and cocaine abuse (Ding *et al.*, 2010). Contrarily, a *lowered* thalamic NAT availability was shown in one study investigating subjects with *mild* obesity (Li *et al.*, 2014). However, in that study, subjects with a BMI >35kg/m^2 were not taken into account. Until the study by Hesse *et al.* in 2017, there were no data on NAT availability in *severe* obesity.

2.6 The serotonergic system in obesity

Serotonin is involved in the regulation of mood, behavioral, autonomic and endocrine responses (Lowry, 2002), and dysregulation of serotonergic signaling is implicated in anxiety-traits and susceptibility for depression (Caspi *et al.*, 2003; Canli and Lesch, 2007). It was discovered at first peripherally in the late 1940s and named after its capacity to increase blood vessel tone (Rapport *et al.*, 1948). Most serotonin is produced in the enterochromaffin cells of the gastrointestinal tract, mediating bowel movement (Berger *et al.*, 2009; Wyler *et al.*, 2017). Analogous to other monoamines such as dopamine, serotonin is not capable of crossing the blood-brain barrier. In the brain, it is synthesized in a two-step enzymatic reaction by the hydroxylation of the essential amino acid L-tryptophan to 5-hydroxy-L-tryptophan and the subsequent decarboxylation finally to 5-hydroxytryptamine (5-HT), synonymously designated *serotonin* (Charnay and Leger, 2010). Centrally, 5-HT synthesizing neurons are almost exclusively located in nine nuclei of the *brainstem raphe* and the *reticular formation*, extending from the caudal-most pole of the medulla oblongata to the mid-level of the mesencephalon (Törk, 1990; Charnay and Leger, 2010, 2010; Yeo, Giles S H and Heisler, 2012). Serotonergic neurons extensively project axons to virtually all brain regions (Jacobs and Azmitia, 1992, 1992; Charnay and Leger, 2010), underlining its involvement in many physiological and neuropsychological processes (Berger *et al.*, 2009; Yeo, Giles S H and Heisler, 2012). There is a smaller *caudal* and a larger *rostral* division of brainstem serotonergic neurons. *Descending projections* originate from the *caudal division* located in the pons and medulla oblongata, comprising 15% of the central serotonergic neurons with projections to the medulla oblongata and spinal cord, where they modulate pain and motor function (Törk, 1990; Charnay and Leger, 2010). The majority of serotonergic projections, however, arise from the *rostral division* of the serotonergic neurons which comprise 85%

of the cell bodies, sending extensive collaterals to the limbic system, the hypothalamus, striatum and the cerebral cortex (Törk, 1990) where they modulate mood, cognitive function, emotion, motivational behavior and reward (Hoebel *et al.*, 1989; Berger *et al.*, 2009; Švob Štrac *et al.*, 2016; Drabe *et al.*, 2017), along with exerting control over the metabolic homeostasis (Nelson and Gehlert, 2006; Wyler *et al.*, 2017) and the neuroendocrine system (Dinan, 1996; Schule, 2007).

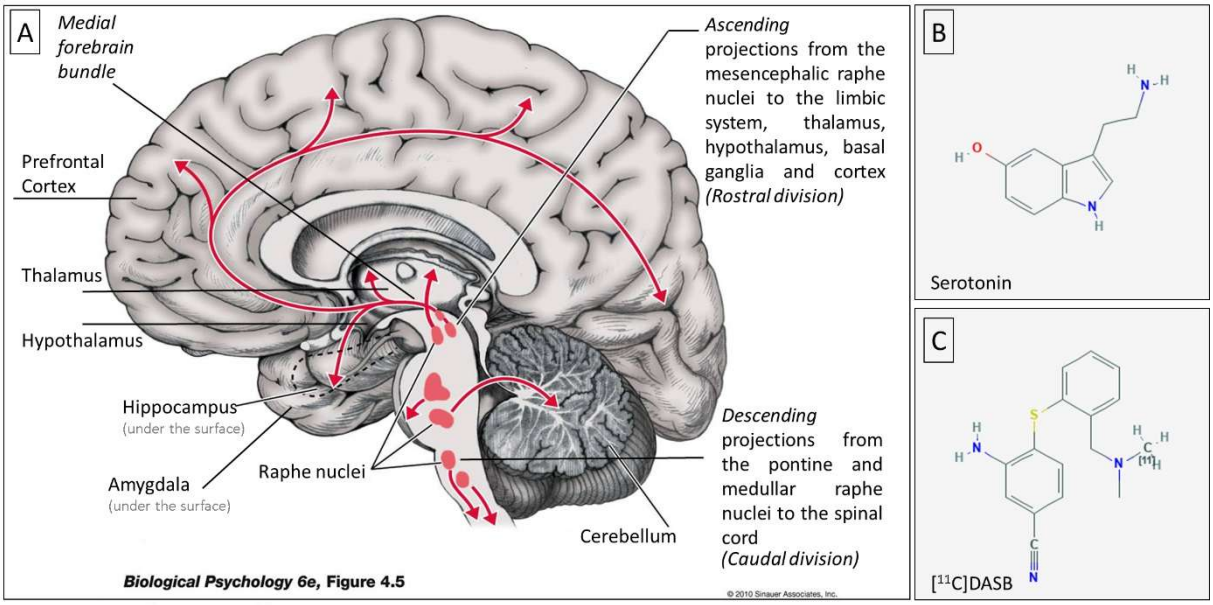


Figure 6. *The brain serotonin system.* Central serotonergic neurons arise from the upper brainstem raphe nuclei through the diencephalon to reach the forebrain. Descending projections originate from the caudal division of the raphe nuclei to the spinal cord. Figure adapted from Kalat, 1997, description according to Törk, 1990. Chemical structures from Pubchem Open chemistry database.

2.6.1 Role of serotonin in the context of feeding behavior and metabolism

Serotonin exerts its effect via at least 14 pre- and postsynaptic 5HT-receptors of 7 receptor families that are primarily expressed in the central nervous system and the gastrointestinal tract, as well as in smooth muscle, the peripheral nervous system, blood vessels and platelets (Charnay and Leger, 2010; Švob Štrac *et al.*, 2016). With the exception of 5-HT_3R which is the only ligand-gated ion channel in the serotonin receptor family, all other members belong to the group of metabotropic g protein-coupled heptahelical transmembrane receptors (Barnes and Sharp, 1999; Švob Štrac *et al.*, 2016), out of which 5-HT_{2C} and 5-HT_{1B} probably play the most important roles in the regulation of metabolism and satiety (Pedigo *et al.*, 1981; Pazos *et al.*, 1984; Bello and Liang, 2011). The relevance of serotonin in the modulation of feeding behavior could be experimentally shown in the 1970s: its depletion leads to hyperphagia and obesity (Breisch *et al.*, 1976; Saller and Stricker, 1976) whereas its central administration results in a reduced food intake (Pollock and Rowland, 1981), indicating an inverse

relationship of brain serotonin concentration and body weight (Lam *et al.*, 2010). The recognition of this relation made the serotonergic system a target in anti-obesity pharmacotherapy, augmenting its synaptic concentration by either serotonin-releasing agents like fenfluramine or with combined monoamine reuptake inhibitors such as sibutramine (Hainer *et al.*, 2006). Both agents were effective in lowering body weight, but suspended by the Federal Drug Administration due to adverse effects (Bello and Liang, 2011), leaving the highly selective 5-HT_{2C} receptor agonist Lorcaserin as the only approved serotonin-modulating anti-obesity-medication in the market (Thomsen *et al.*, 2008; Yeo, Giles S H and Heisler, 2012). Serotonin probably acts through the attenuation of the orexigenic agouti-related peptide and the disinhibition of the proopiomelanocortin system (Heisler *et al.*, 2002; Garfield and Heisler, 2009; Lam *et al.*, 2010; Bello and Liang, 2011).

Magnitude and duration of serotonergic signaling are mainly modulated by the presynaptic *serotonin transporter*, recycling 5-HT into the presynaptic neuron. Similar to NAT, SERT belongs to the SLC6 superfamily (Murphy and Lesch, 2008; Benarroch, 2013). Notably, the use of SERT inhibitors in obesity, but also in mental health disorders such as major depression and obsessive compulsive disorder, was empirically established on the clinical observation of weight loss or clinical improvement in individuals treated with 5-HT(T) affecting medication.

2.6.3 5-HTT imaging

Highly selective radiotracers suitable for SERT imaging were developed in the late 1990s and early 2000s (Wilson *et al.*, 2000), highlighting [^{11}C]DASB *(3-Amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile)* as the most useful radiopharmaceutical for 5-HTT imaging (Wilson *et al.*, 2002). 5-HTT densities in the living human brain vary by region. In healthy individuals, the highest SERT availability is observed in the raphe nuclei, the hypothalamus, thalamus and amygdala, and the lowest concentrations in the prefrontal and cerebellar cortex, of which the latter serves as reference region in PET studies (Cortés *et al.*, 1988; Ichise *et al.*, 2003; Kish *et al.*, 2005; Meyer, 2007). Molecular SERT imaging was performed in major depression, anxiety disorder, bipolar depression and obsessive compulsive disorder, elucidating the involvement of the transporter in the symptomatology of psychiatric illness, albeit results are eventually conflicting (Meyer *et al.*, 2004; Stengler-Wenzke *et al.*, 2004; Hesse *et al.*, 2005; Matsumoto *et al.*, 2010; Spies *et al.*, 2015). In the field of obesity research and eating disorders, there was only one PET study on the role of SERT which showed an inverse relation between the BMI and 5-HTT availability, postulating a potential compensatory downregulation of SERT in order to compensate a *per se* diminished serotonergic signaling which may account for increased appetite in obesity (Erritzoe *et al.*, 2010a). This interpretation, however, was driven by results derived from linear regression analysis of a cohort with widely varying body mass indices, with

only seven individuals actually having a BMI > 30kg/m^2. There was no study comparing SERT availability *in vivo* between normal-weight individuals versus subjects with obesity.

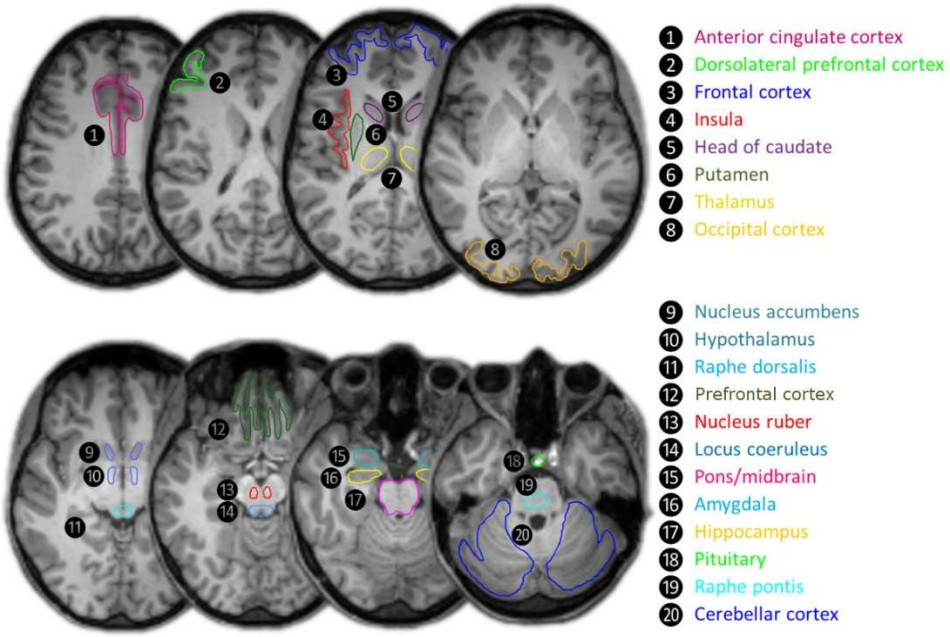


Figure 7. *Regions of interest for the quantification of serotonin and noradrenaline transporter availability.* Figure created by Dr. Julia Luthardt, Department of Nuclear Medicine, University of Leipzig, with friendly permission.

2.7 Objectives and hypotheses

(i) To measure HPA axis responsiveness in subjects with obesity and non-obesity controls, we conducted the combined dexamethasone suppression/CRH stimulation test. ACTH and cortisol curve indicators were derived. Copeptin serum concentrations were measured in the first sample of the test (as a single sample at 1500h, after dexamethasone ingestion and directly before CRH stimulation). Anthropometric data such as the waist-hip-ratio (WHR) were assessed. *We expected* a higher HPA axis responsiveness in subjects with obesity and, correspondingly, higher serum concentrations of the AVP-surrogate copeptin. We assumed serum concentrations of copeptin to be associated with the ACTH and cortisol response of the dex/CRH test. We expected the WHR to be positively associated with stress axis responsiveness.

(ii) To assess the relation of HPA- and neurohypophyseal axes activity to the central noradrenaline system, the dex/CRH test was conducted and brain PET by means of the NAT-selective radiotracer [^{11}C] MRB applied. The approach was exploratory in nature. However, *we expected* relations to be primarily centered in the hypothalamus and the limbic system. We assumed these relations to regionally differ between the obesity group and non-obesity controls.

(iii) To elucidate central serotonin transporter availability *in vivo*, OB and NOC underwent brain PET with the SERT-selective radiopharmaceutical [^{11}C]DASB. We expected SERT availability to be related to the BMI.

2.8 Study design

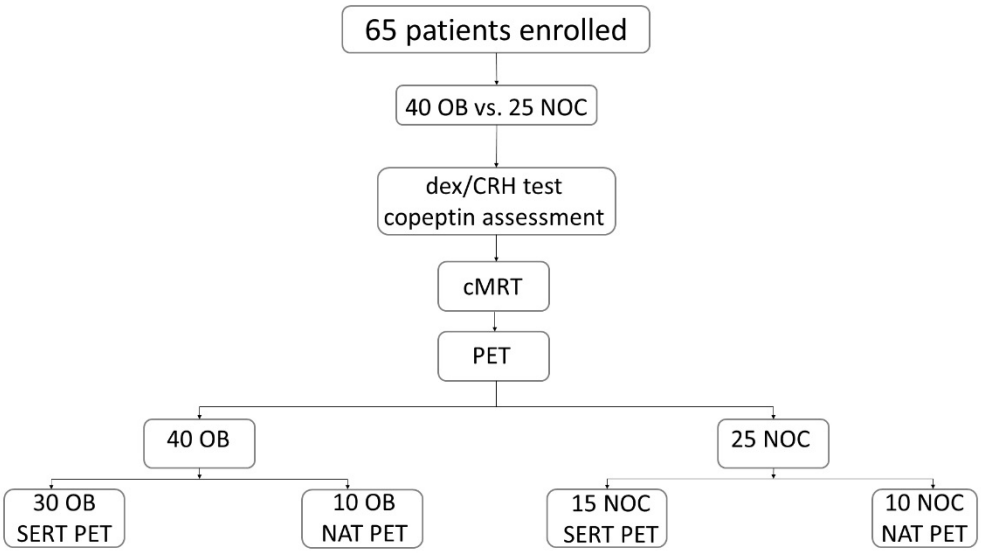
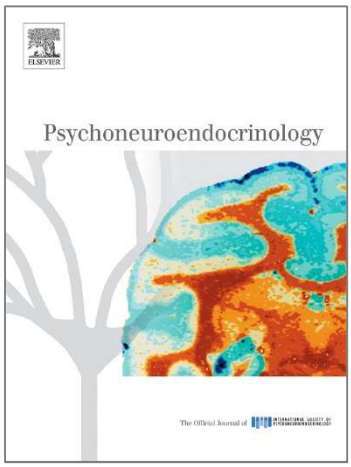


Figure 8. *Study design.* 65 individuals were enrolled, including 40 subjects with obesity (OB) compared to 25 non-obesity controls (NOC). All individuals underwent endocrine testing by means of the dex/CRH test and copeptin assessment, magnetic resonance imaging (MRI) and positron emission tomography (PET) with two different radiotracers (SERT PET: 30 OB vs. 15 NOC, NAT PET: 10 OB vs. 10 NOC). Endocrine parameters of the dex/CRH test were correlated to NAT availability. A detailed description of the methods is included in the publications of the section *III. RESULTS* of this thesis.

III. RESULTS

3. 1 Post-dexamethasone serum copeptin corresponds to HPA axis responsiveness in human obesity.

Schinke C, Hesse S, Stoppe M, Meyer K, Schmidt E, Orthgiess J, Bechmann L, Bresch A, Rullmann M, Luthardt J, Sabri O, Blüher M, Kratzsch J, Then Bergh F.



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Post-dexamethasone serum copeptin corresponds to HPA axis responsiveness in human obesity

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ABSTRACT

Context: Increased activities of the arginine-vasopressin (AVP) system and the hypothalamic-pituitary-adrenal (HPA) axis were shown to be associated with human obesity, but relationships between these systems in obesity remain unclear.
Objectives: To assess HPA axis responsiveness and its relation to serum concentrations of the AVP-surrogate copeptin in subjects with obesity (OB) in comparison to non-obesity controls (NOC).
Methods: In a cross-sectional monocentric study, thirty-nine OB (f/m 25/14; age 36.5 ± 10.0 years; body mass index, BMI, 41.5 ± 4.7 kg/m^2) were compared to twenty-two NOC (f/m 12/10; age 35.3 ± 8.5 years; BMI 23.1 ± 2.4 kg/m^2), matched for age and sex. All individuals underwent the combined dexamethasone/CRH test.
Main outcome measures: Plasma ACTH and cortisol curve indicators derived from the dex/CRH test (post-CRH concentrations 30 min after 100 μg CRH; maximum concentration, MAX; area-under-the-curve, AUC; ACTH/cortisol ratios). Copeptin was assessed in 1500 h samples of the dex/CRH test (after 1.5 mg of oral dexamethasone, prior to CRH administration).
Results: Copeptin serum concentrations were higher in OB (median [IQR]: OB 4.62 [2.60–5.88] vs. NOC 3.04 [2.52–4.29] pmol/l, P = 0.04). Correspondingly, OB showed higher post-CRH cortisol concentrations (OB: 51.5 [25.9–159.3] vs. NOC: 28.6 [20.0–41.6] nmol/l, P = 0.01) and a lower post-CRH ACTH/cortisol ratio (OB: 0.028 [0.016–0.053] vs. NOC: 0.048 [0.034–0.070] pmol/nmol, P < 0.01). Serum copeptin was significantly associated with HPA responsiveness in OB (post-CRH ACTH: R = 0.42, P < 0.01), driven by OB men (post-CRH ACTH: R = 0.76, P < 0.01, post-CRH cortisol: R = 0.64, P = 0.02). All associations withstand adjustments for BMI and age.
Conclusions: The association between increased copeptin with ACTH and cortisol release suggests a potential mechanistic interaction of the AVP system with HPA activation in human obesity. The relation of copeptin and HPA responsiveness should be further validated in situations with pronounced HPA activation, such as depression or multiple sclerosis.

1. Introduction

Stress is an adaptive mechanism countering threats to an individual's homeostasis. Its neuroendocrinological effectors are crucial for maintaining equilibrium in situations of demanding environmental changes (Sinha and Jastreboff, 2013). Finely adjusted endocrine correlates of the stress response are medi-

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ated by the hypothalamic-pituitary-adrenal (HPA) axis. They are intertwined with the regulation of metabolism and affect intake, distribution and expenditure of energy (Torres and Nowson, 2007). Repeated administration of stress leads to increased intake of energy-dense food (Rowland and Antelman, 1976) and obesity is more prevalent in chronically stressed humans (Chandola et al., 2006). These fundamental observations form the foundation of the hypothesis that obesity is, at least partly, a stress-induced phenomenon.

In obesity research, HPA dysregulation is an established contributor in the pathogenesis of the metabolic syndrome (Pasquali et al., 2006; Incollingo et al., 2015). Clinical observations in patients suffering from Cushing's disease support the eminent role of glucocorticoids (Anagnostis et al., 2009), and HPA dysregulation constitutes a risk factor for both obesity and unfavorable metabolic profiles (Incollingo et al., 2015). While hypothalamic CRH is the major regulator of HPA activity, the nonapeptide arginine-vasopressin (AVP) is another hypothalamic stress hormone; it is released by the neurohypophysis into the peripheral circulation to mediate water conservation and vascular regulation (Aguilera, 2011), and exerts effects on glucose homeostasis and fat metabolism (Saleem et al., 2009). In addition, vasopressin enhances the CRH-mediated ACTH release from the anterior pituitary to trigger the adrenal production of cortisol (Antoni, 1993; Sivukhina and Jirikowski, 2016). Large studies showed increased activity of the AVP system to precede the development of diabetes, hypertension and obesity (Enhörning et al., 2010; Enhörning et al., 2011). These data led to the assumption that vasopressin-mediated co-stimulation of the HPA axis may be critically involved in the pathophysiology of the metabolic syndrome (Saleem et al., 2009). However, data on this relationship are sparse, and the interaction of serum AVP and HPA reactivity has not been investigated in human obesity.

To study HPA activity, we conducted the dexamethasone/corticotropin-releasing hormone (dex/CRH) test (Heuser et al., 1994) in highly obese but otherwise healthy subjects. The dex/CRH test combines dexamethasone suppression with CRH stimulation and was originally applied to distinguish Cushing's syndrome from pseudo-Cushing's states, with higher diagnostic accuracy than the dexamethasone suppression test alone (Yanovski et al., 1993). It has been extensively studied in patients with psychiatric and stress related disorders (Heuser et al., 1994), and its ACTH and cortisol response is presumably co-mediated by AVP (Keck et al., 2002). We assumed higher dex/CRH test responses in individuals with obesity compared to non-obesity controls. At the same time, we measured copeptin, the c-terminal fragment of the AVP precursor, expecting its concentrations to be associated with ACTH and cortisol release.

2. Subjects and materials

2.1. Study population

Thirty-nine individuals with obesity, who were otherwise healthy (OB; body mass index [BMI] 41.5 ± 4.7 [35.5–54.1] kg/m^2; age 36.5 ± 10.0 years; f/m 25/14), and twenty-two age- and gender-matched non-obesity (BMI < 30 kg/m^2) controls (NOC; BMI 23.1 ± 2.4 [19.8–28.7] kg/m^2; of whom 18/22 had normal weight [BMI < 25 kg/m^2] and 4/22 overweight [BMI 25–30 kg/m^2]; age 35.3 ± 8.5 years; f/m 12/10) were analyzed. All subjects were metabolically healthy and free from any neurological or psychiatric diseases, centrally acting medications, illicit drugs or glucocorticoid treatment. All subjects received a general physical examination along with neurological status, and were seen by a psychiatrist conducting a semi-structured interview. The degree of depressive symptoms was measured using the Beck Depression Inventory (BDI) (Hautzinger, 1991); subjects with a score higher than 22, with symptoms or signs of clinically relevant depression were excluded. Routine laboratory investigations and urine screening were performed. For the day of neuroendocrine testing, subjects followed their daily routine and presented in normal hydration status. Three additional probands were originally included, but subsequently excluded from the analysis: (i) one female NOC, due to self-reported shivering after dexamethasone ingestion and severe dyslipidemia, suspected to be hereditary, (ii) one male NOC, due to severe psychosocial stress on the day of the dex/CRH test and (iii) one female OB, due to suspected Cushing's disease based on the test results. The study was conducted in accordance with the updated Declaration of Helsinki II and Guidelines for Good Clinical Practice and approved by the local ethics committee. Written informed consent was obtained from all individuals.

2.2. Procedures

All participants underwent the combined dex/CRH test as described previously (Then Bergh et al., 1999). In brief, subjects received 1.5 mg dexamethasone orally at 2300 h the day before CRH application. On the test day, an intravenous catheter was inserted into the cubital vein at 1430 h and kept patent by isotonic saline infusion at a rate of 20 ml/h. The first blood sample was taken at 1500 h. At 1502 h, 100 μg of synthetic humanCRH (Ferring) were applied as an i.v. bolus. Subsequent blood samples were taken at 1530 h, 1545 h, 1600 h and 1615 h. They were stored at 4 degC, centrifuged immediately after the test, serum and plasma, respectively, were taken off and samples were stored at −80 °C until assayed. Copeptin was measured in the 1500 h serum sample after dexamethasone ingestion the night before, prior to CRH administration. Plasma osmolality and sodium concentrations were assessed. ACTH concentrations were measured in EDTA plasma; cortisol, copeptin, osmolality and sodium in serum.

2.3. Assay methodology

Commercial chemiluminescence immunoassays were used to determine hormone concentrations. Copeptin concentrations were measured with Brahms CopeptinUs®, Thermo Scientific, Germany, with a lower detection limit of 0.9 pmol/l, an intra-assay coefficient of variation (CV) of <15% and an inter-assay CV of <17% in the range of 3–4 pmol/l. ACTH concentrations were measured with Liaison® ACTH, DiaSorin, Italy, and cortisol concentrations with Cobas®, Roche Diagnostics, Germany, following the manufacturers' instructions.

2.4. Statistical analysis

For statistical analysis of the dex/CRH test, we compared ACTH and cortisol "post-CRH" (30 min after CRH application), maximum concentration (MAX) and area under the time course curve above zero according to the trapezoid rule ("ground" area-under-the-curve; AUC). In addition, ACTH/cortisol ratios were computed for each indicator.

Data analyses were performed using Microsoft Excel 2010 and SPSS 23. Graphs were created using GraphPad Prism 5. All data are given as median with interquartile range (IQR) or mean ± standard deviation (SD). Since the dex/CRH curve parameters of ACTH, cortisol and copeptin concentrations were not normally distributed (Shapiro-Wilk test $P < 0.05$) and skewed to the right in both groups, non-parametric inference tests were conducted (Mann-Whitney-U test for intergroup differences). To reduce variance and to reach normal distribution, ACTH and cortisol curve indicators were also logarithmically transformed and analyzed using the *t*-test for group

Table 1
Subject characteristics and dex/CRH test indicators.

	Obesity group	Non-obesity controls	*P*-value
Number of subjects	39	22	
Sex, male/female	14/25	10/12	.59[c]
Age (years)	36.5 ± 10.0	35.3 ± 8.5	.63[a]
BMI (kg/m^2)	41.5 ± 4.7	23.1 ± 2.4	**<.0001**[a]
Waist circumference (cm)	121.2 ± 13.1	84.9 ± 7.8	**<.0001**[a]
Waist/Hip ratio	.92 ± 0.09	.88 ± 0.10	.11[a]
Smoking habits, # with score 0/1/2/3	27/3/0/9	16/2/0/4	.90[d]
$ACTH_{1500h}$ [pmol/l]	<0.84 (<0.84–0.93)	<0.84 (<0.84–0.96)	.90[b]
$ACTH_{post\text{-}CRH}$ [pmol/l]	1.71 (1.33–2.51)	1.33 (1.08–1.92)	.08[b]
$ACTH_{MAX}$ [pmol/l]	2.09 (1.55–3.35)	2.11 (1.58–3.21)	.98[b]
$ACTH_{AUC}$	6.70 (5.02–9.92)	6.54 (4.59–9.31)	.65[b]
$Log_{10}ACTH_{post\text{-}CRH}$	.29 ± 0.28	.16 ± 0.19	.07[a]
$Log_{10}ACTH_{MAX}$	.36 ± 0.25	.34 ± 0.21	.69[a]
$Log_{10}ACTH_{AUC}$	.86 ± 0.22	.82 ± 0.19	.52[a]
$Cortisol_{1500h}$ [nmol/l]	19.8 (14.5–23.2)	16.0 (11.7–20.6)	.13[b]
$Cortisol_{post\text{-}CRH}$ [nmol/l]	**51.5 (25.9–159.3)**	**28.6 (20.0–41.6)**	**.01**[b]
$Cortisol_{MAX}$ [nmol/l]	75.5 (33.0–225.2)	59.2 (28.1–106.0)	.27[b]
$Cortisol_{AUC}$	245.3 (108.1–684.3)	152.8 (85.4–271.0)	.15[b]
$Log_{10}Cortisol_{post\text{-}CRH}$	**1.81 ± .48**	**1.49 ± .29**	**.002**[a]
$Log_{10}Cortisol_{MAX}$	1.90 ± 0.47	1.77 ± 0.31	.20[a]
$Log_{10}Cortisol_{AUC}$	2.40 ± 0.46	2.22 ± 0.29	.06[a]
$ACTH/Cortisol_{post\text{-}CRH}$	**.028 (.016–.053)**	**.048 (.034–.070)**	**.009**[b]
$ACTH/Cortisol_{MAX}$	.025 (.014–0.049)	.041 (.027–0.057)	.07[b]
$ACTH/Cortisol_{AUC}$	.025 (.015–0.054)	.043 (.031–0.064)	.06[b]
Osmolality [mosmol/kg]	294.0 (289.0–297.0)	292.5 (289.8–297.0)	.74[b]
Sodium [mmol/l]	140.5 (138.4–142.4)	140.5 (138.9–141.5)	.64[b]
$Copeptin_{1500h}$[pmol/l]	**4.62 (2.60–5.88)**	**3.04 (2.52–4.29)**	**.04**[b]

Data are given as median (interquartile range) or mean ± standard deviation BMI, body mass index; Smoking habits, 0. . .non-smoker, 1. . .occasionally, 2. . .not more than 3 cigarettes/d, 3. . .regular smoker. Dex, dexamethasone; CRH, corticotropin releasing hormone; ACTH, adrenocorticotropic hormone; MAX, maximum; AUC, area under the curve. Post-CRH concentrations for ACTH and cortisol were measured 30 min after 100 μg hCRH. ACTH/cortisol ratios in pmol/nmol, AUC and log_{10} data in arbitrary units. **bold**: significant at $P < 0.05$.

[a] *t*-test.
[b] Mann-Whitney-*U* test.
[c] Fisher's exact test.
[d] Pearson's Chi-Square test.

comparison. Associations between variables were analyzed by Spearman-rank correlation. Two-tailed significance was applied. Results were considered significant at $P < 0.05$ and for trend at $0.05 \leq P < 0.1$.

3. Results

3.1. Study population characteristics

OB and NOC were well matched for age and sex (see Table 1 for probandsí characteristics).

3.2. Post-dexamethasone copeptin concentrations are higher in individuals with obesity

Copeptin concentrations, measured at 1500 h after dexamethasone administration but before CRH application, were significantly higher in OB subjects (see Table 1 and Fig. 2). Copeptin concentration was positively associated with the BMI in the obesity group (Table 2).

3.3. Subjects with obesity show mild HPA axis hyperactivity

OB individuals showed a higher cortisol output in the dex/CRH test, reaching statistical significance in the post-CRH sample (see Table 1 and Figs. 1 and 2). Post-CRH ACTH concentrations and logarithmically transformed cortisol AUC were also higher in OB, reaching trend level (see Table 1). In OB, the ACTH response was independently associated with the waist-hip ratio and with age (see Table 2). Within the NOC group (n = 22), there was no statistical difference between overweight (n = 4) and normal weight (n = 18) individuals.

3.4. ACTH/cortisol ratio is lower in obesity

ACTH/cortisol ratio of the post-CRH sample was significantly lower in OB and reached trend level for ACTH/cortisol AUC (Table 1, Fig. 2). In OB, the ACTH/cortisol ratio was negatively associated with age, whereas in NOC, a positive relation was found (Table 2).

3.5. Association between copeptin and HPA responsiveness

In the OB group, post-dexamethasone copeptin serum concentrations correlated significantly with indicators of ACTH secretion, namely post-CRH ACTH, ACTH AUC and ACTH maximum (see Table 3 and Fig. 3). Since the correlation appeared to be driven by few subjects, we performed correlation analyses in subgroups according to diagnostic group and gender. This revealed that the relationship was driven by OB men (n = 14), with highly significant and substantial associations of circulating copeptin with all ACTH and cortisol indicators (see Table 3, Fig. 3). All associations remained significant after adjusting for BMI and age. Although we are aware that correlations including two diagnostic groups may be vulnerable to false-positive results, we still performed an additional exploratory overall correlation with all subjects (OB and NOC, n = 61) and found the same associations between copeptin with ACTH parameters as in the OB group.

Table 2
Spearman-correlations of anthropometric data with neuroendocrine indicators.

	Obesity group (n = 39)				Non-obesity controls (n = 22)			
	Age	BMI	Waist/Hip ratio	Waist circumference	Age	BMI	Waist/Hip ratio	Waist circumference
$ACTH_{post\text{-}CRH}$	**.41 (.01)**	.09 (.60)	**.42 (.008)**	**.34 (.03)**	−0.14 (.54)	.14 (.52)	−0.20 (.38)	.01 (.98)
$ACTH_{MAX}$	**.44 (.005)**	−0.03 (.86)	**.42 (.008)**	.23 (.15)	−0.06 (.79)	.059 (.79)	−0.15 (.51)	−0.07 (.77)
$ACTH_{AUC}$	**.41 (.009)**	−0.04 (.84)	**.42 (.009)**	.24 (.14)	−0.11 (.64)	.086 (.70)	−0.15 (.52)	−0.01 (.98)
$Cortisol_{post\text{-}CRH}$	**.48 (.002)**	.08 (.64)	.10 (.56)	.21 (.20)	−0.28 (.20)	−0.21 (.36)	−0.24 (.29)	−0.16 (.48)
$Cortisol_{MAX}$	**.42 (.009)**	.01 (.96)	.03 (.87)	.07 (.68)	−0.41 (.06)	−0.13 (.58)	−0.31 (.16)	−0.13 (.56)
$Cortisol_{AUC}$	**.42 (.007)**	.01 (.93)	.04 (.80)	.09 (.58)	−0.36 (.10)	−0.17 (.44)	−0.21 (.36)	−0.11 (.64)
$ACTH/Cortisol_{post\text{-}CRH}$	**−0.40 (.01)**	−0.30 (.86)	.10 (.55)	−0.04 (.81)	.16 (.48)	.04 (.88)	.14 (.55)	.03 (.90)
$ACTH/Cortisol_{MAX}$	−0.27 (.10)	−0.30 (.85)	.22 (.18)	.08 (.63)	**.51 (.02)**	.26 (.25)	.26 (.24)	.15 (.52)
$ACTH/Cortisol_{AUC}$	**−0.32 (.05)**	−0.42 (.80)	.16 (.34)	.03 (.85)	**.44 (.04)**	.20 (.38)	.09 (.70)	.06 (.78)
$Copeptin_{post\text{-}dex}$	.11 (.52)	**.40 (.01)**	.27 (.10)	**.51 (.001)**	.20 (.37)	−0.16 (.47)	.07 (.74)	−0.05 (.84)

Spearman-rho and *p*-value in parantheses. Dex, dexamethasone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; BMI, body mass index; MAX, maximum; AUC, area under the curve; post-CRH concentrations for ACTH and cortisol were measured 30 min after 100 μg hCRH. ACTH and copeptin in pmol/l, cortisol in nmol/l, ACTH/cortisol ratios as pmol/nmol, BMI as kg/m^2; waist circumference in cm; AUC in arbitrary units; **bold**: significant at $P < 0.05$.

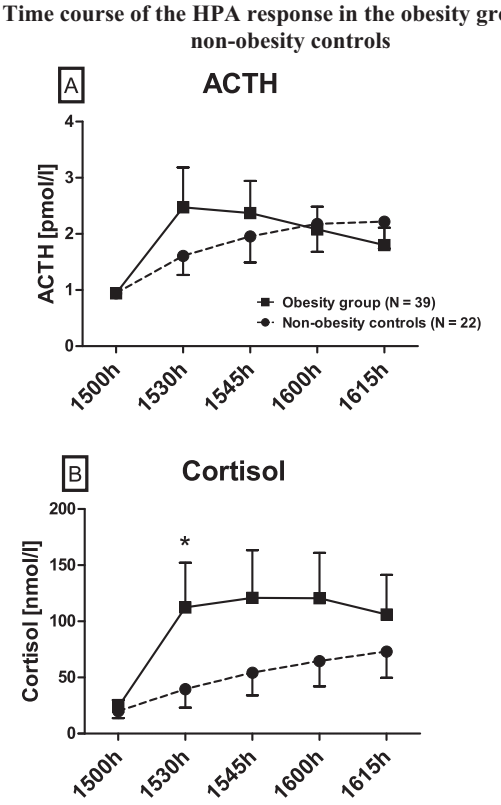

Fig. 1. Time course of ACTH (A) and cortisol response (B) to the combined dex/CRH test in subjects with obesity (N = 39; solid line, squares) and non-obesity controls (N = 22; dashed line, circles). After 1.5 mg dexamethasone, taken orally at 2300 h the night before the test, a bolus of 100 μg CRH was applied i.v. at 1502 h. Post-CRH cortisol (30 min after CRH stimulation, taken at 1530 h) was significantly higher in the obesity group (marked with *, p = 0.01). Data are given as mean with standard error. dex, dexamethasone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone.

Table 3
Spearman-correlations of copeptin with dex/CRH test indicators.

	Obesity group (n = 39)		Non-obesity controls (n = 22)	
$ACTH_{post\text{-}CRH}$	**.42 (.008)**		.05 (.84)	
$ACTH_{MAX}$	**.35 (.03)**		.25 (.27)	
$ACTH_{AUC}$	**.33 (.04)**		.17 (.45)	
$Cortisol_{post\text{-}CRH}$	.29 (.08)		.42 (.05)	
$Cortisol_{MAX}$	.21 (.19)		.28 (.21)	
$Cortisol_{AUC}$	.19 (.24)		.33 (.13)	
	female (n = 25)	male (n = 14)	female (n = 12)	male (n = 10)
$ACTH_{post\text{-}CRH}$	.12 (.58)	**.76 (.002)**	.24 (.46)	−0.01 (.99)
$ACTH_{MAX}$	.04 (.84)	**.67 (.009)**	.13 (.70)	.36 (.31)
$ACTH_{AUC}$	−0.03 (.88)	**.71 (.004)**	.11 (.73)	.20 (.58)
$Cortisol_{post\text{-}CRH}$	.05 (.82)	**.64 (.02)**	.39 (.21)	.48 (.16)
$Cortisol_{MAX}$	.01 (.95)	**.59 (.03)**	.39 (.22)	.49 (.15)
$Cortisol_{AUC}$	−0.03 (.88)	**.60 (.02)**	.41 (.19)	.46 (.19)

Spearman-rho and *p*-value in parentheses. Dex, dexamethasone; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; BMI, body mass index; MAX, maximum; AUC, area under the curve; post-CRH concentrations for ACTH and cortisol were measured 30 min after 100 μg hCRH. ACTH and copeptin in pmol/l, cortisol in nmol/l, BMI in kg/m^2; AUC expressed as arbitrary unit; **bold**: significant at $P < 0.05$.

4. Discussion

4.1. Pathophysiology of HPA axis activation in obesity

We identified higher serum concentrations of the AVP-surrogate copeptin to be associated with mild HPA hyperactivity in the obesity group, driven by men with obesity. The higher HPA responsiveness was shown by the significantly higher cortisol output after CRH stimulation, with the other indicators of cortisol release all pointing in the same direction. ACTH concentrations differed for trend in the post-CRH sample, whereas overall output was similar in both groups. Interestingly, the pituitary ACTH response was associated with the waist-hip ratio, but not with the BMI itself. The lower ACTH/cortisol ratio in subjects with obesity suggests an increased sensitivity of the adrenal cortex to ACTH. This relation was negatively associated with the subjects' age.

In accordance to earlier reports (Vicennati and Pasquali, 2000), these results support the notion of an increased activity of the serum AVP system and HPA axis activation in obesity. To the best of our knowledge, ours is the first study to show that circulating copeptin is associated with the ACTH and cortisol response in human obesity, and that serum copeptin is related to HPA responsiveness as measured by the dex/CRH test. These observations are best explained by a combination of hypothalamic-pituitary escape of ACTH release, adrenal hypersensitivity to ACTH and an accelerated clearance of ACTH and cortisol. All three mechanisms are supported by this study or previous work, as discussed below.

AVP is a nonapeptide produced by parvocellular and magnocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. Parvocellular AVP is released through the external zone of the median eminence into the pituitary portal circulation, stimulating the HPA axis via ACTH release (Dinan and Scott, 2005). AVP from the magnocellular division is transported axonally, stored in the neurohypophysis and secreted into the systemic circulation through fenestrated capillaries to mediate water conservation and vascular regulation (Aguilera, 2011). Magnocellular AVP appears to co-mediate the interaction between the hypothalamic-neuro-pituitary system and the HPA

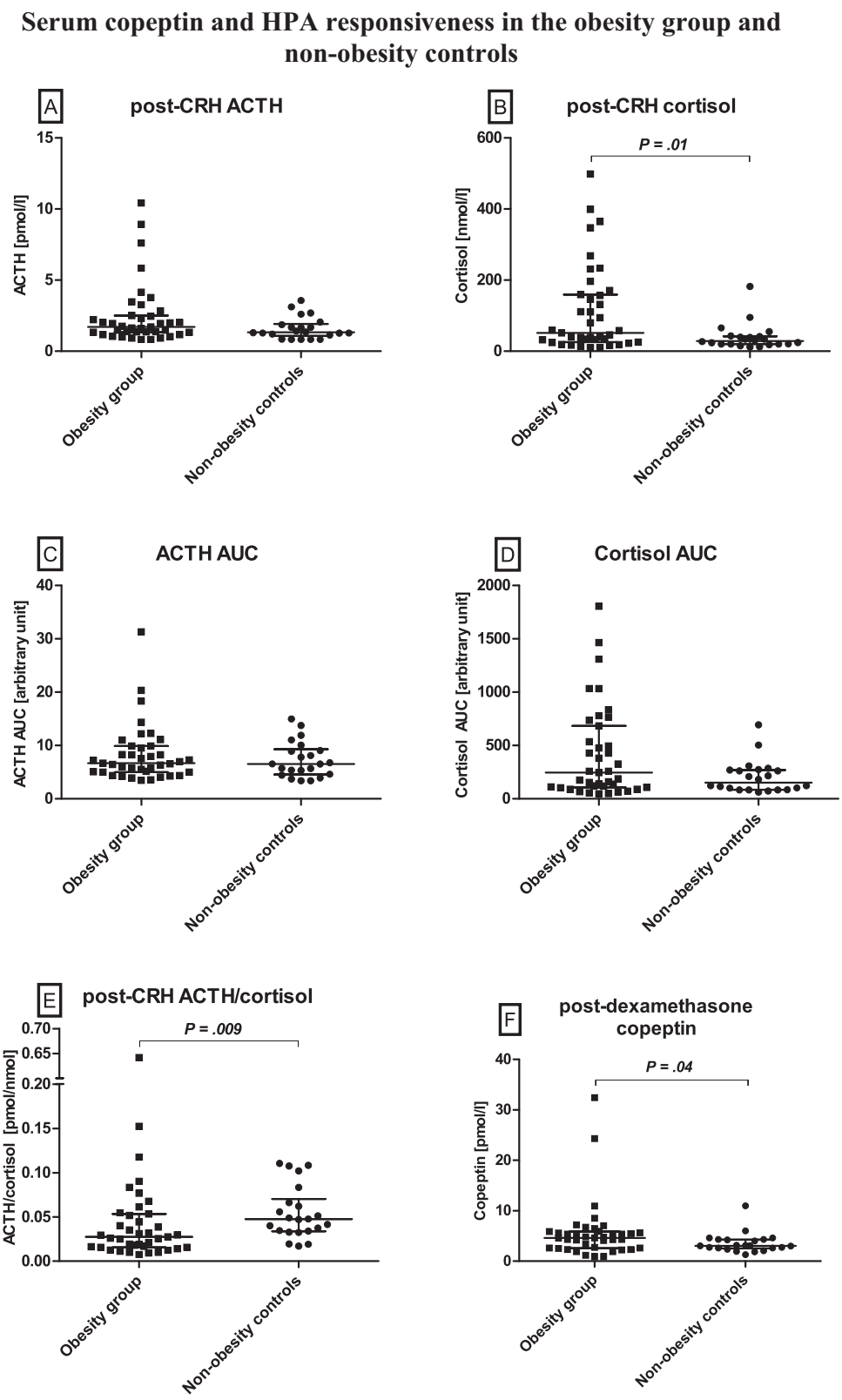

Fig. 2. Group comparison of dex/CRH test indicators and post-dexamethasone serum copeptin. Post-dex copeptin was measured in the sample taken at 1500 h, after ingestion of 1.5 mg dexamethasone the night before (2300 h), but before CRH injection. Post-CRH values were measured in the blood sample 30 min after CRH stimulation (1530 h). Post-CRH cortisol and post-dexamethasone copeptin were significantly higher in the obesity group (B, F). The ACTH/cortisol ratio was significantly lower in subjects with obesity (E). CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone, AUC, area under the curve.

axis (Sivukhina and Jirikowski, 2016) since it reaches the pituitary portal blood system by en passant release through the internal zone of the median eminence or via short portal vessels from the posterior pituitary (Wotjak et al., 1996; Keck et al., 2002), potentiating the CRH-induced ACTH secretion (Watabe et al., 1988). Copeptin is released in a 1:1 ratio to AVP from the same precursor reflect-

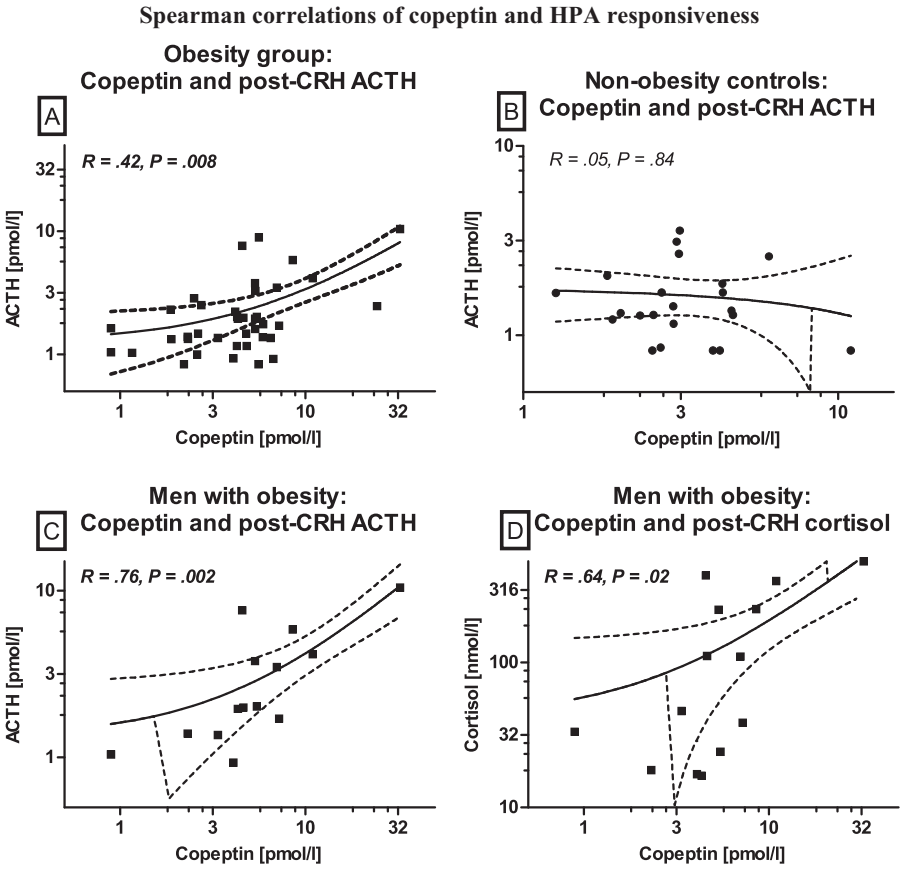

Fig. 3. Spearman correlations of post-dexamethasone copeptin with dex/CRH test indicators. Subjects with obesity, but not non-obesity controls, showed significant positive associations of copeptin with all ACTH indicators (illustrated for post-CRH ACTH, graph A). Associations were most pronounced in men with obesity, with positive associations of copeptin with all ACTH and cortisol parameters, illustrated for post-CRH ACTH (C) and cortisol (D). Data are given with regression line and 95% confidence interval. X- and y-axes are log10-scaled. CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone.

ing recent AVP secretion while representing a more stable analyte (Morgenthaler et al., 2006). Its marked elevation in acute stress has raised the suggestion to use copeptin concentrations as a prognostic marker in patients with acute illness (Katan et al., 2008), e.g. the acute coronary syndrome (Keller et al., 2010) or ischemic stroke (Katan et al., 2009). By means of copeptin, our results indicate an increased serum AVP tone in subjects with obesity, corresponding to mild hypercortisolism, and support the prevailing concept that AVP co-stimulates HPA activity in human obesity (Saleem et al., 2009), along with being a major determinant of exaggerated ACTH and cortisol release in the dex/CRH test (Keck et al., 2002). This concept stems from a wealth of experimental data, showing e.g. that endogenous serum AVP stimulates the CRH-mediated ACTH release (Watabe et al., 1988), or escape from dexamethasone suppression when CRH and lysine-vasopressin are simultaneously administered to healthy humans (Bardeleben et al., 1985). A similar situation appears to apply in genetically anxious rats (Keck et al., 2002), and has been consistently reported in patients with major depression, who display exaggerated ACTH and cortisol secretion in the dex/CRH test (Heuser et al., 1994) and increased spontaneous (van Londen et al., 1997) or post-dexamethasone AVP concentrations (Watson et al., 2006). While a correlation between vasopressin and cortisol was reported in subgroups of depressed patients (de Winter et al., 2003), there is no data on the simultaneous evaluation of AVP or its surrogate copeptin with the more sensitive dynamic dex/CRH challenge. Random copeptin concentrations were reported to be within the reference range in one study in depressed subjects (Krogh et al., 2013). In addition to AVP costimulation, impaired feedback control could be responsible for HPA axis dysregulation. Reduced concentration, affinity or regulatory efficiency of glucocorticoid receptors result in hypo-sensitivity to circulating cortisol, leading to inadequate adaptations to the current glucocorticoid state (Holsboer, 2000).

It is noteworthy that the positive overall correlation between copeptin and the HPA response was essentially driven by OB men alone. Gender-specific differences were previously shown for copeptin and HPA activity, suggesting the involvement of sex hormones in the regulation of both systems (Künzel et al., 2003; Morgenthaler et al., 2006; Taskin et al., 2015; Rothermel et al., 2016). In line with our results, a recent study reported a copeptin-cortisol relation in healthy males (Spanakis et al., 2016), whereas other studies showed associations for the overall group (Lewandowski et al., 2016), only in women (Kacheva et. al., 2015) or avoided potential gender-effects by including only men (Demiralay et al., 2016). It is to be noted that the aforementioned studies applied distinct methodological approaches using psychological or pharmacological stressors and included different patient groups, but not specifically individuals with obesity.

The decreased ACTH/cortisol ratio in the obesity group suggests an adrenal contribution: presumably the result of chronic overstimulation, increased adrenal sensitivity to circulating ACTH leads to a higher relative cortisol secretion. This is in line with a report of enlarged adrenal glands in subjects with abdominal obesity and diabetes mellitus (Godoy-Matos et al., 2006). Interestingly, we found the ACTH/cortisol ratio of obese subjects to be negatively associated with age, suggesing that in obesity the adrenal

sensitivity increases over time. This is in accordance with findings in a longitudinal multiple sclerosis study, showing a decrease of ACTH/cortisol ratios (Kümpfel et al., 2014). It is further notable that in the obesity group the ACTH response was associated with the waist hip ratio, but not with the BMI per se. This corroborates previous studies which showed HPA disturbances to be linked to visceral fat accumulation and unfavorable metabolic risk profiles rather than to obesity itself (Porzezińska-Furtak et al., 2014; Incollingo et al., 2015).

Mild hypercortisolism is detected with the highest diagnostic accuracy by measuring ACTH and cortisol in the early phase after CRH or CRH/AVP stimulation (Pasquali et al., 1999; Erickson et al., 2007). Accordingly, the post-CRH sample showed significant inter-group differences whereas the log-transformed AUC for cortisol only reached trend level. A possible explanation for the latter is that subjects with obesity clear cortisol from the circulation faster than their non-obesity counterparts, as reported earlier using a different paradigm (Pasquali et al., 2006). Altered activity of 11beta-hydroxysteroid dehydrogenase, the enzyme that converts cortisone into cortisol or vice versa, has been discussed as a possible cause of decreased glucocorticoid feedback and the manifestation of type2 diabetes (Cooper and Stewart, 2009); similarly, enhanced glucocorticoid clearance could be mediated by higher hepatic 5alpha reductase activity (Tsilchorozidou et al., 2003), and HPA hyperactivity was even postulated an appropriate compensatory mechanism counteracting the increased cortisol clearance (Vicennati and Pasquali, 2000).

We assessed osmolalities and sodium concentrations to exclude intergroup differences since copeptin correlates strongly with the individual water balance (Balanescu et al., 2011). A possible limitation is that we did not measure dexamethasone concentrations. Sufficient suppression by dexamethasone is indicated since there was no intergroup-difference of pre-CRH cortisol. Previous studies showed a dose-response effect of dexamethasone concentrations on ACTH and cortisol suppression only at very low dosages, but no difference between overweight and normal weight controls after the administration of 1 mg dexamethasone, which was considered a near-maximum dose (Pasquali et al., 2002). Accordingly, no association of serum dexamethasone with the BMI was shown, implicating that its measurement does not improve the performance of the dexamethasone suppression test at doses of 1 mg or higher (Asvold et al., 2012).

4.2. Elevated copeptin – a reasonable surrogate for HPA dysregulation?

HPA assessment using the dex/CRH test is a sensitive and well-validated tool (Heuser et al., 1994), but is burdensome to the proband and requires costly hormone measurement in several blood samples. Identifying a more simply acquired surrogate which outlines patients at risk for diseases with HPA dysregulation would facilitate neuroendocrine assessment, especially in larger populations. Copeptin is stoichiometrically released from the same precursor but more stable than AVP itself (Morgenthaler et al., 2006). Subjects with obesity appeared to be a suitable group to assess the AVP-HPA interplay since exceeding glucocorticoids have long been hypothesized as a mediator in the pathogenesis of obesity (Pasquali et al., 2006) and copeptin has been implicated in the metabolic syndrome (Enhörning et al., 2013). A direct association of copeptin with HPA activity could recently be shown in patients with multiple sclerosis (Baranowska-Bik et al., 2015) and in children with obesity (Rothermel et al., 2016). Our finding of significant correlations of copeptin to ACTH curve indicators supports this approach; further evaluation is required, e.g. to assess the relative sensitivity and specificity of random and post-dexamethasone measurement of copeptin. The latter may in fact add discriminatory power, since it incorporates dynamic testing. We measured copeptin before CRH stimulation with the intention to reflect a higher tone of serum AVP to be associated with HPA reactivity and to explore copeptin as a potential surrogate simplifying HPA assessment. However, the dynamic AVP-HPA interplay after different types of stimulation should be further elucidated. Whereas psychosocial stress (Urwyler et al., 2015; Spanakis et al., 2016), stress by CCK-4 induced panic symptoms (Demiralay et al., 2016), glucagon (Lewandowski et al., 2016) or hypoglycemic states (Kacheva et. al., 2015) induce copeptin and cortisol release, it would be of interest how endocrine stimulation, e.g. by CRH, affects serum copeptin and how this interacts with the HPA axis in healthy controls compared to patients. Such validations should also be performed in people with psychiatric disorders (Heuser et al., 1994; Schmider et al., 1995; Erhardt et al., 2006) or multiple sclerosis (Then Bergh et al., 1999), in whom HPA axis hyperactivity has been reported. More frequent dexamethasone non-suppression in depression (Heuser et al., 1994) and a different HPA response pattern in obesity indicate a distinct pathophysiology, and assessment of copeptin may contribute to understanding the differences.

HPA dysregulation and copeptin are linked to disadvantageous metabolic changes (Anagnostis et al., 2009; Enhörning et al., 2011; Ebert et al., 2016). HPA hyperactivity is pharmacologically modifiable (Then Bergh et al., 2001), including an attenuating effect of a central vasopressin receptor antagonist on HPA activity (Katz et al., 2016). In patients with Cushing's syndrome, eradication of severe hypercortisolism leads to the reversal of their symptoms (Pasquali et al., 2006). It is to question to what extent HPA downregulation, e.g. by targeting the vasopressin system, favors weight loss in common obesity or in how far it has a beneficial metabolic impact. If this turned out to be true, individual stress axis assessment might be a promising investigative tool outlining patients who are at risk of metabolic comorbidities but potentially susceptible for HPA normalizing therapies.

Disclosure statement

The authors have nothing to disclose.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.01.004.

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3. 2 Central noradrenaline transporter availability is linked with HPA axis responsiveness and copeptin in human obesity and non-obese controls.

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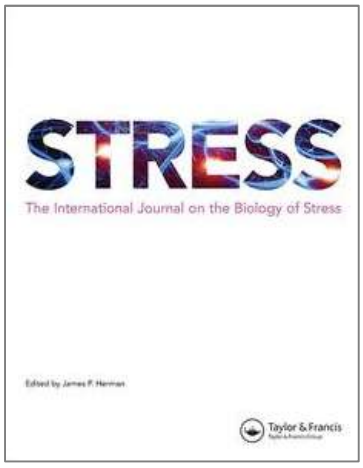

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ORIGINAL ARTICLE

Central noradrenaline transporter availability is linked with HPA axis responsiveness and copeptin in human obesity and non-obese controls

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ABSTRACT

The central noradrenaline (NA) stress-response network co-mediates hypothalamic-pituitary-adrenal (HPA) axis activation and arginine-vasopressin (AVP) release. Dysregulation of these systems contributes to stress-related diseases such as human obesity, but their interrelation remains unclear. The study was aimed to test for the first time *in vivo* whether central noradrenergic activity quantitatively indexed by the availability of the presynaptic NA transporter (NAT) is associated with HPA axis responsiveness as measured with the combined dexamethasone suppression/corticotropin releasing hormone stimulation (dex/CRH) test and copeptin as a surrogate marker of the serum AVP tone in highly obese, otherwise, healthy individuals compared to age- and sex-matched non-obese, healthy controls. In order to assess central NAT availability, positron emission tomography (PET) was applied using the NAT-selective radiotracer S,S-[^{11}C]O-methylreboxetine (MRB) and correlated with curve indicators derived from the dex/CRH test (maximum, MAX, and area under the curve, AUC, for cortisol and adrenocorticotropic hormone, ACTH) as well as with copeptin. In non-obese controls, positive correlations were found between the NAT distribution volume ratios (DVR) of the orbitofrontal cortex (OFC) and the amygdala with the HPA response (OFC: $ACTH_{MAX}$ $r = 0.87$, $p = .001$; $cortisol_{MAX}$ $r = 0.86$, $p = .002$; amygdala: $ACTH_{MAX}$ $r = 0.86$, $p = .002$; $cortisol_{MAX}$ $r = 0.79$, $p = .006$), while in obesity, the hypothalamic DVR correlated inversely with the HPA axis response ($cortisol_{MAX}$, $r = -0.66$, $p = .04$) and with copeptin ($r = -0.71$, $p = .02$). This association of central NAT availability with HPA axis responsiveness and copeptin suggests a mechanistic interaction between noradrenergic transmission with HPA axis activity and the serum AVP system that differs between non-obese individuals with prefrontal-limbic involvement and obesity with a hypothalamic-centered relationship. Whether the latter finding contributes to obesogenic behavior needs to be further explored.

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1. Introduction

Obesity has reached an epidemic scale; however, its neurobiological underpinnings are not entirely understood and sustained treatment is limited or not available. One key assumption includes stress to promote overeating and to increase the vulnerability to obesity and diet-related metabolic risks (Incollingo et al., 2015; Pasquali, Vicennati, Cacciari, & Pagotto, 2006). Stressors activate forebrain, limbic, and brainstem structures (Ulrich-Lai & Herman, 2009), of which the latter directly extends noradrenergic projections to the paraventricular (PVN) nuclei of the hypothalamus as integrational homeostatic relay of the neuroendocrine stress response systems (Plotsky, Cunningham, & Widmaier, 1989; Radant et al., 1992). Noradrenergic neurotransmission exerts control over the endocrine axes (Plotsky et al., 1989; Zhou, 2004) by integrating stress signals at the level of the brainstem cell bodies and noradrenaline (NA) release in the forebrain-limbic and hypothalamic areas (Myers, Scheimann, Franco-Villanueva, & Herman, 2017). NA binding to hypothalamic NA receptors triggers corticotropin-releasing hormone (CRH) secretion into the pituitary-portal circulation (Feldman & Weidenfeld, 2004) and arginine-vasopressin (AVP) liberation from the neurohypophysis into the peripheral circulation (Liu et al., 1994; Radant et al., 1992; Simmler, Hysek, & Liechti, 2011; Spanakis, Wand, Ji, & Golden, 2016). While CRH induces HPA activation, serum AVP mediates water conservation and vascular regulation (Aguilera, 2011) with effects on glucose

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homeostasis and fat metabolism (Saleem et al., 2009). Increased activity of the vasopressin system relates to HPA activity (Rothermel et al., 2016; Schinke et al., 2017) and seems causally linked with obesity (Enhörning et al., 2011). Thus, dysregulation of stress-activated neuromodulatory systems that involve (hypothalamic) NA signaling together with hypothalamic-pituitary-adrenal (HPA) axis activity and AVP is thought to be implicated in overeating and the pathophysiology of obesity (Boundy & Cincotta, 2000; Incollingo et al., 2015; Li et al., 2014; Pasquali et al., 2006; Plotsky et al., 1989).

The NA transporter (NAT) is a critical modulator of noradrenergic transmission since it limits NA concentrations in the synaptic cleft by NA reuptake into the presynaptic neuron (Mandela & Ordway, 2006; Torres, Gainetdinov, & Caron, 2003) to terminate its action. NAT alterations are implicated in stress-related disorders (Li et al., 2014; Moriguchi et al., 2017), while NATs represent a major pharmacological target for the treatment of obesity as well (Astrup et al., 2008). The pharmacological decrease of NAT availability by NA reuptake inhibitors leads to higher concentrations of NA in the synaptic cleft, which in turn stimulates HPA activity (Schule, 2007) and vasopressin release (Simmler et al., 2011). Therefore, it is likely that NAT plays a pivotal role in the regulation of these neuroendocrine systems. Understanding perturbations of this interplay between central NA and the neuroendocrine stress response system is particularly important, given that HPA dysregulation and increased AVP tone are linked to obesity and unfavorable physical health conditions (Enhörning et al., 2011; Incollingo et al., 2015).

In accordance with this, we recently showed that increased HPA axis responsiveness is associated with enhanced concentrations of the AVP-surrogate copeptin in human obesity (Schinke et al., 2017). In another study, we furthermore assessed brain NAT availability in both highly obese, otherwise healthy individuals and non-obese, healthy controls by using NAT-selective (S,S)-[^{11}C]O-methylreboxetine ([^{11}C]MRB) positron emission tomography (PET) (Hesse et al., 2017). These data indicated a decrease in hypothalamic NAT availability with an increase in body mass index (BMI), which was previously shown to be related to emotional well-being (Melasch et al., 2016).

To further investigate whether there is an association of central NAT availability with HPA and AVP activity in human obesity, we applied both PET imaging with [^{11}C]MRB and the combined dexamethasone/CRH (dex/CRH) test for HPA responsiveness (Heuser, Yassouridis, & Holsboer, 1994; Schinke et al., 2017; Then Bergh, Kumpfel, Trenkwalder, Rupprecht, & Holsboer, 1999) in highly obese but otherwise healthy individuals compared to non-obesity, healthy controls which were carefully matched for age and sex. At the same time, we measured copeptin, the c-terminal precursor fragment of vasopressin, as a surrogate of the serum AVP tone (Enhörning et al., 2011). We hypothesized that NAT availability in brain regions relevant for stress control, i.e. the prefrontal cortex (PFC), the amygdala, and the hypothalamus (Arnsten, 2009), is related to HPA axis activity and copeptin and that these relations regionally differ between individuals with obesity compared to their non-obesity counterparts.

2. Material and methods

2.1. Participants and ethical approval

The study was conducted in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use Guideline for Good Clinical Practice and the declaration of Helsinki and approved by the local ethics committee (registration number 206-10-08032010) and the German Bundesamt für Strahlenschutz/Federal Office for Radiation Protection (Z5-22461-2-2011-002). The study was registered at the European clinical trial database EudraCT 2012-000568-32 and the German Clinical Trials Register (DRKS). Written informed consent was obtained from all participants.

Twenty individuals were prospectively included in the study, which included ten obese, otherwise healthy individuals with a BMI $>35\,kg/m^2$ and aged over 18 years. The participants with obesity were recruited from the outpatient clinic of the Integrated Research and Treatment Center AdiposityDiseases (IFB) Leipzig, which is a dedicated university clinic for obesity and associated disorders. Ten non-obese, healthy individuals carefully matched for age and sex and free of any medication or illicit drugs were recruited using flyers and advertisements on the webpage of the IFB (see Table 1 for subject characteristics). Exclusion criteria for both cohorts were current psychiatric disease, i.e. psychosis, depression, and anxiety disorders. To ensure the psychiatric health, the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) was performed by an experienced psychiatrist during the first visit. In addition, self-rating questionnaires were applied to screen for subthreshold depression and anxiety (Beck Depression Inventory, BDI, German Version, Hautzinger, 1991) and the Symptom Checklist-90-Revised version (SCL-90-R, Derogatis, Lipman, & Covi, 1973). Binge-eating disorder was excluded based on the use of the Eating Disorder Examination (Hilbert & Tuschen-Caffier, 2006). Head trauma or vascular encephalopathy, malignant hypertension, insulin-dependent diabetes, or other general medical conditions that may alter brain function, the use of anorectic medication or other interventions for weight loss, centrally acting medication or nutrition supplements over the last 8 weeks, past or present history of alcohol misuse and/or illicit drug abuse, pregnancy and breastfeeding were also defined as exclusion criteria. The intake of oral contraceptives was not defined as an exclusion criterion and applied in only one female with obesity and normal cortisol suppression after dexamethasone intake. Compared to the group of highly obese participants, the BMI of controls was significantly lower (Table 1).

All study participants underwent a general physical examination, including weight and length measurement for BMI calculation. They also underwent magnetic resonance imaging (MRI) (Magnetom Trio, 3 T, Siemens, Germany; T1-weighted 3 D magnetization prepared rapid gradient echo (MP-RAGE); time of repetition 2300 ms, time of echo 2.98 ms, 176 slices, field of view (FoV) 256×240 mm, voxel size $1 \times 1 \times 1$ mm) for PET-MRI co-registration (Hesse et al., 2017) and for exclusion of brain pathologies such as diffuse or confluent white matter hyperintensities in T2-weighted images,

Table 1. Subject characteristics and dex/CRH test indicators.

	Non-obesity controls	Obesity group	*p*-Value
Number of subjects	10	10	
Sex, male/female	6/4	6/4	1.0b
Age (years)	33.3 ± 10.0	34.4 ± 9.0	0.80a
Body mass index (kg/m^2)	23.9 ± 2.5 (21.7 - 28.7)	42.4 ± 3.7 (35.7 - 47.8)	**<0.0001**a
Waist circumference (cm)	89.3 ± 6.3	128.9 ± 13.4	**<0.0001**a
Waist/Hip ratio	0.91 ± 0.1	0.96 ± 0.09	0.27a
Ethnicity		Caucasian	
$ACTH_{MAX}$	2.69 (1.89–4.30)	1.86 (1.34–6.28)	0.47c
$ACTH_{AUC}$	7.85 (5.44–14.05)	6.26 (4.32–18.82)	0.76c
$Cortisol_{MAX}$	77.8 (36.6–193.4)	123.2 (29.0–284.7)	0.71c
$Cortisol_{AUC}$	226.1 (85.4–552.2)	378.2 (97.8–918.5)	0.55c
$ACTH/Cortisol_{MAX}$	0.037 (0.023–0.051)	0.022 (0.017–0.046)	0.17c
$ACTH/Cortisol_{AUC}$	0.035 (0.027–0.055)	0.023 (0.016–0.044)	0.13c
$Copeptin_{post\text{-}dex}$	3.10 (2.01–4.15)	4.32 (2.10–6.54)	0.41c
Osmolality (mosmol/kg)	293.9 ± 4.6	290.8 ± 6.6	0.33a
Sodium (mmol/l)	140.8 ± 2.6	139.5 ± 3.1	0.60a

[a]*t*-test;
[b]Pearson's Chi-Square test;
[c]Mann–Whitney *U*-test. Data are given as mean ± standard deviation (range) or median (interquartile range). BMI: body mass index. **Bold:** significant at $p < .05$.

tumors, or stroke. Dex/CRH test and [^{11}C]MRB PET imaging were performed within a median of 17.5 days.

2.2. Dex/CRH test

The dex/CRH test was performed according to the standard protocols described previously (Heuser et al., 1994; Schinke et al., 2017; Then Bergh et al., 1999). Briefly, all participants received 1.5 mg dexamethasone orally at 2300 h on the day before CRH administration. Subjects were advised to come in a relaxed state and to avoid psychological or physical stress exceeding their daily routine and to have a light lunch before the test. On the test day, an intravenous cannula was inserted into the cubital vein at 1430 h and kept patent by isotonic saline infusion at a rate of 20 ml/h. The first blood sample was taken at 1500 h. At 1502 h, an i.v. bolus of 100 µg of synthetic human CRH (Ferring, Kiel, Germany) was applied. Subsequent blood samples were taken at 1530 h, 1545 h, 1600 h, and 1615 h. The filled tubes were stored at 4 °C, centrifuged immediately after the test, serum and plasma, respectively, were taken off and samples were stored at -80 °C until assayed. Copeptin was measured in the 1500 h sample after dexamethasone ingestion the night before, prior to CRH application. Sodium concentrations and plasma osmolality were assessed. Copeptin, cortisol, osmolality, and sodium concentrations were measured in serum; ACTH concentrations in EDTA plasma.

2.3. Assay methodology

Commercial chemiluminescence immunoassays were used to determine ACTH (Liaison® ACTH, DiaSorin, Saluggia, Italy), and cortisol (Cobas Cortisol I®, Roche, Basel, Switzerland) concentrations. Respective intra- and inter-assay coefficients of variation (CV) for ACTH were below 7.7% for a target value of 9.53 pmol/L and below 7.3% for a target value of 62.3 pmol/L. Representative intra- and interassay CVs for cortisol were below 3.2% for a target value of 86.2 nmol/L and below 2.0% for a target value of 1120 nmol/L. The functional sensitivity of 20% CV was set to be 0.84 pmol/L for ACTH and 8.5 nmol/L for cortisol, according to the manufacturer's instruction. Copeptin concentrations were measured with Brahms CopeptinUs® (ThermoScientific, Hennigsdorf, Germany) with a lower detection limit of 0.9 pmol/l, an intra-assay coefficient of variation (CV) of <15% and inter-assay CV <17% in the range of 3–4 pmol/l, according to the manufacturer's instruction.

2.4. Radiotracer synthesis and PET imaging

[^{11}C]MRB was synthesized with [^{11}C]methyliodide ([^{11}C]MeI) as previously described (Hesse et al., 2017). Dynamic PET was performed between 1000 h and 1200 h after intravenous bolus injection (90 sec) of 359 ± 11 MBq [^{11}C]MRB (average injected mass: 0.027 ± 0.023 µg/kg) using the ECAT EXACT HR + scanner in three-dimensional acquisition mode (Siemens, Erlangen, Germany; intrinsic resolution at the center 4.3 mm (full-width at half maximum, FWHM), axial resolution: 5–6 mm, field of view: 15.5 cm). Emission scan duration was 120 min acquiring 26 frames (4 × 0.25, 4 × 1, 5 × 2, 5 × 5, 8 × 10 min). Immediately before the application of the radiotracer, a 10-min-transmission scan (from three ^{68}Ge/Ga sources) was performed for attenuation correction and iterative data reconstruction was applied (Hesse et al., 2017).

2.5. Imaging data processing

For PET data processing, individual MRI data sets of the subjects were spatially reoriented onto a standard brain dataset similar to the Talairach space using the image processing software PMOD version 3.3 (PMOD Technologies, Zurich, Switzerland). Hereafter, volumes of interest (VOIs) were manually drawn atlas-based on consecutive transversal slices of the reoriented individual MRI data sets by the consensus of two experienced readers. The VOI set included the NAT-rich thalamus, the hypothalamus, and the LC, but also regions of moderate-to-low NAT density of the prefrontal-limbic brain, which are the orbitofrontal cortex (OFC), the insula, the hippocampus, and the amygdala. PET data were corrected for head motion artifacts with the help of SPM2 software (Statistical Parametric Mapping; Wellcome Trust Centre

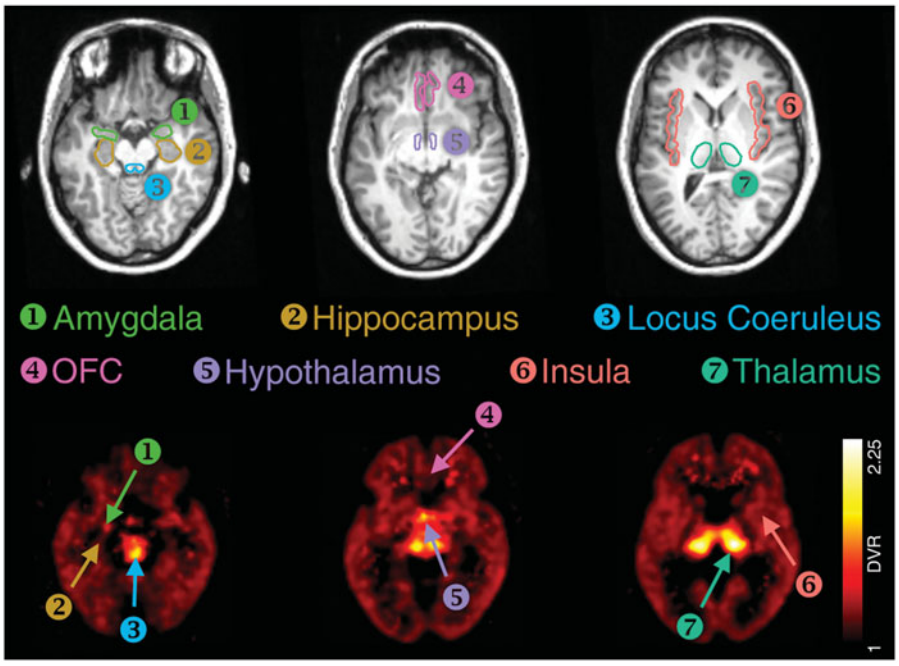

Figure 1. Regions-of-interest for the quantification of noradrenaline transporter availability. The outlined regions-of-interest are exemplarily shown on an individual MR (top row). The same regions are depicted with arrows on an averaged parametric PET (NAT DVR) image (bottom row).

for Neuroimaging, London, UK) and co-registered to the individual MRI including VOIs (Figure 1). Corresponding tissue time activity curves (TACs) were obtained from the dynamic PET data via PMOD and kinetic modeling of these regional brain TACs was performed using the multilinear reference tissue models MRTM2 (2 parameters) with the occipital cortex as a reference region for the calculation of the DVR (Hesse et al., 2017).

2.6. Statistical analysis

PASW/SPSS 25 was used for statistical analysis. Graphs were created with GraphPad Prism 5 (La Jolla, USA). All data are given as median with interquartile range or mean ± standard deviation (SD). After excluding asymmetries of corresponding brain regions, DVR was averaged side-by-side to reduce the number of variables and multiple comparisons. For statistical analysis of the dex/CRH test results, "post-CRH" concentrations for ACTH and cortisol (30 min after CRH application), maximum concentration (MAX) and area under the time course curve above zero according to the trapezoid rule ("ground" area-under-the-curve; AUC) were calculated from the plasma hormone concentrations measured at the five time points mentioned above and shown in Figure 2. In addition, ACTH/cortisol ratios were computed for each indicator. The Shapiro–Wilk test was performed to test if data were normally distributed and yielded $p < .05$ for all neuroendocrine data. Hence, relationships between DVR and dex/CRH test parameters, i.e. ACTH and cortisol MAX and AUC, respectively, were analyzed using Spearman-rank correlation for categorical data. To correct the putative DVR-copeptin correlation for sodium and osmolality, copeptin data were logarithmically transformed so they reached normal distribution (Shapiro–Wilk test: $p = .5$). Then, partial correlation was applied, correcting for sodium and osmolality as covariates. Two-tailed significance was applied. The Mann–Whitney *U*-test (not-normally distributed data) or unpaired *t*-test (data with normal distribution) was conducted for group comparison. Results were considered significant at $p < .05$.

3. Results

3.1. Group statistics

Individuals with obesity and non-obese participants did not differ in demographical variables (with the exception of BMI and waist circumference), see Table 1.

3.2. Individuals with obesity exhibit a tendency to higher HPA responsiveness and copeptin concentrations

Figure 2 shows the time course of ACTH and cortisol response to the combined dex/CRH. On a group level, the obesity group tended to show higher cortisol secretion and post-dexamethasone copeptin concentrations, and lower ACTH/cortisol ratios, albeit not reaching statistical significance (Table 1).

3.3. Correlative analyses revealed associations between NAT DVR of selected brain areas with the stress response, which are different between obese and non-obese individuals

In non-obesity controls, NAT DVRs of the OFC and the amygdala, but not in the hypothalamus or the midbrain, showed significant positive correlations with neuroendocrine stress test indicators (Table 2; Figure 3). This includes both the ACTH and the cortisol response (MAX, AUC). No association between NAT and copeptin was found in non-obese controls.

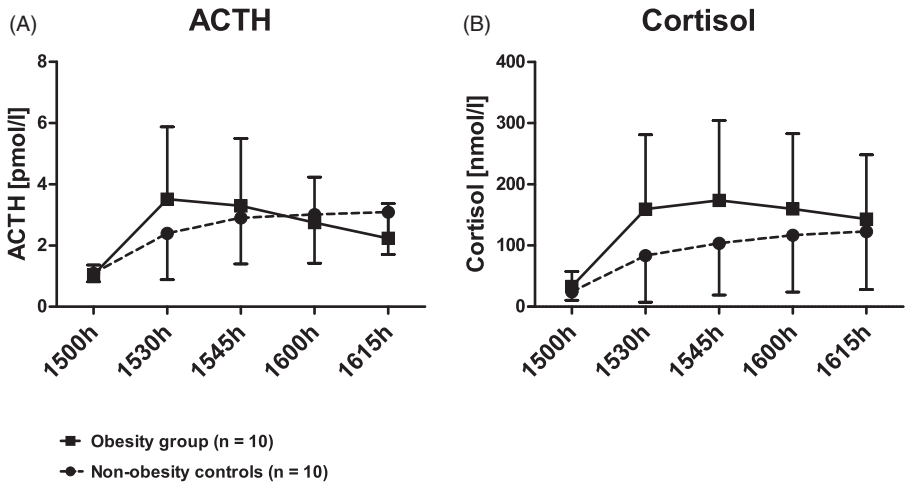

Figure 2. HPA responsiveness in the course of time. Obesity group vs. non-obesity controls. Time course of ACTH (A) and cortisol response (B) to the combined dex/CRH test in subjects with obesity ($N = 10$; solid line, squares) and non-obesity controls ($N = 10$; dashed line, circles). After 1.5 mg dexamethasone, taken orally at 2300 h, the night before the test, a bolus of 100 ug CRH was applied i.v. at 1502 h. Data are given as mean with standard error. Dex: dexamethasone; CRH: corticotropin-releasing hormone; ACTH: adrenocorticotropic hormone.

Table 2. Spearman correlation of dex/CRH test indicators and noradrenaline transporter availability.

	Obesity group					Non-obesity controls				
	$ACTH_{MAX}$	$ACTH_{AUC}$	$Cortisol_{MAX}$	$Cortisol_{AUC}$	Copeptin	$ACTH_{MAX}$	$ACTH_{AUC}$	$Cortisol_{MAX}$	$Cortisol_{AUC}$	Copeptin
OFC	0.21 (0.56)	0.21 (0.56)	0.05 (0.88)	0.05 (0.88)	−0.35 (0.33)	**0.87 (0.001)**	**0.86 (0.002)**	**0.86 (0.002)**	**0.84 (0.002)**	−0.24 (0.51)
Insula	−0.22 (0.53)	−0.22 (0.53)	−0.21 (0.56)	−0.21 (0.56)	−0.44 (0.21)	0.32 (0.37)	0.18 (0.63)	0.38 (0.28)	0.24 (0.51)	−0.21 (0.56)
Hippocampus	−0.30 (0.40)	−0.30 (0.40)	−0.44 (0.20)	−0.44 (0.20)	**−0.65 (0.04)**	0.16 (0.65)	0.14 (0.70)	0.12 (0.75)	0.10 (0.78)	0.30 (0.41)
Amygdala	−0.01 (0.99)	−0.01 (0.99)	−0.07 (0.85)	−0.07 (0.85)	−0.05 (0.89)	**0.86 (0.002)**	**0.88 (0.001)**	**0.79 (0.006)**	**0.75 (0.013)**	0.08 (0.83)
Thalamus	−0.50 (0.14)	−0.50 (0.14)	−0.15 (0.68)	−0.15 (0.68)	−0.35 (0.33)	0.13 (0.73)	0.03 (0.93)	−0.01 (0.99)	0.10 (0.78)	−0.27 (0.45)
Hypothalamus	−0.50 (0.14)	−0.50 (0.14)	**−0.66 (0.04)**	**−0.66 (0.04)**	**−0.71 (0.02)**	0.14 (0.70)	−0.02 (0.96)	0.09 (0.80)	−0.01 (0.99)	0.04 (0.91)
Locus coeruleus	0.16 (0.65)	0.16 (0.65)	−0.19 (0.60)	−0.19 (0.60)	0.24 (0.51)	0.31 (0.38)	0.15 (0.68)	0.30 (0.40)	0.24 (0.51)	−0.10 (0.78)

Spearman-rho coefficients and significance values (p). MAX: maximum; AUC: area under the curve; ACTH: adrenocorticotropic hormone; OFC: orbito-frontal cortex. ACTH in pmol/l, cortisol in nmol/l, AUC expressed as arbitrary unit. Noradrenaline transporter availability was indexed by distribution volume ratios. **Bold:** significant at $p < .05$.

In the obesity group, there was no association between prefrontal and limbic NAT, respectively. Instead, hypothalamic NAT correlated negatively with cortisol and copeptin (Table 2, Figure 4). A moderate negative association between NAT and copeptin was also found for the hippocampus. No other significant correlations were detected. The associations between hypothalamic NAT and copeptin remained significant after correcting for sodium concentrations and osmolality ($r = -0.79$, $p = .02$).

4. Discussion

The current study demonstrates for the first time *in vivo* a putative association between regional noradrenergic activity as measured by means of NAT-selective [^{11}C]MRB PET with neuroendocrine stress response indicators. These data add value to our previous findings of an increased HPA axis responsiveness in highly obese individuals, which was related to higher concentrations of the AVP-surrogate copeptin in human obesity (Schinke et al., 2017). These first data on an *in vivo* association between NAT availability with HPA axis parameters and the AVP system indicate that in non-obese controls, noradrenergic activity in the prefrontal-limbic brain (that is, in the OFC and the amygdala) is *positively* related to stress responsivity while in obese individuals, we observed that noradrenergic activity of the hypothalamus is *negatively* associated with HPA axis responsiveness and copeptin. This observed pattern of a distinct neuroendocrine stress adaptation suggests a switch from forebrain (prefrontal) and limbic NAT-HPA associations towards a bottom-up regulation primarily involving the hypothalamic neural-NA system together with HPA and neuro-hypophyseal axes regulation (Bains, Wamsteeker Cusulin, & Inoue, 2015; Arnsten, 2009).

4.1. Potential role of hypothalamic NA(T) in the regulation of the activity of the HPA axis and the vasopressin system

Hence, the present work supports both a physiological and pathophysiological role of the noradrenergic system in the regulation of the HPA axis and the vasopressin system in obesity and potentially in other stress-related diseases. Given that the activation of the neuroendocrine stress systems involves overlapping circuits of the limbic forebrain, the brainstem, and the hypothalamus (Ulrich-Lai & Herman, 2009), the positive association between prefrontal-limbic NAT availability with the HPA axis response in non-obese, healthy individuals is in line with a top-down control of HPA axis activity mediated by the forebrain and limbic system. The prefrontal brain is extensively connected with subcortical structures inhibiting HPA axis responses by limiting glucocorticoid secretion (Aihara et al., 2007; Arnsten, 2009; Ulrich-Lai & Herman, 2009) while the amygdala presumably

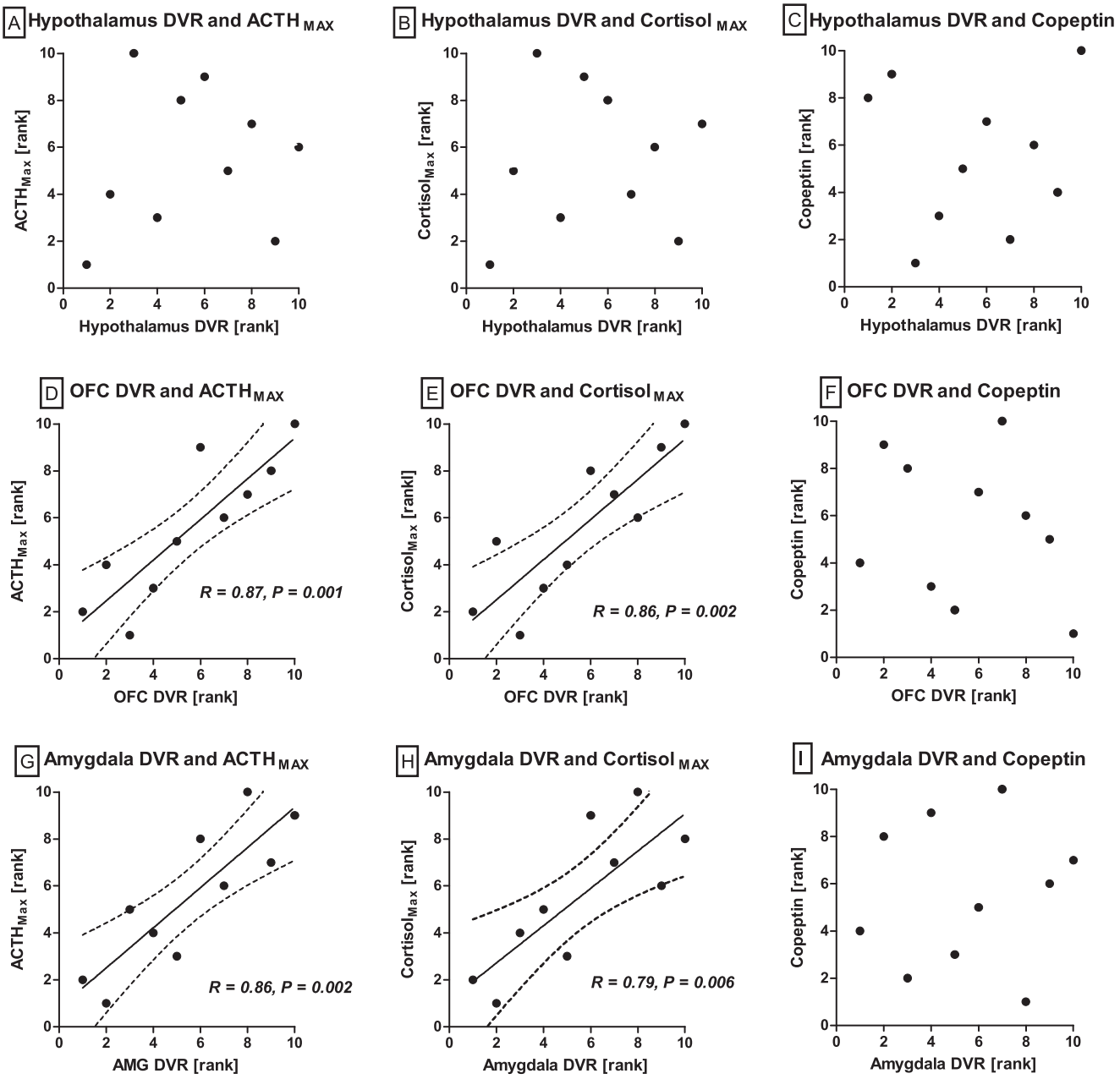

Figure 3. Non-obesity controls. NAT DVR in relation to HPA axis responsiveness and serum copeptin. Noradrenaline transporter distribution volume ratios and neuroendocrine parameters in non-obesity controls. Spearman-rho and *p*-value given for significant correlations. Data are presented in ranks with regression line and 95% confidence interval. MAX: maximum; ACTH: adrenocorticotropic hormone. Significant positive correlations were found between noradrenaline transporter (NAT) distribution volume ratios (DVR) of the orbitofrontal cortex (OFC) and the amygdala with ACTH and cortisol maxima (D, E, G, H). No correlation of copeptin with NAT DVR was found (C, F, I).

mediates HPA axis responsiveness via intervening hypothalamus-projecting neurons (Ulrich-Lai & Herman, 2009).

The hypothalamus is a central homeostatic control region for both the regulation of weight and the neuroendocrine axes (Farr, Li, & Mantzoros, 2016; Radant et al., 1992) that has dense noradrenergic innervation. As shown by preclinical experiments, hypothalamic noradrenergic activity is closely related to energy expenditure, feeding behavior, and the pathogenesis of obesity (Boundy & Cincotta, 2000; Nelson, Gehlert, & Gehlert, 2006; Paeger et al., 2017; Robertson et al., 2010). These hypothalamic centers stimulate ACTH release to trigger the adrenal production of cortisol (Aguilera, 2011). Furthermore, hypothalamic neurons contain AVP, which is transported axonally into the posterior pituitary and released into the systemic circulation in response to stress (Aguilera, 2011; Katan et al., 2009), but also stimulates HPA axis activity (Keck et al., 2002; Sivukhina & Jirikowski, 2016). Alterations of these endocrine stress systems have been frequently associated with obesity and health impairment (Enhörning et al., 2011; Incollingo et al., 2015; Pasquali et al., 2006). However, there is still an inconsistency in the literature on the relation of cortisol to metabolic parameters (Abraham, Rubino, Sinaii, Ramsey, & Nieman, 2013). This is partly based on the variety of methodological approaches and the high inter-individual

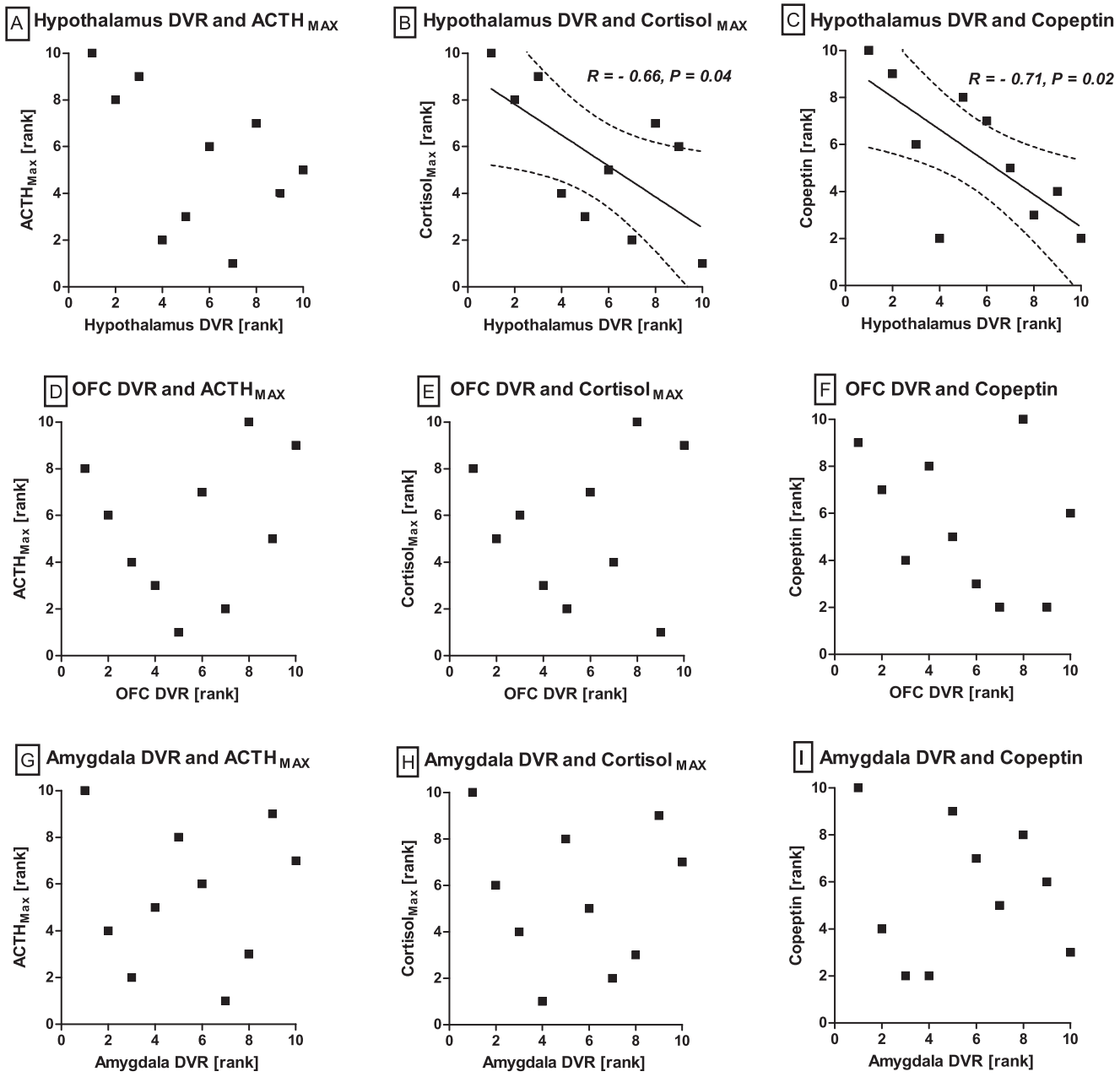

Figure 4. Obesity group. NAT DVR in relation to HPA responsiveness and serum copeptin. Noradrenaline transporter distribution volume ratios and neuroendocrine parameters in the obesity group. Spearman-rho and *p*-value given for significant correlations. Data are presented as ranks with regression line and 95% confidence interval. MAX: maximum; ACTH: adrenocorticotropic hormone. Significant negative correlations were found between noradrenaline transporter (NAT) distribution volume rations (DVR) of the hypothalamus with cortisol maximum (B) and copeptin, measured after dexamethasone ingestion, prior to CRH administration (C). No correlations between the neuroendocrine parameters with NAT DVR of the orbitofrontal cortex (OFC) or amygdala DVR were found (D–I).

variability of cortisol secretion (Incollingo et al., 2015). Further, peripheral determinants of cortisol concentration such as the activity of the 11-beta-hydroxysteroid dehydrogenases 1 and 2, enzymes converting active cortisol into inactive cortisone and vice versa, contribute to the complex regulation of circulating cortisol concentrations (Bailey, 2017; Cooper & Stewart, 2009). While basal hypercortisolism is not supported by the majority of the literature (Abraham et al., 2013; Bailey, 2017; Incollingo et al., 2015), it seems that rather alterations of HPA reactivity in response to different kinds of stressors are associated especially to abdominal obesity (Bjorntorp & Rosmond, 2000; Incollingo et al., 2015; Schinke et al., 2017; van der Valk, Savas, & van Rossum, 2018).

4.2. Relation of NAT to noradrenergic function and an approach to explain the inverse relation to the HPA axis and copeptin

Considering the positive relation between NAT availability and HPA axis responsiveness in non-obese controls in contrast to absent or, respectively, negative relations in obese individuals, the following assumptions can be made: (1) Normally, NAT density depends on synaptic NA concentration in a homeostatic attempt to normalize noradrenergic transmission (Lee, Javitch, & Snyder, 1983), indicating that a higher NAT density is associated with higher synaptic NA concentrations and therefore with a higher HPA response, as reflected by the close association between prefrontal and

limbic NAT availability with HPA axis responsiveness observed in healthy, non-obesity controls. (2) The absence of a significant association between prefrontal-limbic NAT availability and HPA axis activity in individuals with obesity hint towards a loss (or gradual reduction) of the prefrontal top-down control to a distress-induced regulation mediated by the subcortical noradrenergic system. In keeping with this hypothesis, one may speculate that lowered NAT in obesity is accompanied by higher intra- or extra-synaptic NA concentrations as an indicator of a higher noradrenergic tone that increases HPA axis activity and changes AVP tone (as indexed by copeptin). This is in accordance with pharmacological studies showing that NAT inhibition by reboxetine leads to higher ACTH and cortisol responses in the dex/CRH test (Schule et al., 2006), or to higher copeptin serum concentrations after the consumption of amphetamines (Simmler et al., 2011). It has to be noted that only the short-term application of NAT inhibitors produce enhanced HPA activity, whereas long-term administration leads to a gradual normalization of HPA activation by the restoration of feedback control, partly explaining the effectiveness of NAT inhibitors in the treatment of stress-related disorders (Schule, 2007).

4.3. Limitation

This is an observational trial. From the pilot character of the study, we cannot conclude whether the individual stress response helps to predict phenotype; this has to be elucidated in larger, longitudinal samples that reduce the relatively high variance in HPA axis activity, which was driven by a few subjects with pronounced stress reactivity. Another limitation, which was yet beyond the score of this research project, is that the number of study participants appeared inappropriate to differentiate effects of age and sex on the outcome measures of the stress test results or NAT DVR (Bangasser et al., 2013; Künzel et al., 2003; Rothermel et al., 2016).

The time interval between PET scan and the dex/CRH test was within a median of 17.5 days. A possible limitation, however, is that two participants out of the non-obesity controls underwent NAT PET and neuroendocrine testing within >2 months due to logistic difficulties, maybe partly explaining the high variance. Moreover, a challenging aspect of any PET study on central NAT availability is that NAT levels are low and changes in modulatory systems to which the noradrenergic fibers belong are rather low and difficult to balance for accuracy and noise. Hence, the interpretability of data, in particular, of areas with low NAT expression, which includes the prefrontal cortex, is limited and the results need replication in studies with large sample sizes.

5. Conclusion

Stress has long been associated with obesity (Spencer & Tilbrook, 2011; van der Valk et al., 2018). Previous literature suggested individual alterations of the HPA and AVP stress response to predispose to an obesogenic phenotype or behavior in some individuals (Enhörning et al., 2011; Epel, Lapidus, McEwen, & Brownell, 2001; Rosmond & Bjorntorp, 2000; Schinke et al., 2017). Our work aimed to add new insights into how the HPA and AVP axes are related to the NA neurotransmitter system in the living human brain in obesity and potentially in other stress-associated diseases. Altogether, this study combined for the first time *in vivo* PET measures of brain noradrenergic transmission with an assessment of HPA axis activity and the AVP system. The findings suggest a distinct NA modulated stress response in human obesity, which is centered in the hypothalamus and which is different from NA-mediated stress adaptation in healthy, non-obese individuals. If this points to a noradrenergic dysregulation or an alteration of homeostatic control together with changes in eating behavior that lead to obesity remains to be elucidated in a larger population or in diseases with pronounced stress axis dysregulation. If confirmed, the findings of our study may support pharmacological or behavioral treatment strategies to normalize NA transmission in individuals susceptible to compensate with overeating.

Disclosure statement

No potential conflict of interest was reported by the authors.

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3. 3 Central serotonin transporter availability in highly obese individuals compared with non-obese controls: A [^{11}C] DASB positron emission tomography study.

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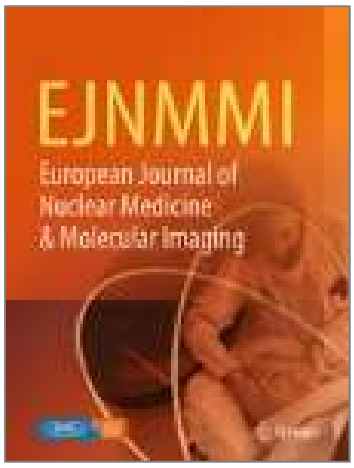

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ORIGINAL ARTICLE

Central serotonin transporter availability in highly obese individuals compared with non-obese controls: A [^{11}C] DASB positron emission tomography study

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Abstract

Purpose The role of the central serotonin (5-hydroxytryptamine, 5-HT) system in feeding has been extensively studied in animals with the 5-HT family of transporters (5-HTT) being identified as key molecules in the regulation of satiety and body weight. Aberrant 5-HT transmission has been implicated in the pathogenesis of human obesity by in vivo positron emission tomography (PET) and single-photon emission computed tomography (SPECT) imaging techniques. However, results obtained thus far from studies of central 5-HTT availability have been inconsistent, which is thought to be brought about mainly by the low number of individuals with a high body mass index (BMI) previously used. The aim of this study was therefore to assess 5-HTT availability in the brains of highly obese otherwise healthy individuals compared with non-obese healthy controls.

Methods We performed PET using the 5-HTT selective radiotracer [^{11}C] DASB on 30 highly obese (BMI range between 35 and 55 kg/m^2) and 15 age- and sex-matched non-obese volunteers (BMI range between 19 and 27 kg/m^2) in a cross-sectional study design. The 5-HTT binding potential (BP_{ND}) was used as the outcome parameter.

Results On a group level, there was no significant difference in 5-HTT BP_{ND} in various cortical and subcortical regions in individuals with the highest BMI compared with non-obese controls, while statistical models showed minor effects of age, sex, and the degree of depression on 5-HTT BP_{ND}.

Conclusion The overall finding of a lack of significantly altered 5-HTT availability together with its high variance in obese individuals justifies the investigation of individual behavioral responses to external and internal cues which may further define distinct phenotypes and subgroups in human obesity.

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Keywords Serotonin · Serotonin transporter · Positron emission tomography (PET) · Obesity · Body mass index (BMI) · Depression

Introduction

Obesity rates are currently at pandemic levels and if not curbed, the disease is on course to becoming the major preventable public health threat of the 21st century. A better understanding of the causative biological factors of obesity undoubtedly would aid both to prevent the development and the perpetuation of the disease and to identify potential treatment targets.

Studies in rodents have for decades implicated the central monoamine system in the regulation of energy homeostasis [1]. Only relatively recently this system has been suggested to play a role in the pathogenesis of human obesity. Focus has predominantly been placed so far on brain dopamine signaling and much less is known about the function of serotonin (5-HT) in the development of overweight and obese phenotypes. Central serotonergic mechanisms are prime candidates since there are numerous drugs that target 5-HT transmission, in particular 5-HT transporter (5-HTT) inhibitors such as sibutramine and fenfluramine, with proven clinical efficacy [2]. This includes the novel US Food and Drug Administration (FDA)-approved appetite-suppressing drug lorcaserin which is a 5-HT_{2c} receptor agonist [3].

The use of mouse and rat strains that are resistant or prone to diet-induced obesity when placed on a high fat diet has provided insight into the role of central 5-HT transmission in body weight regulation. Diet-induced obese mice have higher levels of 5-HTTs in the nucleus accumbens, a brain region involved in the motivational aspects of feeding, compared to diet resistant mice [4]. Additionally, diet-induced obese rats have higher levels of 5-HT_{2A} and 5-HT_4 receptors [5], which is in general agreement with in vivo human PET studies [6–8].

To date, there have been several in vivo human PET studies using the highly selective 5-HT radioligand [^{11}C] DASB examining the association between brain 5-HTT levels with obesity; however, findings so far have been inconsistent. Our preliminary data hinted at a positive correlation between BMI (range between 18 and 32 kg/m^2) and 5-HTT availability in the insula, the ventral striatum, and the hypothalamus [9]. A subsequent PET study showed a negative correlation between subcortical (caudate-putamen-thalamus) [^{11}C] DASB binding potential with BMI. These results were likely skewed by the low number of participants with higher BMI (>30 kg/m^2) used. Thus, correlation curves may have been driven by outliers in the upper range. Recently it was shown that there is no difference in subcortical 5-HTT availability in obese patients before and after gastric bypass surgery or compared to lean controls, although no detailed correlation analysis was performed [10].

To more comprehensively assess central 5-HTT availability in human obesity, we aimed to investigate highly obese (BMI>35 kg/m^2), otherwise healthy, non-depressed individuals compared with non-obese (BMI <30 kg/m^2), healthy controls in various cortical, subcortical, and limbic regions using PET and [^{11}C] DASB. Based on rodent andour own preliminary data, we hypothesized that higher BMI is associated with higher 5-HTT availability in brain areas involved in feeding.

Material and methods

Subjects

Thirty obese individuals with a BMI>35 kg/m^2 and aged over 18 years were included. After obtaining informed consent, screening for inclusion/exclusion criteria, a general physical examination (including weight and height measures for BMI calculation) and magnetic resonance (MR) imaging, eligible study participants underwent PET with [^{11}C] DASB as well as a comprehensive psychiatric and neuropsychological assessment. This included validated German language versions of the Beck depression inventory (BDI) [11]. Exclusion criteria were current or past neurological or psychiatric illness, i.e., depression (as assessed by a psychiatrist at the first visit), head trauma or vascular encephalopathy, resistant hypertension, insulin-dependent diabetes, or other medical conditions that may alter brain function, the use of anorectic medication or other interventions for weight loss, centrally acting medication, over-the-counter-medication or nutrition supplements over the last 8 weeks, past or present history of alcohol misuse and/or illicit drug abuse, pregnancy, and breast-feeding. Fifteen non-obese subjects, carefully matched for age and sex and free of any medication or illicit drugs, participated as controls. The study participants were Caucasians; none of the subjects fulfilled the criteria for binge-eating disorder nor were there participants with reported glucose intolerance. The amount of alcohol and/or nicotine consumption was recorded for both cohorts. One normal-weight control was excluded due to insufficient PET data statistics (less counts). Additionally, we investigated a length polymorphism of the 5-HTT coding gene (5-HTTLPR) as the potential influencing factor on in vivo 5-HTT PET signal [12].

Magnetic resonance (MR) imaging

Structural MR images were acquired using a 3T Siemens scanner and a T1-weighted 3D magnetization prepared rapid gradient echo (MP-RAGE) sequence (repetition time 2, 300 ms, echo time 2.98 ms, 176 slices, field of view (FOV)

256×240 mm, voxel size 1×1×1 mm) for PET-MRI co-registration and (with other sequences based on the Alzheimer's Disease Neuroimaging Initiative protocol) for exclusion of brain pathologies such as diffuse or confluent white matter hyperintensities in T2-weighted images, tumors, and stroke but not malformation without functional impairment.

Positron emission tomography (PET) imaging

[^{11}C]DASB was synthesized according to a previous publication [13]. Dynamic PET was performed for 90 min after intravenous bolus injection (90 s) of (mean±SD) 484±10 MBq [^{11}C]DASB using the ECAT EXACT HR+ scanner (Siemens, Erlangen, Germany; intrinsic resolution at the centre: 4.3 mm, axial resolution: 5–6 mm FOV 5.5 cm, 3–4 mm full width at half maximum) in three-dimensional (3D) acquisition mode. Emission scan acquired 23 frames (4×0.25, 4×1, 5×2, 5×and 5, 5×10 min). We used a 10-min transmission scan (from 3 ^{68}Ge sources), which was performed prior to the emission scan, for attenuation correction and iterative reconstruction (ten iterations, 16 subsets) in transverse image series (63 slices, 128×128 matrix, voxel size 2.6×2.6×2.4 mm^3) with a Hann filter (cutoff 4.9 mm) for image reconstruction. PET data were corrected for head motion artifacts using SPM2 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 7.3.0 (The MathWorks Inc., Natick, MA, USA). Then a summed image was built of the first 13 frames and co-registered with the individual 3D MRI data using PMOD software (Version 3.4) for re-alignment and stereo-tactical normalization (according to the anterior commissure-posterior commissure line). The same transformation was applied to the dynamic PET data. Parametric images of 5-HTT binding potential (BP_{ND}) were generated from the PET data by the multi-linear reference tissue model with two parameters (MRTM2) and the cerebellar cortex as the reference tissue [14, 15].

Imaging data analysis

Regional analyses of BP_{ND} values were consecutively performed atlas-based [16] by manual delineation of the volumes of interest (VOIs) for cortical, subcortical, and limbic areas including the amygdala, the hippocampus, the striatal subregions the head of the caudate and putamen, the nucleus accumbens (Acb), the thalamus, the hypothalamus, and the midbrain including the substantia nigra (SN) and ventral tegmental area (VTA) (Fig. 1). A voxelwise statistical analysis was performed using SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK) also implemented in Matlab 7.3.0 (MathWorks Inc., Natick, MA, USA). BP_{ND} maps were spatially normalized on the SPM8 integrated PET template and smoothed with 8 mm full-width-at-half-maximum (FWHM) on a Gaussian Filter. Group comparisons (two-sample *t*-test, obese vs. normal-weight controls) were considered significant for $p<0.005$, unadjusted for multiple comparisons ($T>3.5$) and a minimum of 30 voxels / cluster.

Statistical analysis

The data were tested for normal distribution using the Shapiro-Wilks test and the Levene test for homogeneity of variance. After thorough review and tests excluding asymmetries between corresponding left and right brain regions, VOIs were averaged side-by-side to reduce the number of dependent variables within the models. To estimate the difference in variance between and within the groups, an analysis of variance (ANOVA) was performed. For correlative analysis the Pearson product–moment correlation was applied (two-sided) and partial analysis if univariate analysis of covariance (ANCOVA) indicated a significant covariate effect by comparing the groups of obese and non-obese subjects regarding differences between the measured BP_{ND} within all brain VOIs and epidemiological factors (age, sex, and smoking habits), seasonal data (day length and sunshine duration), BDI, and 5-HTTLPR genotypes as covariates (complete data set for $n=44$). Additionally, a mixed linear modeling analysis was performed including the regions with high BP_{ND} (e.g., grouping the subcortical and the limbic regions in two separate analyses) to test whether there is a significant difference in subcortical or limbic regions between obese and non-obese subjects. If many relationships were tested, correction for multiple comparisons using a false discovery rate was applied to adjust the significance level.

Results

Subject characteristics and epidemiological data are summarized in Table 1. Obese and non-obese study participants not only differed in BMI but also in BDI and the length of the scanning day. All other parameters (sex, smoking habits, and 5-HTTLPR) were well matched.

Serotonin transporter (5-HTT) binding potential (BP_{ND}) in obese versus non-obese individuals

The BP_{ND} values in selected regions relevant for appetite control are shown in Fig. 2. Overall, no significant group differences in mean BP_{ND} were found (ANOVA); the entire BP_{ND} data are summarized in Table 2. Mean between-subject variability was 12.1 % in obese and 9.5 % in non-obese individuals, respectively, but this was not significantly different (for region-specific variability see Table 3). Results also indicate no significant differences

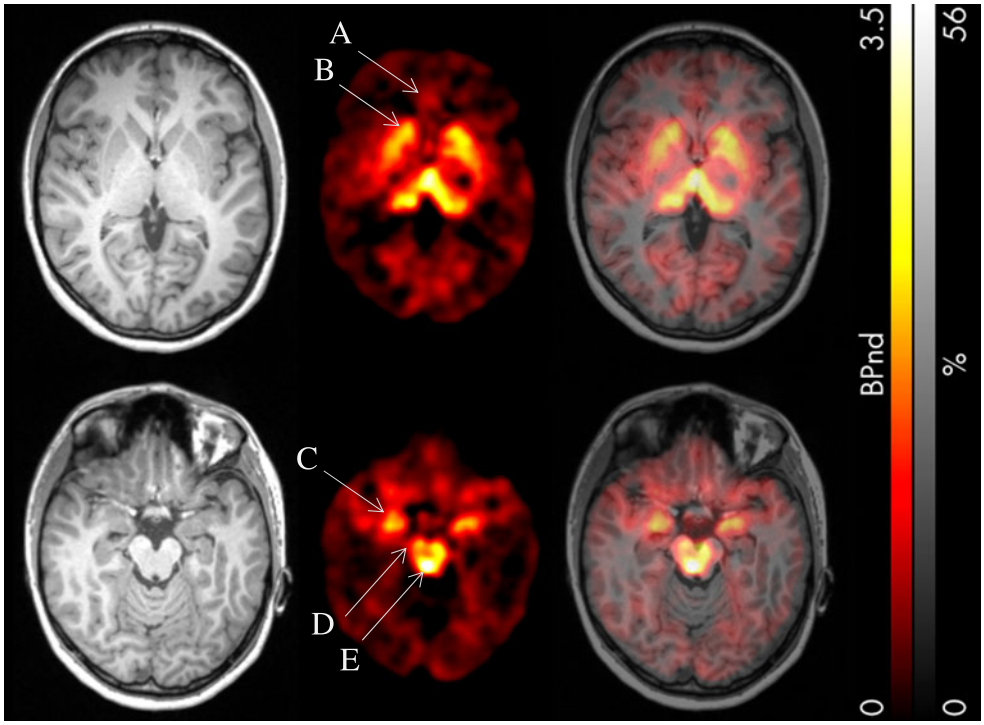

Fig. 1 Parametric maps of binding potentials (BP_{ND}) and co-registered magnetic resonance images (MRIs) at two different levels, the striatum/thalamus (*upper row*) and the brainstem (*bottom row*). *Arrows* indicate (**A**) anterior cingulate cortex, (**B**) head of the caudate, (**C**) amygdala, (**D**) substantia nigra, and (**E**) periaqueductal *grey*

in the test for homogeneity of variances between the groups. In the analyses of each sub-cohort, BMI and BP_{ND} did not correlate (voxel-wise and VOI-based analyses), ranging from r=0.27 (p=0.14) for the thalamus to r=0.03 (p=0.89) for the hippocampus in the obese study group, and from r=0.28 (p=0.33) for the head of the caudate to r=−0.33 (p=0.24) for the insula in the non-obese study group (Table 3). Neither did we find a significant difference between obese and non-obese subjects by grouping either the subcortical or the limbic regions in a mixed linear model analysis (p=0.53, and p=0.51, vs. BMI p=0.51, and p=0.25, respectively). Inter-regional correlation analyses of BP_{ND} revealed more robust associations in obese compared with non-obese controls as this mainly included the insula and the ACC (Fig. 3).

Table 1 Subject characteristics and seasonal data (n=44)

	Obese subjects	Non-obese controls	p-value
Number of subjects (complete data sets)	30	14	
Sex, male/female	8/22	5/9	0.72[c]
Age (years), mean±SD (range)	36.5±10.2 (21–59)	36.1±7.2 (21–49)	0.89[a]
BMI (kg/m^2), mean±SD (range)	41.2±4.9 (35.5–54.1)	22.5±2.6 (19.1–26.9)	**<0.0001**[a]
Smoking habits, #with score 0 / 1 / 2 / 3	21 / 2 / 0 / 7	12 / 1 / 0 / 1	0.54[c]
BDI, mean±SD (range)	9.8±6.9 (0–25)	2.4±2.7 (0–7)	**<0.0001**[a]
Injected activity (MBq), mean±SD	481.9±10.8	488.2±6.2	**0.02**[a]
Sunshine (h), median (IQR)	4.8 (0.2–9.75)	1.9 (0.0–6.6)	0.15[b]
Outside temperature (°C), median (IQR)	13.7 (7.6–18.2)	5.6 (0.8–8.2)	**0.003**[b]
Day length (h), mean±SD	13.0±2.5	11.0±2.2	**0.02**[a]
5-HTTLPR: SS / SL / LL	2 / 13 / 15	0 / 8 / 6	1[c]

[a] t test

[b] Mann-Whitney test

[c] Fisher's exact test

SD standard deviation, *IQR* interquartile range, *BMI* body mass index, *BDI* Beck Depression Inventory, *5-HTTLPR* serotonin-transporter-linked polymorphic region, *S* short allele, *L* long allele, *bp* base pairs

Bold values indicate statistical significance (p<0.05) and italics borderline significance (0.05<p<0.1)

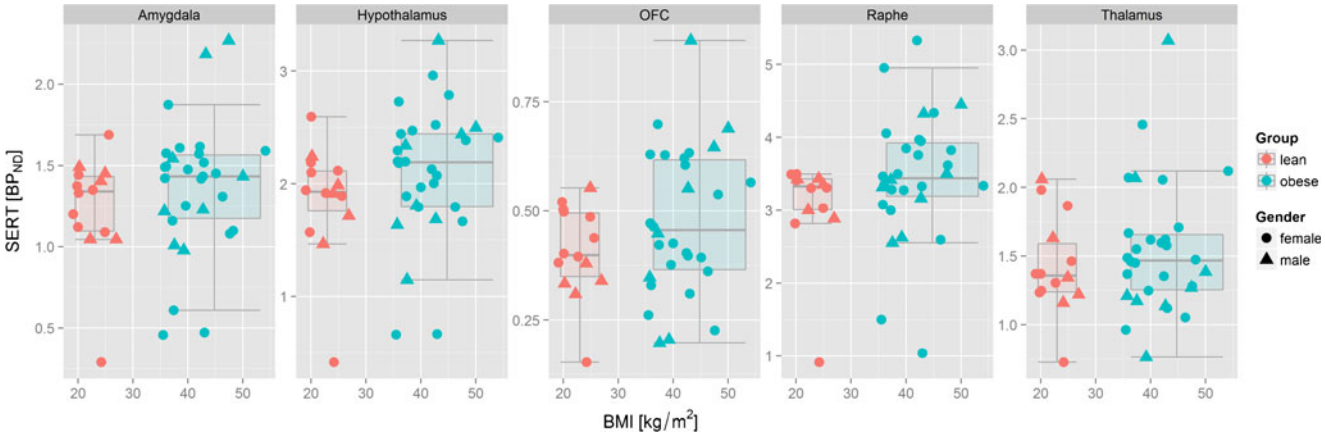

Fig. 2 Individual binding potential BP_{ND} values (correlation coefficients *r* and *p*-values are: 0.19, 0.20 for the amygdala; 0.23, 0.13 for the hypothalamus; 0.27, 0.08 for the orbito-frontal cortex, OFC; 0.23, 0.14 for the raphe; and 0.12, 0.42 for the thalamus)

Corrected model of body mass index (BMI)-associated 5-HTT binding potential (BP_{ND})

Bivariate correlative analyses indicated an effect of age (in the thalamus $r=-0.35$; $p=0.02$) and of BDI (in the OFC $r=0.30$; $p=0.05$) on BP_{ND}. Considering all covariates in an analysis of covariance, no overall significant effect on obese versus non-obese subjects were detected (Table 4). For distinct VOIs, however, covariates showed significant or borderline-significant effects, e.g., age in the dorsolateral PFC, the ACC, the thalamus, and 5-HTTLPR in the hippocampus, respectively (Table 4). By using these variables as covariates in SPM analyses, however, we found either no additional effect on the relationship between BMI and BP_{ND} or small clusters of BP_{ND}versus BMI correlation when considering BDI as covariate (Fig. 4).

Discussion

Among the various drugs available that target central biogenic amine pathways, the combined 5-HTT and norepinephrine transporter (NET) inhibitor sibutramine has been demonstrated to be the most effective for both the long-term treatment of obesity and amelioration of obesity-related health risks [2, 17]. Hence, brain 5-HTTs have been implicated in the etiology of obesity; the evidence obtained from in vivo human imaging data thus far, however, are sparse and contradictory [8–10]. This is the first study to comprehensively compare highly

Table 2 Serotonin transporter (5-HTT) binding potential (mean±SD) in obese subjects compared with non-obese controls

Volume of interest	Obese subjects (n=30)	Non-obese controls (n=14)	Levene-F (p)	ANOVA-F (p)
Medial prefrontal cortex	0.24±0.13	0.21±0.07	1.88 (0.18)	0.59 (0.45)
Orbitofrontal cortex	0.47±0.17	0.41±0.11	3.83 (0.06)	1.92 (0.17)
Dorsolateral prefrontal cortex	0.26±0.13	0.22±0.07	4.54 (0.04)	1.16 (0.29)
Anterior cingulate cortex	0.42±0.14	0.39±0.10	3.37 (0.07)	0.59 (0.45)
Insula	0.68±0.21	0.64±0.14	1.06 (0.31)	0.45 (0.50)
Hippocampus	0.59±0.27	0.53±0.15	1.33 (0.26)	0.78 (0.38)
Amygdala	1.36±0.41	1.24±0.33	0.58 (0.45)	0.98 (0.33)
Nucleus accumbens	1.60±0.44	1.60±0.35	1.58 (0.22)	0.01 (0.93)
Head of the caudate	1.48±0.36	1.43±0.38	0.01 (0.92)	0.18 (0.67)
Putamen	1.29±0.32	1.28±0.33	0.04 (0.85)	0.01 (0.96)
Thalamus	1.55±0.47	1.43±0.36	0.57 (0.46)	0.69 (0.41)
Hypothalamus	2.10±0.59	1.86±0.50	0.79 (0.38)	1.74 (0.19)
Substantia nigra/ventral tegmental area	1.62±0.25	1.55±0.46	1.58 (0.22)	1.36 (0.71)
Raphe	3.46±0.87	3.09±0.67	1.05 (0.31)	1.90 (0.18)

Levene test of homogeneity of variances; *ANOVA* analysis of variance

Table 3 Coefficients of correlation (*r,* corrected for age) and variance (COV) between serotonin transporter (5-HTT) binding potential (mean±SD) and body mass index (BMI) in each of the two sub-cohorts (obese and non-obese)

Volume of interest	Obese subjects (n=30)		Non-obese controls (n=14)	
	r	*COV*	*r*	*COV*
Medial prefrontal cortex	0.12	10.7	−0.28	6.0
Orbitofrontal cortex	0.25	11.2	−0.17	7.5
Dorsolateral prefrontal cortex	0.24	10.7	−0.23	5.9
Anterior cingulate cortex	0.29	10.0	−0.28	7.3
Insula	0.13	12.3	−0.33	8.5
Hippocampus	0.03	16.8	−0.21	9.5
Amygdala	0.21	17.3	−0.16	14.9
Nucleus accumbens	0.16	17.1	−0.21	13.5
Head of the caudate	0.08	14.7	−0.28	15.0
Putamen	0.14	14.1	−0.11	14.4
Thalamus	0.08	18.6	−0.26	14.7
Hypothalamus	0.22	18.9	−0.32	17.6
Substantia nigra/ventral segmental area	0.22	21.2	−0.25	17.9
Raphe	0.18	19.6	−0.30	16.4

Correlations are not statistically significant (p>0.05); %: between-subject variability for every region, which is defined as the coefficient of variation: COV=SD/Mean * 100 %

obese yet metabolically healthy and non-depressed individuals with non-obese healthy controls using in vivo 5-HTT brain PET. The findings obtained here indicate that there is no significant effect of BMI on regional 5-HTT availability on a group level, in line with a recent PET analysis on patients undergoing bariatric surgery [10]. In particular, there was no indication that in cortical, subcortical, or limbic regions 5-HTT availability is higher in obese subjects. Notably, the group difference was comparatively large and approaching statistical significance in the OFC, a brain region strongly activated by the appetite suppressant and 5-$HT_{1B/2C}$ receptor agonist mCPP [18].

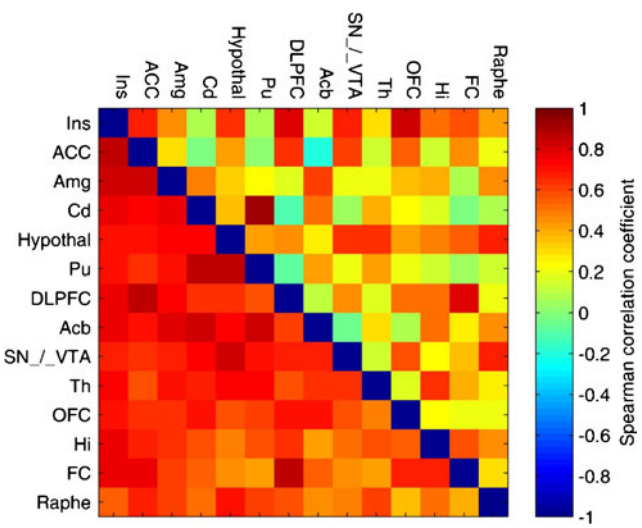

Fig. 3 Spearman rank correlation coefficients matrix of serotonin transporter (5-HTT) binding potentials between every volume of interest for the obese subjects (*lower left triangle*) and non-obese controls (*upper right triangle*). Regions are sorted based on the mean correlation coefficient per volume of interest. *Ins* insula, *ACC* anterior cingulate cortex, *Amg* Amygdala, *Cd* head of the caudate, *Hypothal* hypothalamus, *Pu* putamen, *DLPFC* dorsolateral prefrontal cortex, *Acb* nucleus accumbens, *SN_/_VTA* substantia nigra/ventral tegmental area, *Th* thalamus, *OFC* orbito-frontal cortex, *Hi* hippocampus, *FC* medial prefrontal cortex, *Raphe* raphe nuclei)

In terms of pathophysiology, a change in serotonergic tone or compensatory up-regulation of 5-HTT may determine the individual variance in response to favorite-food or stress cues, and in appetitive or defensive motivated behavior. In our previous study, the high 5-HTT availability associated with high BMI was hypothesized to lead to or result from low synaptic 5-HT levels underlying hyperphagia and weight gain [9, 19]. The higher 5-HTT availability in the OFC of obese subjects in the present study is in keeping with this theory. In contrast, a SPECT study exclusively focusing on the hypothalamus in human subjects revealed reduced 5-HTT availability that occurred within 6 weeks of consuming a high calorie diet associated with modest weight gain [20]. It is possible that these inconsistencies can be explained by the quadratic relationship between 5-HTT and 5-HT implied by the study of Haahr and colleagues [10]. Thus, expected group differences will be harder to prove and are meaningful only in relation to other parameters such as stress or behavioral responses, similar to that that has been discussed for NET [21]. We surmise that since the 5-HT system is a tonic, modulatory network with widespread serotonergic innervation from the raphe nuclei, 5-HTT availability does not necessarily simply reflect either decreases or increases in synaptic 5-HT concentrations. One can assume rather that input from internal and external sources differentially activate serotonergic tone in obese versus non-

Table 4 Results of the post-hoc univariate analysis of covariance (ANCOVA)

Model (n=44)	mPFC	OFC	dlPFC	ACC	Insula	Hippoc.	Amygd.	Acb	Caudate	Putamen	Thal.	SN/VTA	Raphe	Hypoth.
Factors														
Obese vs. non-obese	0.23	0.31	0.11	0.32	0.45	0.65	0.30	0.73	0.69	0.90	0.35	0.79	0.06	0.41
Covariates														
5-HTTLPR	0.96	0.74	0.99	0.46	0.58	**0.04**	0.65	0.81	0.47	0.95	0.63	0.30	0.18	0.16
Sex	0.15	0.69	0.18	0.25	0.67	0.45	0.18	0.33	*0.07*	0.14	0.42	0.40	0.79	0.78
Age	*0.06*	0.48	**0.03**	**0.04**	*0.08*	0.24	*0.09*	0.13	0.54	0.33	**0.01**	0.13	0.19	0.19
Smoking	0.10	0.25	0.21	0.19	0.23	0.83	0.55	0.18	0.53	0.41	0.61	0.94	0.56	0.99
BDI	0.90	0.67	0.67	0.90	0.82	0.62	0.84	0.98	0.79	0.91	0.48	0.77	0.47	0.73
Sunshine	0.28	0.78	0.30	0.27	0.64	0.52	0.41	0.53	0.92	0.87	0.49	0.97	0.95	0.78
Day length	0.95	0.94	0.99	0.98	0.51	0.53	0.73	0.92	0.94	0.78	0.76	0.81	*0.08*	0.64

P-values of between-group effects observed are presented in the table (**bold:** significant: $p<0.05$; *italics:* borderline significant: $0.05<p<0.1$)

5-HTTLPR insertion/deletion polymorphism in the 5-HTT-linked promoter region, *BMI* body mass index, *BDI* Beck depression inventor, *sunshine* sunshine duration, *mPFC* medial prefrontal cortex, *OFC* orbito-frontal cortex, *dlPFC* dorsolateral prefrontal cortex, *ACC* anterior cingulate cortex, *Hippoc*. hippocampus, *Amygd.* amygdala, *Acb* nucleus accumbens, *Caudate* nucleus caudate, *Thal* thalamus, *SN/VTA* substantia nigra/ventral tegmental area, *Hypoth* hypothalamus

obese controls which can be addressed by dedicated challenge studies when probes sensitive for endogenous 5-HT fluctuations become available.

Studies on high fat diet-resistant mice have previously shown increased 5-HTTs in the nucleus accumbens compared to diet-induced obese mice [4]. This finding was not reproduced when comparing high fat diet-resistant with diet-induced obese rats or in other brain regions including the hypothalamus [5]. Long-term manipulation of 5-HTT function in knockout (KO) animals has also yielded conflicting findings. Such animals develop paradoxical sex-specific forms of obesity not attributable to changes in feeding and which may be due to changed 5-HT signaling in the periphery [22, 23]. These findings nevertheless suggest that 5-HTTs play redundant roles in the control of feeding behavior. It seems instead that 5-HT receptors are indispensable for normal appetite regulation. For instance, 5-HT_{2C} receptor KO mice are hyperphagic and obese and when 5-HT_{2C} receptors are restored in a subpopulation of hypothalamic neurons, this leads to reversal of obesity and in re-sensitization to the anorectic effect of 5-HTT inhibitors [24]. The importance of 5-HT receptors in body-weight regulation is also supported by the consistent observations of changes in levels of 5-HT_{2A} and 5-HT_4 receptors in human obesity [6, 7].

We did not find major effects of potential covariates (sex, age, 5-HTTLPR, or seasonal data) on 5-HTT binding potential within the age range and sample size of our cohort; mainly age interferes with BP in parts of the

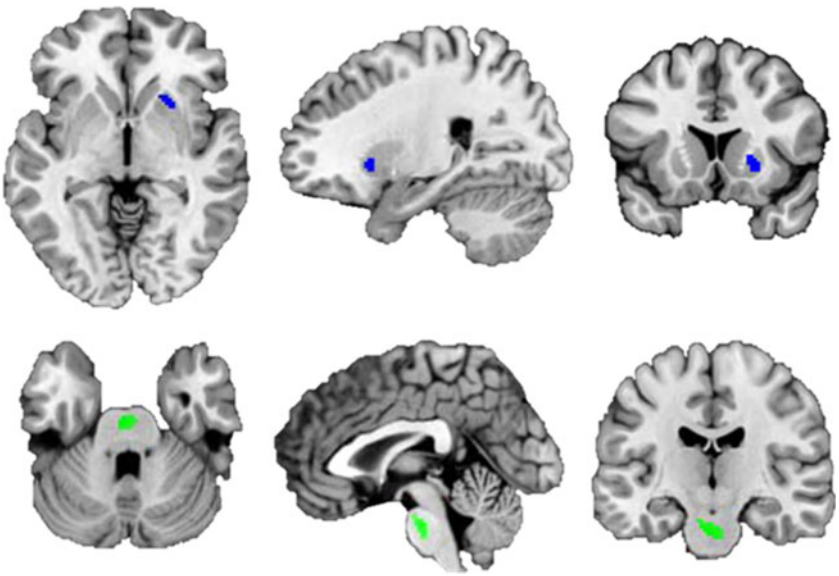

Fig. 4 Statistical parametric mapping (SPM8) projections superimposed on representative magnetic resonance imaging slices, small clusters with significant decreased values (*blue/top; putamen*) and increased values (*green/bottom; pons*) in obese subjects (n=30) compared with normal-weight (n=14) controls (using Beck depression inventory as a covariate) ($p<0.005$, uncorrected, 30 voxel/cluster minimum)

forebrain. On a voxel-level, depression was demarked by smaller clusters in the brainstem and the striatum (but not amygdala) which requires further validation with clinically depressed participants [24]. Given that the current BDI version includes items for weight loss, body shape, or libido, the validity and performance of the questionnaire for mood disorders (i.e., subsyndromal depression) in obese patients is likely to be modest [25]. Regression analysis was hampered by the fact that weight/BMI is a continuous variable but we did not include here subjects between 30 and 35 kg/m^2 due to the intended extreme cohort design of the study; thus BMI appears rather to be dichotomous and correlative analysis needs further prospective validation by including subjects with a BMI range of between 30 and 35 kg/m^2.

In order to obtain first insights on connectivity between the regions as an index of obesity beyond regional 5-HTT availability, we further inter-correlated regional BP_{ND}. This analysis revealed that there is a higher degree of correlation in the obese cohorts with a larger number of significant associations between regions as compared with non-obese controls (and possibly indicating an altered tone as well). Connectivity within this 5-HTT network seems to be significantly stronger in areas of saliency attribution and interoception with the insula and the ACC demonstrated to be robustly connected in the obese but not in the non-obese. However, we cannot rule out a specific network pattern in either obese individuals or non-obese controls as the correlation matrix was obtained solely from BP_{ND} and not from dynamic data. Future studies on network correlates may include covariant information using sparse inverse covariance estimation [26]. It is hence of further interest how the resting-state data of the study participants are modulated by individual 5-HTT availability, both at baseline and with interventions such as testing for food-cue reactivity. From functional MRI (fMRI) data an enhanced reactivity and a more effortful strategy for appetite control in obese individuals [27] corroborates the idea of higher 5-HT-mediated arousal, in particular in areas of affective-cognitive processing, in such persons. It is of further interest that the BP_{ND} of the obese group is highly correlated but still exhibits a high degree of variation (although the difference in variance is not significant). Future studies therefore will focus on this variability by entering additional parameters such as obesity-related leptin, ghrelin, and stress-related indicators.

The present findings may have implications in designing optimal treatment strategies for obesity. For instance targeting 5-HTTs may not prevent obesity development per se in susceptible individuals. However, inhibiting 5-HTTs in established obesity still remains an attractive therapeutic option. This is further supported by the recent finding that changes in subcortical 5-HTTs inversely correlate with extent of body weight loss in obese individuals after gastric bypass surgery [10]. The role of the other biogenic amines in this context are also of interest and dedicated studies (e.g., by dual-tracer approaches) warrant consideration. Additionally, serotonergic mechanisms have been implicated in obesity-related medical conditions like depression where resting-state hyperactivity in subcortical core–paracore regions was found to be related to abnormal function of the neuromodulatory systems located in the raphe nucleus and the locus coeruleus with 5-HT and NE (and other transmitters like dopamine and acetylcholine) [28, 29]. In keeping with this, preclinical findings in 5-HTT knock-out mice point to modulation of the limbic cortical-ventral striatopallidal pathway as a consequence of perturbed 5-HTT function. Thus, molecular disruption of 5-HTT that produce behavioral changes alter the functional anatomy of the reward circuitry in which all the monoamine systems are involved [30].

One further limitation in the interpretation of our study results is the fact that we did not implement a 1:1 case-cohort design, although we carefully matched obese and non-obese samples on an individual basis. Although large for a PET study, the sample size was relatively small, which may have obscured individual differences (e.g., sex, age, and 5-HTTLPR) that have been linked to important aspects of eating behaviors such as impulse control and obesity.

In conclusion, we showed no significant alterations of 5-HTT availability in obese compared with non-obese individuals but, seemingly, there is high variance of 5-HTT availability. These results may provide a useful platform upon which to further investigate 5-HT functions in human obesity.

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Compliance with ethical standards

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Conflict of interest The authors report no conflicts of interest relating to the content of this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ICH Guideline for Good Clinical Practice (GCP) and with the 1964 Helsinki declaration and its later amendments. The study was approved by the ethics committee of the Medical Faculty of the University of Leipzig (registered under the number 206-10-08032010) and the German Bundesamt für Strahlenschutz/ Federal Office for Radiation Protection (number Z5-22461-2-2011-002), and registered at the European clinical trial database EudraCT 2012-000568-32) and the German Clinical Trials Register (DRKS).

Informed consent Informed consent was obtained from all individual participants included in the study.

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IV. SUMMARY

The present work elucidates *neuroendocrine correlates of stress responsiveness in human obesity and non-obesity controls,* with focus on HPA reactivity and its potential modulation by the AVP system and central monoamine signaling. To the best of my knowledge, this cumulative thesis includes the first study to dynamically assess stress axis *responsiveness* by means of the combined dex/CRH test in obesity and the first to show a relation of HPA axis reactivity to the serum AVP tone as measured by its surrogate copeptin. A further novel finding is the association of the HPA axis and copeptin with the noradrenergic system *in vivo*. This thesis includes the largest PET study on serotonin transporter availability and connectivity in the living human brain of individuals with severe obesity.

4.1 Subjects with obesity show an enhanced HPA axis responsiveness which correlates to serum concentrations of the AVP surrogate copeptin and abdominal fat distribution

The ACTH and cortisol response of the dex/CRH test is determined by the integrity of feedback mechanisms (Holsboer, 2000; Watson *et al.*, 2006; Laryea *et al.*, 2013), the sensitivity to applied CRH (Nussey *et al.*, 1991), a co-stimulation of the HPA axis by the AVP system (de Goeij, D C *et al.*, 1992; Bardeleben *et al.*, 1985; Keck *et al.*, 2002, 2002) and the strength of the adrenocortical response to circulating ACTH (Holsboer *et al.*, 1984; Kümpfel *et al.*, 2014). In line with previous literature (Yanovski *et al.*, 1993; Pasquali *et al.*, 2002), we found *basal* ACTH and cortisol concentrations after dexamethasone suppression not to differ between the groups. The novel finding, however, is that OB subjects show a higher HPA axis *responsiveness* as measured by cortisol concentrations after CRH stimulation, and that the ACTH and cortisol curve indicators in OB seem to relate to the serum tone of the AVP-surrogate copeptin. Further, subjects with obesity tend to have a higher adrenal sensitivity to ACTH, as indicated by a lower ACTH/cortisol ratio. The HPA response increases with the waist-hip-ratio as a marker of visceral fat in *abdominal* obesity.

Our results are best explained by a pituitary-escape from dexamethasone suppression due to insufficient feedback control, a facilitation of HPA reactivity by AVP and a hypersensitivity of the adrenal glands to ACTH. The apparent HPA-copeptin relation emphasizes that not only AVP from parvocellular hypothalamic neurons stimulates the HPA axis *in vivo*, but that also magnocellular AVP released into the peripheral circulation augments HPA reactivity. The enhanced AVP tone can be measured by its surrogate copeptin, and this approach may now outline patients which are more likely to show an enhanced stress axis reactivity.

Albeit the median of post-CRH cortisol values differ between the groups, it is noteworthy, however, that the majority of subjects with obesity show a normal or only slightly enhanced cortisol reactivity in the dex/CRH test, as the right-skewed distribution in *Figure 2b* of the first article indicates (Schinke *et al.*, 2017). This underlines that an enhanced endocrine stress response is not a *conditio sine qua non* in obesity, which is in accordance with the assumption of its etiological heterogeneity (Heymsfield and Wadden, 2017).

On a morphological level, the finding of sensitized adrenal glands in OB *functionally* corroborates previous reports on enlarged adrenal glands in major depression (Nemeroff *et al.*, 1992), multiple sclerosis (Reder *et al.*, 1994) and obesity (Godoy-Matos *et al.*, 2006). This indicates a rather long-term stimulation of the adrenal cortex by ACTH in these situations (Hoeflich and Bielohuby, 2009), thereby pointing towards chronic HPA axis hyperactivity in the OB cohort. The association of an enhanced HPA activity with an increasing WHR but not with the BMI supports previous findings (Porzezińska-Furtak *et al.*, 2014), and emphasizes the necessity to better understand this relation since a high WHR is a stronger predictor of cardiovascular mortality than the BMI *per se* (Despres and Lemieux, 2006; Katzmarzyk *et al.*, 2012; Tchernof and Després, 2013).

Programming effects on the HPA axis which may lead to its dysregulation occur throughout the whole lifespan. Exposure to cortisol or repeated stress during susceptible developmental stages - prenatally, the childhood and adolescence - leads to long-lasting increases of stress-induced glucocorticoid secretion (Lupien *et al.*, 2009). A genetic pre-determination is suspected since healthy first-degree relatives of patients with major depression show higher cortisol reactivity in the dex/CRH test than individuals without familial risk (Holsboer *et al.*, 1995), and epigenetic modifications moderate the association of life trauma and cortisol reactivity (Houtepen *et al.*, 2016). Despite this long-term imprinting effects of internal and environmental factors, HPA axis responsiveness is modifiable by pharmacotherapy (Then Bergh *et al.*, 2001; Schule *et al.*, 2006), and a few studies investigated the effect of lifestyle interventions (Sarubin *et al.*, 2014b; Deuschle *et al.*, 2017) or the influence of dietary behavior (Dallman *et al.*, 2005; Tryon *et al.*, 2015; Hryhorczuk *et al.*, 2017). This is of interest since the unspecific *endophenotype* of the dysregulated HPA axis has clinical implications, predisposing to physical health conditions such as diabetes and cardiovascular disease (Pasquali *et al.*, 2006; Bose *et al.*, 2009; Incollingo *et al.*, 2015; Jackson *et al.*, 2017), as well as to depressive and anxiety-like behaviors (Erhardt *et al.*, 2006; Lupien *et al.*, 2009). It is to be noted that these conditions more frequently co-occur in obesity (Incollingo *et al.*, 2015). However, our cohort had a higher prevalence of an enhanced cortisol reactivity but without signs of clinically relevant psychiatric disorders. It is hence to be speculated if unhealthy behaviors such as the consumption of highly palatable *rewarding* food constitutes a resilience factor relieving from endocrine stress upon chronic stimulation as a form

of self-medication (Dallman *et al.*, 2005; Adam and Epel, 2007; Jackson *et al.*, 2010), of which higher calorie intake and obesity are the flip-side of the coin.

4.2 HPA axis responsiveness and copeptin concentrations are differentially related to central NAT availability in subjects with obesity compared to non-obesity controls

We found hints for a dysregulation of the HPA axis which is related to enhanced copeptin concentrations as a serum marker of the AVP system in obesity (Schinke *et al.*, 2017). To further elucidate the neurobiological underpinnings of this endophenotype, we assessed the brain noradrenaline system as a likely modulator of the stress response. Our results indicate an association between HPA axis responsiveness and central noradrenergic signaling, with a prefrontal-limbic control of HPA reactivity in NOC versus a loss of this relation in favor of a hypothalamic-centered relation in OB.

Only one previous study investigated central NAT availability in obesity, hinting to decreased NAT binding potentials in the thalamus of obese subjects, suggesting a noradrenergic dysfunction (Li *et al.*, 2014). In the hypothalamus as a regulation center of appetite, hunger, satiety and endocrine function, however, no differences could be detected, and the finding of changed NAT availability in OB could not be replicated by our working group (Hesse *et al.*, 2017). An interesting additional finding, however, was that Hesse *et al.* reported OB subjects to have a higher symmetry of NAT availability among the hemispheres, especially in the putamen, and higher inter-regional associations as the correlative matrix of NAT BP_{ND} revealed (Hesse *et al.*, 2017). The latter also held true for the connectivity of the serotonin transporters in OB, as discussed below (Hesse *et al.*, 2015). This indicates subtle changes in the monoamine system, and that a higher lateralization of monoamine transporter availability is a characteristic of the brain of non-obese individuals.

There was one previous study which investigated the HPA response in relation to in vivo monoamine signaling, but with focus on the brain's *serotonin system* in depressed individuals. A *negative* association between SERT availability and HPA reactivity was found, centered in the thalamus (Reimold *et al.*, 2011).

The NAT regions we found to be involved in HPA axis modulation are best explained by anatomic studies on the noradrenaline-endocrine stress network: Ascending noradrenergic fibers project from the Locus coeruleus to the cortex and amygdala, controlling the level of alertness and arousal (Samuels and Szabadi, 2008). The prefrontal cortex is a highly evolved brain region regulating thought and emotion, and is extensively connected with subcortical structures inhibiting the HPA axis indirectly (Aihara *et al.*, 2007; Ulrich-Lai and Herman, 2009; Arnsten, 2009), whereas the amygdala, a center

regulating fear and anxiety, probably affects the HPA axis more directly via intervening hypothalamus-projecting neurons (Ulrich-Lai and Herman, 2009). Interestingly, the expected relation of the HPA response to the noradrenergic prefrontal cortex could not be shown in the obesity group, in which we reported a dysregulation of the HPA and neurohypophyseal axes. Previous literature provides strong hints that especially chronic uncontrollable stress impairs prefrontal cortex function which may lead to a disinhibition of the stress response network. It is to be speculated if the enhanced HPA axis responsiveness we found in OB also constitutes such a type of chronic *endocrine* stress, or if it is rather just the *endocrine result* of chronic perceived distress in obesity. Either way, our findings indicate a switch from the normal *top-down* control of HPA axis activity by the forebrain and limbic system in NOC towards a *bottom-up* control of HPA reactivity in OB, driven by the hypothalamus (Radant *et al.*, 1992; Robbins and Arnsten, A F T, 2009; Farr *et al.*, 2016).

A potential pitfall of the NAT PET approach is the fact that *in vivo* it is not entirely clear yet how NAT actually relates to the concentration or turnover of NA in the synaptic cleft. The classic point of view rather supports the notion of NAT to homeostatically adapt to NA concentrations in order to normalize NA transmission than the other way around (Lee *et al.*, 1983). Another missing piece is how NAT relates to NA *receptors* (or even more downstream, to their affinity to NA, its intracellular signal cascades and so forth), which cannot be addressed *in vivo* yet due to the lack of suitable radiotracers (Finnema *et al.*, 2015). Such knowledge would enable a better understanding of actual NA activity.

In a nutshell, our cross-sectional approach highlights two findings: *First*, the stress response and copeptin are linked with noradrenergic activity, which has not been shown in vivo. *Second*, the observation that the HPA-AVP axes are related to NAT in different brain regions supports the hypothesis on changes of stress regulating systems in obesity.

4.3 Central serotonin transporter availability does not significantly differ in subjects with obesity compared to their non-obesity counterparts

Various drugs targeting monoamine transporters or receptors showed clinical efficacy achieving weight loss in subjects with obesity (Hainer *et al.*, 2006; Smith *et al.*, 2010) or were lately proven to decrease the incident of metabolic complications such as diabetes (Bohula *et al.*, 2018). Based on these clinical observations and well-established models of the role of serotonin in the regulation of energy homeostasis (Breisch *et al.*, 1976), altered 5-HT(T) signaling would be expected in obesity. The few previous studies on the *in vivo* situation of SERT availability in OB, however, revealed inconsistent findings (Kuikka *et al.*, 2001; Erritzoe *et al.*, 2010a; Hesse *et al.*, 2015; Haahr *et al.*, 2015).

Ours was the largest PET study to investigate serotonin transporter availability in non-depressed subjects with severe obesity. In this exploratory approach, unexpectedly, no differences of 5-HTT BP_{ND} were found on a group level. Possibly, there are simply no changes of serotonergic signaling in OB, and weight loss success upon SERT inhibitors is solely based on a post-synaptic 5-HT_{2C}, 5-HT_{2A} or 5-HT_4 receptor stimulation, decreasing the motivation for food intake (Jean *et al.*, 2007; Erritzoe *et al.*, 2009; Halford and Harrold, 2012; Valencia-Torres *et al.*, 2017). However, a complicating factor studying the brain serotonin system is the non-linear relation of the pre-synaptic serotonin transporter to its post-synaptic receptor which is characterized by an inverted U-shape (Erritzoe *et al.*, 2010b). This makes it more sophisticated to detect changes in serotonergic signaling if only one determinant is investigated. Moreover, there is quite convincing evidence that lowered central SERT levels predispose to obesity: in SERT knockout mice, locomotor activity is decreased which leads to late onset obesity (Uceyler *et al.*, 2010), and carriers of the short SLC6A4 allele of the SERT promotor region are prone to overweight in adolescence (Sookoian *et al.*, 2007) and adulthood (Sookoian *et al.*, 2008). Further, on an epigenetic level, the methylation status of the serotonin transporter promoter is associated with an attenuated reward sensitivity in obese subjects (Drabe *et al.*, 2017) which may reinforce a hedonic drive for highly palatable food. Analogous to the various diverging SERT PET studies performed in major depression, a similar etiological and symptomatic heterogeneity is observed in obesity (Heymsfield and Wadden, 2017), and may contribute to the high variance of serotonergic signaling. This underlines the need for a more accurate stratification of patients into different clinical subsets facilitating future research (Spies *et al.*, 2015).

Albeit our study failed to detect inter-group differences of SERT BP_{ND}, *associative patterns* were not the same between the groups: the correlation matrix revealed a higher inter-regional connectivity for SERT, which corroborates analogues findings of a higher inter-hemispheric symmetry for NAT in OB (Hesse *et al.*, 2017). This indicates different inter-regional associations in NOC compared with OB, hinting towards changes of SERT on a network level. Interestingly, this correlative SERT network pattern is modifiable by pharmacotherapy, as it was shown in a longitudinal PET study including patients with major depressive disorder who were followed up after SSRIs treatment (James *et al.*, 2017). If future studies prove the observed network changes to be of clinical relevance in OB, e.g. predicting weight loss *failure* despite lifestyle interventions, it may constitute an easily accessible target for pharmacological treatment.

4.4 Future direction

In summary, we found hints for dysregulated HPA axis responsiveness in OB that is associated with copeptin as a serum marker of the AVP system. These stress axes seem to be differentially linked to the central noradrenaline system in OB compared to NOC. From the cross-sectional approach of our study, we cannot tell if HPA dysregulation is cause or consequence of obesity. However, this endocrine phenotype has previously been shown to be associated with physical and mental illness in other entities (Heuser *et al.*, 1994; Then Bergh *et al.*, 1999), which raises the need to better characterize the subset of OB patients with pronounced HPA reactivity – e.g., with respect to their propensity to develop affective disorders, or their metabolic risk profile of inflammatory markers, blood glucose and lipids. The observed association of HPA reactivity with the WHR, a strong risk factor of morbidity and mortality in OB, already hints to a pathophysiological link of HPA axis dysregulation with metabolic complications. Since HPA reactivity is modifiable by pharmacotherapy and potentially by lifestyle interventions (Then Bergh *et al.*, 2001; Schule *et al.*, 2006; Wirtz *et al.*, 2010; Sarubin *et al.*, 2014a), it constitutes a promising target for experimental treatment strategies in susceptible individuals.

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6.2 Publications

A case report of delayed cortical infarction adjacent to sulcal clots after traumatic subarachnoid hemorrhage in the absence of proximal vasospasm.

Schinke C, Horst V, Schlemm L, Wawra M, Scheel M, Hartings JA, Dreier JP.

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Central noradrenaline transporter availability is linked with HPA axis responsiveness and copeptin in human obesity and non-obese controls.

Schinke C, Hesse S, Rullmann M, Becker GA, Luthardt J, Zientek F, Patt M, Stoppe M, Schmidt E, Meyer K, Meyer PM, Orthgieß J, Blüher M, Kratzsch J, Ding YS, Then Bergh F, Sabri O.

Stress. 2018 Oct 29:1-10. doi: 10.1080/10253890.2018.1511698.

Post-dexamethasone serum copeptin corresponds to HPA axis responsiveness in human obesity.

Schinke C, Hesse S, Stoppe M, Meyer K, Schmidt E, Orthgiess J, Bechmann L, Bresch A, Rullmann M, Luthardt J, Sabri O, Blüher M, Kratzsch J, Then Bergh F.

Psychoneuroendocrinology. 2017 Apr;78:39-47. doi: 10.1016/j.psyneuen.2017.01.004. Epub 2017 Jan 16.

Serotonin transporter gene promoter methylation status correlates with in vivo prefrontal 5-HTT availability and reward function in human obesity.

Drabe M, Rullmann M, Luthardt J, Boettcher Y, Regenthal R, Ploetz T, Becker GA, Patt M, Schinke C, Then Bergh F, Zientek F, Hilbert A, Bresch A, Fenske W, Hankir MK, Sabri O, Hesse S

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Central serotonin transporter availability in highly obese individuals compared with non-obese controls: A [(11)C] DASB positron emission tomography study.

Hesse S, Rullmann M, Luthardt J, Winter K, Hankir MK, Becker GA, Zientek F, Reissig G, Regenthal R, Drabe M, Schinke C, Bresch A, Arelin K, Lobsien D, Patt M, Meyer PM, Fasshauer M, Fenske WK, Blüher M, Stumvoll M, Sabri O.

Eur J Nucl Med Mol Imaging. 2016 Jun;43(6):1096-104. doi: 10.1007/s00259-015-3243-y. Epub 2015 Nov 18.

6.3 Scientific contribution of the doctoral candidate to the publications

Erklärung über den wissenschaftlichen Beitrag des Promovenden zur Publikation

Folgende wissenschaftliche Veröffentlichungen sind die Grundlage der von mir, Christian Schinke, vorgelegten Dissertation:

Post-dexamethasone serum copeptin corresponds to HPA axis responsiveness in human obesity. Schinke et al., *Psychoneuroendocrinology*. 2017 Apr;78:39-47.

Central noradrenaline transporter availability is linked with HPA axis responsiveness and copeptin in human obesity and non-obese controls. Schinke/Hesse et al., *Stress*. 2018 Oct 29:1-10.

Central serotonin transporter availability in highly obese individuals compared with non-obese controls: A [(11)C] DASB positron emission tomography study. Hesse et al., *Eur J Nucl Med Mol Imaging*. 2016 Jun;43(6):1096-104.

Für die beiden erstgenannten Arbeiten führte ich die Experimente im Rahmen des Dexamethason-CRH Testes eigenständig durch, erhob und analysierte die Daten inklusive ihrer statistischen Auswertung und graphischer Darstellung und verfasste und überarbeitete die Manuskripte. In drittgenannter Studie von Prof. Dr. Swen Hesse leistete ich einen Beitrag zur Verfassung und Überarbeitung des Manuskriptes.

Christian Schinke | Prof. Dr. Florian Then Bergh | Prof. Dr. Swen Hesse

Dr. Julia Luthardt | Dr. Georg-Alexander Becker | Dr. Johannes Orthgieß

Dr. Michael Rullmann | Elisa Schmidt | Klara Meyer

6.4 Declaration of the independent writing of this thesis

Erklärung über die eigenständige Abfassung der Arbeit

Hiermit erkläre ich, dass ich die vorliegende Arbeit selbstständig und ohne unzulässige Hilfe oder Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe. Ich versichere, dass Dritte von mir weder unmittelbar noch mittelbar eine Vergütung oder geldwerte Leistungen für Arbeiten erhalten haben, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen, und dass die vorgelegte Arbeit weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt wurde. Alles aus anderen Quellen und von anderen Personen übernommene Material, das in der Arbeit verwendet wurde oder auf das direkt Bezug genommen wird, wurde als solches kenntlich gemacht. Insbesondere wurden alle Personen genannt, die direkt an der Entstehung der vorliegenden Arbeit beteiligt waren. Die aktuellen gesetzlichen Vorgaben in Bezug auf die Zulassung der klinischen Studien, die Bestimmungen des Tierschutzgesetzes, die Bestimmungen des Gentechnikgesetzes und die allgemeinen Datenschutzbestimmungen wurden eingehalten. Ich versichere, dass ich die Regelungen der Satzung der Universität Leipzig zur Sicherung guter wissenschaftlicher Praxis kenne und eingehalten habe.

Berlin, den 06.03.2019 Christian Schinke

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