Improving metabolic control in humans by intranasal neuropeptide administration

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Um, so, we asked ourselves, internally we asked ourselves over here, "okay, what does a pancreas do?" And the answer was, "does it make pirates?" No, it makes insulin, you know?

Alejandro, *Rick and Morty (2013)*

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Abstract

The prevalence of obesity, defined as a body mass index (BMI) $\geqslant 30$, has been rising on a worldwide scale over recent decades. Obesity and the metabolic syndrome are associated with health impairments, e.g., insulin resistance and hypothalamic-pituitary-adrenal (HPA) axis hyperactivity, as well as reduced quality of life. Therefore, the physiological systems that regulate energy intake and body weight are of great interest not only to basic research but also as possible targets of interventions to improve metabolic control. Several endogenous neuropeptides released in association with metabolic function have been investigated for their potential to block orexigenic (appetite-stimulating) and to promote anorexigenic (appetite-suppressing) signaling cascades in the brain as well as for their insulin-sensitizing and stress-regulating properties. The present thesis aims at characterizing the role of three of these neuropeptides, i.e., oxytocin, insulin, and orexin A, in the control of food intake, HPA axis activity, and, respectively, glucose metabolism in humans. For these investigations, the intranasal administration paradigm is the method of choice because it enables neuropeptides to bypass the blood-brain barrier and reach the central nervous system quickly, efficiently, and non-invasively while peripheral uptake is minimized. Three studies were performed in which we investigated the impact of oxytocin via the intranasal route (24 IU) on eating behavior and metabolic function in obese men (mean BMI of 32.10) in comparison to normal-weight men (mean BMI of 22.66; Study I), the influence of intranasal

insulin administration (160 IU) in elderly (70.0 years) and young subjects (23.6 years) on early-sleep nadir concentrations of adrenocorticotropin and cortisol, i.e., indicators of baseline HPA axis activity (Study II), and the acute glucoregulatory effect of intranasal orexin A administration (500 nmol) during an oral glucose tolerance test in healthy young men (Study III). In Study I, intranasal oxytocin exerted an acutely inhibitory impact on food intake that was enhanced in obese compared with normal-weight men in that hunger- as well as reward-driven eating was attenuated by oxytocin in obese participants, whereas normal-weight subjects only showed a reduction in reward-driven snack intake. HPA axis secretion and the postprandial rise in plasma glucose were blunted by oxytocin in both groups. In Study II, intranasal insulin reduced cortisol levels during early sleep in elderly but not young participants, indicating that central nervous insulin can act as an inhibitory signal in basal HPA axis activity regulation. In particular, insulin may normalize sleep-associated HPA axis activity in elderly subjects who show relatively increased nadir values of stress hormones during early sleep. In Study III, intranasal orexin A attenuated the peak excursion of plasma glucose and lactate levels and blunted the initial increases in insulin and C-peptide concentrations in response to a glucose challenge, findings that are in line with respective animal studies. Taken together, these results indicate that oxytocin, insulin, and orexin A, in addition to their well-characterized role in other organ systems and bodily functions, contribute to the regulation of, respectively, feeding behavior, stress axis activity, and glucose homeostasis in humans. These results bode well for potential clinical applications of oxytocin, insulin, and orexin A in the treatment of metabolic disorders.

Zusammenfassung

Die Prävalenz von Adipositas, definiert als Body Mass Index (BMI) $\geqslant 30$, hat weltweit in den letzten Jahrzehnten kontinuierlich zugenommen. Adipositas und das Metabolische Syndrom sind mit gesundheitlichen Beeinträchtigungen (z. B. einer Insulinresistenz und überhöhter Hypothalamus-Hypophysen-Nebennierenrinden-(HHN)-Achsenaktivität) sowie einer verminderten Lebensqualität verbunden. Deshalb sind die physiologischen Mechanismen, die die Energiehomöostase und das Körpergewicht regulieren, nicht nur für die Grundlagenforschung sondern auch im Rahmen der medikamentösen Therapieansätze bei Adipositas von großem Interesse. In diesem Zusammenhang wurden verschiedene endogene Neuropeptide, die an der metabolischen Regulation beteiligt sind, hinsichtlich ihres Potenzials, orexigene (appetitsteigernde) Signalkaskaden im Gehirn zu blockieren bzw. anorexigene (appetitmindernde) zu verstärken sowie ihrer insulinsensitivierenden und stressregulierenden Eigenschaften untersucht. Die vorliegende Arbeit zielt darauf ab, die Rolle dreier Neuropeptide – Oxytocin, Insulin und Orexin A – in der Steuerung des Essverhaltens, der HHN-Achsenaktivität und des Glukosestoffwechsels beim Menschen zu charakterisieren. Um dies zu untersuchen, hat sich die intranasale Applikation als praktikabler Ansatz erwiesen, weil er die Blut-Hirn-Schranke umgeht und dem Gehirn Neuropeptide schnell, effektiv und nicht-invasiv zuführen kann, während die periphere Aufnahme dabei minimiert wird. Es wurden drei Studien durchgeführt, in denen wir

einerseits die Auswirkung von intranasal verabreichtem Oxytocin (24 IE) auf das Essverhalten und den Metabolismus bei adipösen Männern (mittlerer BMI von 32.10) im Vergleich zu normalgewichtigen männlichen Probanden (mittlerer BMI von 22.66) untersuchten (Studie I). Des Weiteren wurde der Einfluss von intranasalem Insulin (160 IE) bei älteren (im Mittel 70.0 Jahre) und jungen Probanden (im Mittel 23.6 Jahre) auf die frühnächtlichen Nadir-Konzentrationen von Adrenocorticotropin und Cortisol, welche Indikatoren der basalen HHN-Achsenaktivität sind (Studie II), sowie die glucoregulatorische Wirkung von intranasal appliziertem Orexin A (500 nmol) während eines oralen Glukosetoleranztests bei gesunden, jungen Männern untersucht (Studie III). In Studie I wurde die Nahrungsaufnahme insbesondere bei adipösen im Vergleich zu normalgewichtigen Männern durch die intranasale Gabe von Oxytocin gehemmt. Adipöse Probanden zeigten eine Reduktion im hunger- und belohnungsgesteuerten Essverhalten, wobei normalgewichtige Probanden lediglich eine Reduktion im belohnungsassoziierten Snackverzehr aufwiesen. Die Sekretion der HHN-Achse sowie der postprandiale Glukoseanstieg im Plasma wurden in beiden Gruppen durch Oxytocin gesenkt. In Studie II senkte intranasal verabreichtes Insulin die frühnächtlichen Cortisolspiegel bei älteren Probanden, nicht aber bei jungen Studienteilnehmern, was darauf hinweist, dass die zentralnervöse Insulinsignalübertragung die basale HHN-Achsenaktivität hemmt. Insbesondere kann intranasales Insulin dazu beitragen, die schlaf-assoziierte Stress-Achsenaktivität bei älteren Probanden zu normalisieren, welche erhöhte frühnächtliche Nadir-Konzentrationen von Stresshormonen aufweisen. In Studie III führte die intranasale Gabe von Orexin A zu einer Senkung der Glukosespitzen und Laktatspiegel und schwächte die anfänglichen Insulin- und C-Peptid-Ausschüttungen als Reaktion auf die Glukosebelastung ab. Diese Befunde stimmen mit jenen aus tierexperimentellen Studien überein. Zusammengefasst zeigen die Ergebnisse, dass Oxytocin, Insulin und Orexin A, neben dem bereits gut untersuchten Einfluss auf andere Organsysteme und Körperfunktionen, an der Regulation des Essverhaltens, der Stressachsenaktivität und der Glukosehomöostase

beim Menschen beteiligt sind. Die Ergebnisse können hierbei zur Erkundung des therapeutischen Potenzials von Oxytocin, Insulin und Orexin A in der Behandlung von Stoffwechselstörungen beitragen.

Contents

List of Relevant Publications

This dissertation is based on the following research papers:

I **Thienel, M.**, Fritsche, A., Heinrichs, M., Peter, A., Ewers, M., Lehnert, H., Born, J., and Hallschmid, M. (2016). Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men. *International Journal of Obesity*, 40(11):1707–14.

II **Thienel, M.**, Wilhelm, I., Benedict, C., Born, J., and Hallschmid, M. (2017). Intranasal insulin decreases circulating cortisol concentrations during early sleep in elderly humans. *Neurobiology of Aging*, 54:170–4.

III **Thienel, M.**, Elsässer, T., Lamprinou, A., Klement, J., Peter, A., and Hallschmid, M. (2017). Intranasal orexin A acutely improves glucose tolerance in healthy men. *in preparation*

List of Figures

List of Tables

Abbreviations

α-MSH	α-melanocyte-stimulating hormone
ACTH	Adrenocorticotropic hormone
AgRP	Agouti-related peptide
ANCOVA	Analysis of covariances
ANOVA	Analysis of variances
ARC	Arcuate nucleus
ASD	Autism spectrum disorder
AUC	Area under the curve
BBB	Blood-brain barrier
BMI	Body Mass Index
BPD	Borderline personality disorder
BST	Bed nucleus of the stria terminalis
CART	Cocaine and amphetamine-regulated transcript
CCK	Cholecystokinin
CNS	Central nervous system
CRH	Corticotropin-releasing hormone
CSF	Cerebrospinal fluid
DEBQ	Dutch Eating Behavior Questionnaire
FDA	Food and Drug Administration
GLP-1	Glucagon-like peptide-1
HPA	Hypothalamic-pituitary-adrenal

ICD	International Classification of Diseases
ICV	intracerebroventricular
IDF	International Diabetes Federation
IE	Internationale Einheit
IN	intranasal
IR	Insulin receptor
IU	International Unit
IV	intravenous
LHA	Lateral hypothalamic area
Log*P*	Logarithm of the partition coefficient
MC3R	Melanocortin-3 receptor
MC4R	Melanocortin-4 receptor
MCH	Melanin-concentrating hormone
MDBF	Mehrdimensionaler Befindlichkeitsfragebogen (Multidimensional Mood State Questionnaire)
mRNA	Messenger ribonucleic acid
NAcc	Nucleus accumbens
NEFA	Non-esterified fatty acid
NGT	Normal glucose tolerance
NPY	Neuropeptide Y
NTS	Nucleus of the solitary tract
OGTT	Oral glucose tolerance test
OX1R	Orexin receptor type 1
OX2R	Orexin receptor type 2
OXTR	Oxytocin receptor
PFA	Perifornical area
PFS	Power of Food Scale
POMC	Proopiomelanocortin
PVN	Paraventricular nucleus
$PYY_{3\text{-}36}$	Peptide $YY_{3\text{-}36}$
REM	Rapid eye-movement

rPFC	Right prefrontal cortex
SAD	Social anxiety disorder
SC	subcutaneous
SCN	Suprachiasmatic nucleus
SGLT1	Sodium-glucose linked transporter 1
SON	Supraoptic nucleus
TRH	Thyrotropin-releasing hormone
VAS	Visual analog scale
VMH	Ventromedial nucleus of the hypothalamus
VTA	Ventral tegmental area
WHO	World Health Organization

1 Introduction

1.1 Obesity and metabolic syndrome on the rise

Over the last forty years, obesity and hyperalimentation (overeating) have become a major public health concern that is being increasingly seen throughout the world (Finucane et al., 2011) and are strongly associated with health impairment and reduced quality of life (Taylor et al., 2013). The World Health Organization (WHO) reported that more than 1.9 billion of the adult population worldwide was overweight or obese in 2014, i.e., exhibiting a body mass index (BMI) $\geqslant 25\,\mathrm{kg/m^2}$ (WHO, 2015). In Germany, 55 % of adults are currently overweight (BMI 25.0 to $29.9\,\mathrm{kg/m^2}$), and another 20 % are obese (BMI $> 30\,\mathrm{kg/m^2}$) (WHO, 2015). The interpretation of BMI gradings in relation to health risk, i.e., increased mortality and chronic morbidity, is proposed in Table 1.1.

Obesity independently contributes to a cluster of risk factors that commonly appear together and have been termed the 'metabolic syndrome.' The metabolic syndrome found its way into the International Classification of Diseases (ICD-10; code E88.9), but it is not unambiguously defined. Different metabolic disturbances including impaired glucose metabolism, dyslipidemia (a condition characterized by an abnormal amount of blood lipids), high blood pressure, and visceral obesity are subsumed under the medical term metabolic syndrome (Alberti et al., 2009), which eventually leads to an increased risk

Table 1.1 Classification of overweight in adults by the WHO.

Classification	BMI	Associated health risks
Underweight	< 18.5	Low (but risk of other clinical problems increased)
Normal range	18.5–24.9	Average
Overweight	25.0 or higher	
Preobese	25.0–29.9	Increased
Obese class I	30.0–34.9	Moderately increased
Obese class II	35.0–39.9	Severely increased
Obese class III	40.0 or higher	Very severely increased

Body mass index (BMI) values are both age- and sex-independent. Note that BMI may not correspond to the same degree of adiposity in different populations due, in part, to differences in body proportions. The risks associated with increasing BMI are continuous and graded and begin at a BMI above 25. However, for calculating the risk of obesity comorbidites, a measure of fat distribution (body fat, waist circumference or waist-to-hip ratio) must be consulted in addition to BMI (adapted from WHO, 2000).

of cardiovascular disease and type 2 diabetes mellitus (Kaur, 2014). According to estimates of the International Diabetes Federation (IDF), 8.8 % of the world's population suffer from diabetes in 2015; this number is projected to increase to 10.4 % by 2040 (IDF, 2015).

Associated with obesity, impaired insulin sensitivity or insulin resistance, respectively, appear as critical factors in the pathophysiology of the metabolic syndrome (Haffner et al., 1992; Hu et al., 2004). Insulin resistance is defined as a pathological condition in which cells fail to respond normally to the insulin-mediated glucose disposal into skeletal muscle and other tissues. As a consequence, glucose remains in the blood, triggering the need for more and more insulin (hyperinsulinemia) to be produced in an attempt to process the glucose (Lebovitz, 2001). Hyperinsulinemia is also involved in obese hypertension by increasing renal sodium retention (Modan et al., 1985).

Prolonged chronic (psychosocial) stress is discussed as a further critical factor in the pathogenesis for metabolic syndrome by perturbation of the hypothalamic-pituitary-adrenal axis (HPA axis, see section 1.2.2) manifested in raised basal cortisol and corticotropin-releasing hormone (CRH) levels (Brunner et al., 2002; Gohil et al., 2001; Kaufman et al., 2007). The glucocorticoid hormone cortisol raises blood pressure (Whitworth et al., 1984), stimulates hepatic gluconeogenesis (generation of glucose) (Argaud et al., 1996), promotes adipogenesis (adipocyte differentiation) (Hauner et al., 1989), inhibits insulin-stimulated glucose utilization by adipocytes (Livingston and Lockwood, 1975), and increases lipolysis or non-esterified fatty oxidation, which is associated with peripheral insulin resistance (Guillaume-Gentil et al., 1993). Excessive levels of cortisol over extended periods of time, so called chronic hypercortisolism, therefore consequently promotes insulin resistance, hypertension, and visceral obesity (Tsigos and Chrousos, 2002). Thus, HPA axis dysregulations contribute significantly to the reported risk indication of obesity to cardiovascular disease and type 2 diabetes (Rosmond and Björntorp, 2000).

Considering the multifactorial pathogenesis of obesity and metabolic syndrome, respectively, three major risk domains can be identified: genetics (i.e., heredity), lifestyle habits (e.g., eat high-caloric foods) and medical factors (e.g., psychological issues/stress, age; Marti et al., 2004). The present thesis focuses on distinct risk determinants covering metabolic as well as behavioral aspects, i.e., food intake, stress axis regulation, and insulin sensitivity in particular. Unhealthy diet, chronic stress, and disturbed insulin sensitivity as pivotal risk determinants in the development of obesity present conventional therapeutic programmes with huge challenges on the one hand but also appear to offer a wide range of possibilities for new additional and supportive interventions on the other. For instance, several endogenous peripheral and central neuropeptides released in the context of eating have already been investigated as potential treatments for obesity, e.g., blocking orexigenic peptide signals such as neuropeptide Y and melanin-concentrating hormone, and are still be-

ing explored (Boughton and Murphy, 2013). Improving metabolic function by intranasal administration of neuropeptides involved in metabolic control may be an innovative approach in the treatment of metabolic ailments (Chapman et al., 2013; Spetter and Hallschmid, 2015). In the present thesis, the role of the pancreatic hormone insulin and the hypothalamic neuropeptides oxytocin and orexin A in food intake, stress axis regulation, and glucose metabolism as well as their therapeutic potential shall be further elucidated.

1.2 Neuroendocrine regulation of metabolic function

The hypothalamus is recognized as the main region in the brain regulating food intake by a complex integration of peripheral and central neuropeptide signals, such as leptin, insulin, ghrelin, orexins, oxytocin, and through the sensing of nutrients, such as glucose, amino acids, and fatty acids (Blouet and Schwartz, 2010; Coll et al., 2007). Besides hypothalamic nuclei regulating hunger and satiety, several limbic and cortical brain regions regulate energy intake and are critically involved in the pathophysiology of obesity (Petrovich et al., 2005). The following sections give a brief and basic overview of the brain's role in the control of body weight regulation.

1.2.1 Central nervous regulation of food intake and glucose metabolism: homeostatic and non-homeostatic pathways

Body weight, energy expenditure, and food intake are tightly regulated by complex homeostatic mechanisms. Besides local paracrine actions and peripheral endocrine effects mediated through the bloodstream, especially central circuits are significantly involved in the control of metabolic processes. Peripheral, humoral signals indicating information about nutritional status

interact with several neural signals, e.g., neuropeptides, monoamines, and endocannabinoids in the brain (Valassi et al., 2008). The main regions and pathways regulating food consumption are illustrated in Figure 1.1.

Cholecystokinin (CCK), peptide $YY_{3\text{-}36}$ ($PYY_{3\text{-}36}$), glucagon-like peptide-1 (GLP-1), and ghrelin, are referred to as 'satiety signals' generated in the gastrointestinal tract during food intake. These signals reach the caudal brainstem nucleus of the solitary tract (NTS) via the vagus nerve, where they influence meal size. Afferent nerves from NTS project to the arcuate nucleus (ARC), the primary hypothalamic area involved in the control of food intake. The ARC, contains two distinct neuropeptidergic pathways with interconnected but functionally discrete populations of 'first-order' neurons. One group synthesizes the anorexigenic prepropeptide pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). The precursor polypeptide POMC is cleaved into α-melanocyte-stimulating hormone (α-MSH) which binds to melanocortin-3 and -4 receptors (MC3R and MC4R) on neurons in other hypothalamic areas and elsewhere in the brain (Fan et al., 1997). The second group of arcuate neurons co-express the orexigenic molecules neuropeptide Y (NPY) and agouti-related peptide (AgRP). AgRP serves as a non-selective endogenous antagonist of MC3R and MC4R which thereby reduces the anorexigenic effect of α-MSH (Rossi et al., 1998).

The ARC also receives peripheral information via 'adiposity signals,' i.e., leptin and insulin, secreted by white adipose tissue and the pancreas, respectively. Leptin and insulin circulate in the blood at concentrations proportionate to body-fat mass and enter the brain independently via saturable mechanisms (Banks et al., 1996; Baura et al., 1993). When these adiposity signals reach the ARC, a catabolic circuit is activated due to α-MSH signaling which results in an anorexigenic response (decrease in food intake and meal termination) together with increased energy expenditure (Morton et al., 2006). In contrast, low levels of adiposity signals in the brain indicating a need for food and for replenishing energy stores lead to an activation of an anabolic pathway which enhances food intake (orexigenic response) and

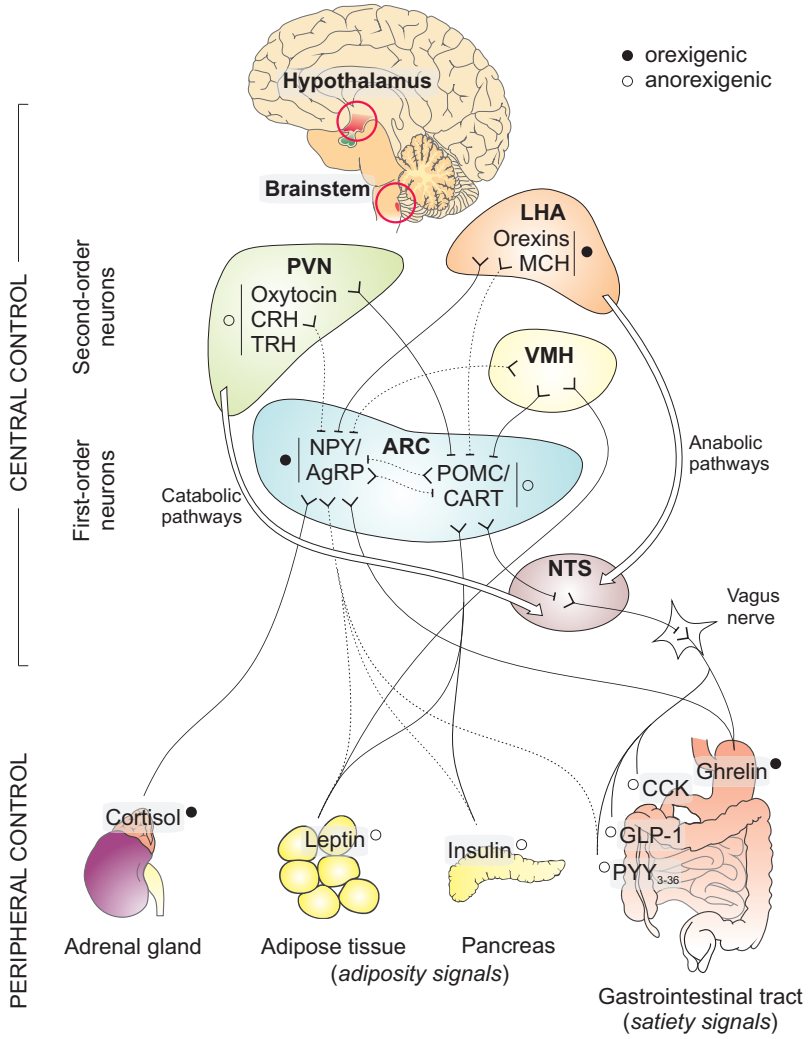

Figure 1.1 Chief hypothalamic pathways in the regulation of food intake. (Caption on next page.)

Figure 1.1 (Previous page.) Diagram showing the interrelationship between orexigenic (black circles) and anorexigenic neuropeptides (white circles) and their major sites of origin and action. Solid and dotted lines indicate a stimulatory and inhibitory effect, respectively. Abbreviations: ARC, arcuate nucleus; NTS, nucleus of the solitary tract; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; $PYY_{3\text{-}36}$, peptide $YY_{3\text{-}36}$; PVN, paraventricular nucleus; LHA, lateral hypothalamic area; NPY, neuropeptide Y; AgRP, agouti-related peptide; POMC, pro-opiomelanocortin; CART, cocaine- and amphetamine-regulated transcript; CRH, corticotropin-releasing hormone; TRH, thyrotropin-releasing hormone; MCH, melanin-concentrating hormone; VMH, ventromedial nucleus of hypothalamus.

reduces energy expenditure, respectively (Morton et al., 2006). NPY/AgRP neurons are stimulated by ghrelin (Cowley et al., 2003) and are inhibited by leptin, insulin, and $PYY_{3\text{-}36}$ (Morton et al., 2006), whereas POMC neurons are stimulated by circulating concentrations of leptin but inhibited by neighboring NPY/AgRP neurons (Arora and Anubhuti, 2006; Cowley et al., 2001). Arcuate neurons are also sensitive to local levels of energy-dense nutrients, including glucose (Levin, 2006), fatty acids (Lam et al., 2005), and some amino acids (Cota et al., 2006). Low glucose levels activate NPY neurons (Beck et al., 1990), but inhibit POMC signaling activity (Brady et al., 1990). Taken together, the POMC-originating tract has an overall catabolic effect, whereas the NPY/AgRP-originating tract is anabolic.

The ARC is extensively connected with other hypothalamic appetite-regulating regions in a reciprocal manner, where two nearby target areas, namely the paraventricular nucleus (PVN) and the lateral hypothalamic area (LHA), are thought to be especially important (Harrold et al., 2012). The axons of arcuate NPY/AgRP and POMC/CART neurons project to 'second-order' neurons partly located in the PVN, where the anorexigenic neuropeptides thyrotropin-releasing hormone (TRH), CRH and oxytocin (see section 1.3.3) are secreted. The PVN represents a major integratory region in the brain with recognized neuroendocrine, autonomic, and behavioral functions. Preau-

tonomic PVN neurons project to the liver through both sympathetic and parasympathetic pathways indicating a pivotal role of the PVN in hepatic glucose production (O'Hare and Zsombok, 2016). Signals from the PVN are catabolic and enhance the potency of satiety signals in the NTS leading to reduced food intake and increased energy expenditure (Woods et al., 2004). Moreover, the PVN modulates the response to stressors by controlling the activity of the HPA axis (see section 1.2.2). Lesions of the PVN result in hyperphagia, reduced energy expenditure, as well as obesity (Shor-Posner et al., 1985) and PVN dysfunction is also associated with conditions such as hypertension and congestive heart failure (Ferguson et al., 2008).

The LHA, known as the classical 'feeding center,' also receives direct inputs from the ARC and has a contrasting profile from the PVN (Broberger et al., 1998). Glucose-sensing neurons in the LHA express the orexigenic peptides melanin-concentrating hormone (MCH) and orexins (see section 1.3.2) in presence of hypoglycemia (Burdakov et al., 2005). Signals from the LHA, contrary to the PVN, are anabolic, i.e., suppressing the activity of the satiety signals in the NTS (Woods et al., 2004). The ventromedial nucleus of the hypothalamus (VMH), on the other, has been identified as a key target of leptin and therefore referred to as 'satiety center' acting on the hypothalamus to inhibit feeding, increase energy expenditure, and cause weight loss (Schwartz et al., 1996).

To maintain metabolic homeostasis, the central nervous system requires a continuous supply of glucose supporting energy requirements of the brain. An extended network of glucose-sensing neurons detects decreases in glucose levels and serves to mediate counterregulatory responses restoring euglycemia. Neurons and neuronal circuits that may be directly or indirectly activated or inhibited by glucose are located at peripheral sites, e.g., pancreatic α- and β-cells, liver, carotid body, and gastrointestinal tract, as well as several brain regions such as the hindbrain, ventromedial hypothalamus, lateral hypothalamus, the ARC, and the suprachiasmatic nucleus (SCN). The SCN of the hypothalamus, known as 'the biological clock' controlling circadian rhythms,

is responsible for the 24-h rhythm in plasma glucose concentrations with elevated glucose levels toward the end of the light period and just before the onset of activity ('dawn phenomenon;' La Fleur et al., 1999). The majority of the glucose-excited neurons are distributed in the lateral hypothalamus, whereas glucose-inhibited neurons are located ventromedially (Verberne et al., 2014).

Aside from staving off hunger, i.e., refilling energy stores to ensure an adequate balance, eating also provides feelings of gratification. The consumption of energy-dense palatable food activates brain circuits of reward and motivation in limbic and paralimbic areas, e.g., nucleus accumbens (NAcc), ventral tegmental area (VTA), amygdala, hippocampus, cingulate cortex, insular cortex, and the striatum. Among several neurotransmitters, e.g., endorphins involved in the processing of hedonic aspects of food intake (Volkow et al., 2011), dopamine is a key neurotransmitter modulating reward mainly through its projections from the VTA into the NAcc (Wise, 2006). Palatable foods, which are high in sugar and fat, serve as potent rewards (Lenoir et al., 2007) that promote food consumption or even overeating, respectively. This complementary non-homeostatic pathway in the regulation of eating behavior is considered particularly important in the development of obesity (Alonso-Alonso and Pascual-Leone, 2007).

However, homeostatic and non-homeostatic pathways are not completely separated circuits but rather intricately involved with each other (Rutters et al., 2012). Neuropeptides involved in homeostatic regulation of food intake are also implicated in reward-driven, hedonic regulation. For instance, the adiposity feedback signal leptin (serving as a 'satiety hormone'), which informs the hypothalamus of the size of fat stores, has been shown to decrease food intake after injection into the VTA of rodents (Hommel et al., 2006). Also ghrelin (referred to as 'hunger hormone') influences both homeostatic and hedonic pathways. As an orexigenic factor synthesized both peripherally in the gastric mucosa and, to a lower amount, centrally in hypothalamic nuclei (Cowley et al., 2003; Kojima et al., 1999), ghrelin mediates not only sensations

of hunger during fasting, but also modulates hedonic aspects of eating, as it directly targets the VTA (Skibicka et al., 2011).

Apart from these homeostatic and reward circuits which tend to favor food intake (so called reflexive eating mode), brain areas involved in cognition, especially the right prefrontal cortex (rPFC), tend to decrease food intake (reflective eating mode; Alonso-Alonso and Pascual-Leone (2007)). The rPFC is important for decision making according to social conduct and comprehension of body-state information at a higher level (Tranel et al., 2002). This non-homeostatic top-down control from reflective areas can suppress reflexive areas (hypothalamus, brainstem, limbic and paralimbic structures) and overrule metabolic needs and homeostatic regulatory mechanisms (Berthoud, 2004), e.g., avoiding certain food products because of personal convictions or judging what is appropriate to eat according to long-term effects on health and body shape (Alonso-Alonso and Pascual-Leone, 2007). This cognitive dimension of eating is also assumed to play an essential role in obesity. Lesions in the right anterior PFC can cause the 'gourmand syndrome,' a passion for eating (Regard and Landis, 1997), whereas hyperactivity of the rPFC, as found in patients with right prefrontal focal epilepsy, can lead to anorexia-like symptoms (Uher and Treasure, 2005).

1.2.2 The hypothalamic-pituitary-adrenal (HPA) axis

The HPA axis is a major neuroendocrine system that controls reactions to physical and emotional stressors and regulates many homeostatic processes, including digestion, the immune system, mood and emotions, sexuality, as well as energy storage and expenditure. Activation of the HPA axis causes hypophysiotropic neurons localized in the medial parvocellular subdivision of the PVN of the hypothalamus to produce vasopressin and CRH (Rivier and Vale, 1983). Vasopressin and CRH stimulate corticotropic cells in the adenohypophysis to secrete the POMC derivative adrenocorticotropic hormone (ACTH) into the systemic regulation. In response to stimulation by ACTH,

the zona fasciculata of the adrenal cortex within the adrenal gland produce and secrete cortisol, a glucocorticoid steroid hormone. Elevated peripheral cortisol concentrations in turn suppress CRH and ACTH production in the PVN and the pituitary gland, respectively, in a delayed negative feedback cycle (De Kloet et al., 1998; Keller-Wood and Dallman, 1984).

Stress in general may lead either to under- or overeating (Razzoli and Bartolomucci, 2016; Torres and Nowson, 2007). Responses to acute stress result in reduced food intake in the short term due to physiologic changes, e.g., slowed gastric emptying and shunting of blood from the gastrointestinal tract to muscles (Torres and Nowson, 2007). However, prolonged exposure to chronic stress, e.g., social stress (Scott et al., 2012) or sleep deprivation (Spiegel et al., 1999), elicits a more passive response driven by the HPA axis. In the ARC, NPY neurons express glucocorticoid receptors (Hisano et al., 1988) and chronic central infusion of glucocorticoids induced obesity as well as increased NPY levels (Zakrzewska et al., 1999).

There is large body of evidence that basal HPA axis function and stress responsiveness increase during aging. This alteration is possibly the result of a loss of negative feedback to glucocorticoids, primarily through a decline of glucocorticoid receptors in hippocampal circuits (Sapolsky, 1999; Sapolsky et al., 2000), that exert a tonic, inhibitory effect on CRH production by the hypothalamus (Jacobson and Sapolsky, 1991). In humans, aging is associated with increased cortisol production (Purnell et al., 2004), and increased cortisol responses to cognitive challenge (Seeman and Robbins, 1994) resulting in elevated peripheral cortisol levels which is associated with increased cognitive decline in elderly humans (Li et al., 2006; Lupien et al., 1999). Apart from that, HPA axis hyperactivity is also linked to several other medical conditions, such as major depressive disorder (Keller et al., 2016; McEwen, 2000; Vreeburg et al., 2009), insomnia (Roth et al., 2007), or pain chronification (Li and Hu, 2016; Sarzi-Puttini et al., 2006). Overall, the data suggest that inappropriate control of HPA axis function predisposes to metabolic and cognitive disorders.

1.3 Insulin, orexin A, and oxytocin in the control of metabolic function

The peptidergic systems that regulate body weight and energy expenditure described in section 1.2.1 and 1.2.2 are of great interest as targets for anti-obesity agents. A staggering number of neuropeptides has been postulated as playing a role in the orchestration of metabolic processes within the hypothalamus (Coll and Yeo, 2013), and have been investigated as potential treatments for obesity (Boughton and Murphy, 2013; Paz-Filho et al., 2015; Sato et al., 2009). While insulin given by injection is the most common type of medication used in diabetes treatment, orexin A and oxytocin appear as relatively new targets in the context of clinical interventions in patients with metabolic disorders and obesity, respectively. Details about site of synthesis and action, as well as physiological effects covering their involvement in food intake, insulin sensitiviy, and HPA axis activity in particular, are provided below.

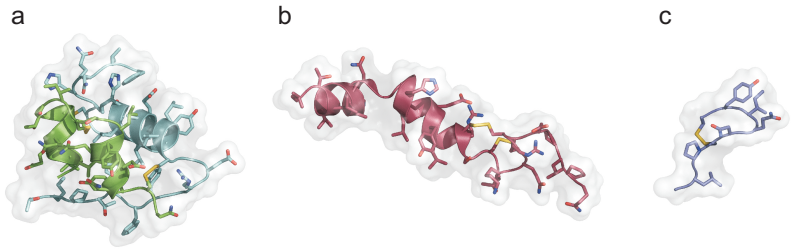

Figure 1.2 Peptide crystal structures. Representations of insulin (**a**), orexin A (**b**), and oxytocin (**c**) showing main chains (ribbons), side chains (sticks), and solvent-accessible surface areas (transparent).

Table 1.2 Physicochemical properties and pharmaceutical information of applied neuropeptides.

	Insulin	Orexin A	Oxytocin
Molecular formula	$C_{257}H_{383}N_{65}O_{77}S_6$	$C_{152}H_{243}N_{47}O_{44}S_4$	$C_{43}H_{66}N_{12}O_{12}S_2$
Mass (g mol^{-1})	5807.63	3561.14	1007.19
Length (aa)	51	33	9
Log*P*	−13.1	−10.1	−2.6
Synthesis	pancreas	hypothalamus	hypothalamus
Receptor	IR	OX1R and OX2R	OXTR
Receptor type	tyrosine kinase	G protein-coupled	G protein-coupled
Appetite regulation	anorexigenic	orexigenic	anorexigenic
Brand	Insulin Actrapid®	–	Syntocinon®
Supplier	Novo Nordisk	Bachem	Defiante Farmacêutica
Indication	diabetes	–	labor induction, lactation stimulation

Physicochemical data are obtained from the PubChem Compound Database (NCBI, 2016). Abbreviations: aa, amino acids; IR, insulin receptor; Log*P*, logarithm of the partition coefficient between *n*-octanol and water as a predictor for intrinsic hydrophobicity; OX1R, orexin receptor type 1; OX2R, orexin receptor type 2; OXTR, oxytocin receptor.

1.3.1 Insulin

Insulin (from Latin *insula* meaning 'island') is a peptide hormone (see Figure 1.2a and Table 1.2) secreted by β-cells of the pancreatic islets in response to nutritional stimuli and its antidiabetic properties were firstly described by Paulesco (1921) under the designation 'pancrein.' Peripherally, the hormone promotes the absorption of glucose from the blood into skeletal muscle, white adipose tissue, and liver while reducing hepatic gluconeogenesis and triglyceride secretion. Insulin triggers lipogenesis in white adipose tissue and liver, thereby boosting fat storage. Thus, systemic insulin has anabolic properties by promoting the growth of skeletal muscle and fat mass, but once it has been transported into the brain compartment, leads to converse catabolic effects by reducing food intake and adipose mass (Air et al., 2002; Woods et al., 1979). Insulin enters the brain via saturable transport proportionate to respective peripheral concentrations (Baura et al., 1993). Insulin receptors (IR) are expressed in several brain regions, mainly the olfactory bulb, hippocampus, cerebral cortex, as well as hypothalamic nuclei, e.g., ARC and PVN (Devaskar et al., 1994; Marks et al., 1990). In the insulin synthesis pathway, proteases split the precursor proinsulin into equal amounts of insulin and C-peptide. Thus, C-peptide serves as a measure of endogenous insulin secretion (Jones and Hattersley, 2013). The insulin monomer consists of two chains, a 21 amino acid residue A-chain and 30 amino acid residue B-chain, linked by disulfide bonds.

Central insulin administration during food deprivation inhibits the fasting-induced increase of NPY levels in paraventricular and preproNPY mRNA in arcuate regions of the hypothalamus (Schwartz et al., 1992). Accordingly, the catabolic effect of centrally administered insulin is prevented by a POMC antagonist suggesting that the hypothalamic melanocortin system mediates the anorexigenic effects of central insulin (Benoit et al., 2002). Neuron-specific disruption of the IR gene in mice results in diet-sensitive obesity

with increases in body fat, elevated plasma and leptin levels, and mild insulin resistance (Brüning et al., 2000).

Beside its involvement in body weight regulation, insulin also has stress-regulating properties when acting centrally. HPA axis activity strongly depends on the availability of food and energy stores. Basal activity is lowest in the absence of hunger, whereas fasting is accompanied by elevated basal levels of the stress hormone corticosterone (Dallman et al., 1995). Insulin is reciprocally regulated with corticosterone during stress, suggesting that insulin provides inhibitory tone to HPA axis activity (Dallman et al., 1995). However, peripheral insulin administration, e.g., during an euglycemic hyperinsulinemic clamp, has been repeatedly shown to stimulate HPA axis activity (Chan et al., 2005; Fruehwald-Schultes et al., 1999, 2001). Intranasal administered insulin, in contrast, dampened HPA axis secretion (Benedict et al., 2004; Böhringer et al., 2008; Hallschmid et al., 2008), which points towards divergent central and peripheral impacts of insulin on HPA axis regulation. HPA axis hyperactivity associated with obesity is likely due to alterations in central signaling pathways, as obese patients exhibit normal pituitary sensitivity to negative feedback from glucocorticoids (Pasquali et al., 2002).

1.3.2 Orexin A

Orexins (also referred to as hypocretins) were simultaneously discovered by two independent research groups as hypothalamus-specific peptides with a weak homology to the gastrointestinal peptide secretin (de Lecea et al., 1998; Goodrick, 2015; Sakurai et al., 1998). Orexin A (see Figure 1.2b and Table 1.2) and orexin B are a pair of 33- and 28-amino acid neuropeptides, respectively, with 46 % sequence identity, produced by proteolytical cleavage of the common precursor protein prepro-orexin (Taheri and Bloom, 2001). Orexin-synthesizing neurons are located in the LHA, perifornical area (PFA), and the posterior hypothalamus and send projections throughout the brain, e.g., hippocampus, amygdala, thalamus, NTS, and cerebral cortex (Mieda

et al., 2013; Sakurai et al., 1998) suggesting that orexins are involved in various metabolic and behavioral processes. Orexins act via two G protein-coupled receptors, orexin receptor type 1 (OX1R) and orexin receptor type 2 (OX2R). Orexin A is a high-affinity ligand for both OX1R and OX2R, whereas orexin B specifically binds to OX2R (Sakurai et al., 1998). In rats, both orexin receptors are widely expressed in the hypothalamus, including ARC, PVN, the locus coeruleus, and dorsal raphe nucleus (Trivedi et al., 1998). In rats, food intake is acutely increased by central administration of orexin A and, to a lesser extent, by orexin B leading to the initial assumption that both orexins principally control food intake (Haynes et al., 1999; Sakurai et al., 1998) which eventually inspired their joint name (derived from ancient Greek ὄρεξις *órexis* meaning 'appetite'). Orexin A may stimulate food intake by directly activating NPY neurons, which express OX1R (Suzuki et al., 2002), and suppressing POMC-containing neurons in the ARC (Muroya et al., 2004).

Moreover, the orexin system is strongly implicated in the maintenance of alertness and wakefulness as well as in the regulation of the autonomic nervous system, reward system, and glucose/energy homeostasis (Sakurai, 2014b; Saper et al., 2005; Tsuneki et al., 2012). Orexin neurons are most active during wakefulness and relatively inactive during sleep (Estabrooke et al., 2001) and a deficiency in the orexin system is supposed to be the cause for narcolepsy, a chronic neurodegenerative disease chracterized by excessive daytime sleepiness, periods of rapidly falling asleep, and cataplexies (De la Herrán-Arita et al., 2011). Narcolepsy in dogs is caused by a mutation of the OX2R gene (Lin et al., 1999) and genetic ablation of orexin neurons in mice leads to narcolepsy but also late-onset obesity, despite the presence of hypophagia (Hara et al., 2001). Accordingly, cerebrospinal fluid (CSF) orexin A levels are undetectably low in patients with narcolepsy compared to healthy controls (Ripley et al., 2001).

Orexin mRNA expression correlates negatively with changes in blood glucose, food intake, and the adipocyte-derived hormone leptin, that provides the brain with negative feedback on body fat stores (Yamanaka et al., 2003).

Orexin neurons are inhibited by high extracellular glucose levels which induce hyperpolarization and decrease membrane resistance (Burdakov et al., 2005). Food deprivation up-regulates both OX1R and OX2R receptor expression in the rat hypothalamus (Karteris et al., 2005), whereas genetically obese mice with elevated basal glucose concentrations show decreased prepro-orexin gene expression in the LHA compared to controls (Yamamoto et al., 1999). Accordingly, there is a strong negative correlation between plasma orexin A and BMI (Adam et al., 2002).

Both orexins stimulate insulin release from pancreatic cells in vitro and in vivo (Nowak et al., 2000, 2005) and orexin A accordingly inhibits secretion of glucagon in perfused rat pancreas in situ and isolated pancreatic islets in vitro (Göncz et al., 2008). Glucagon is synthesized by pancreatic α-cells in response to hypoglycemia and stimulates hepatic gluconeogenesis to keep blood glucose levels stable by insulin-antagonistic effects. Moreover, orexin A stimulates feeding-associated glucose utilization in skeletal muscle via the sympathetic nervous system (Shiuchi et al., 2009). Thus, orexin A appears to be simultaneously involved in systemic insulin sensitivity and hepatic glucose production (Karnani and Burdakov, 2011; Yi et al., 2009) which seems plausible from an evolutionary point of view; during periods of starvation, which are accompanied by low energy levels, hypothalamic orexin neurons stimulate glucose production in the liver to provide energy for the search for food (Tsuneki et al., 2012; Yi et al., 2009). Furthermore, orexin A bidirectionally regulates hepatic gluconeogenesis via control of autonomic balance, contributing to the SCN-controlled daily blood glucose oscillation (Foppen et al., 2016; Tsuneki et al., 2015; Yamamoto et al., 1984). Orexin A appears to regulate insulin sensitivity, because orexin knockout mice fed with normal chow diet show an age-related systemic insulin resistance without changes in body weight (Tsuneki et al., 2008). Accordingly, patients with narcolepsy with cataplexy develop metabolic alterations including insulin resistance, independent of body weight and BMI, respectively (Poli et al., 2009). Nevertheless, the role of orexin A in glucose homeostasis appears

contradictory since orexin A is both exerting blood glucose-lowering as well as -elevating effects seemingly depending on different experimental conditions (Shiuchi et al., 2009; Tsuneki et al., 2012; Yi et al., 2009).

1.3.3 Oxytocin

The nonapeptide oxytocin (see Figure 1.2c and Table 1.2) is synthesized in magnocellular neurosecretory cells of the PVN and, to a lesser extent, supraoptic nuclei (SON) of the hypothalamus. Stored in large dense-core vesicles in the neurohypophysis (posterior pituitary gland), oxytocin is released both into the periphery and to different brain areas by axonal projections and, more locally, dendritic release and diffusion (Landgraf and Neumann, 2004; Ludwig and Leng, 2006), thereby acting both as a neuromodulator and a hormone. Oxytocin neurons are also found in the parvocellular neurons of the PVN and SCN, in the bed nucleus of the stria terminalis (BST), the medial amygdala, the dorsomedial hypothalamus, and the locus coeruleus (Stoop, 2012). Oxytocin is a highly conserved neuropeptide in mammals and closely related to vasopressin which controls water balance and blood pressure, and only differs by two substitutions in the amino acid sequence at the 3rd and 8th position. Initially, oxytocin has been known for its contribution to labor induction and lactation. Its cyclic structure and amino acid sequence was first described by du Vigneaud et al. (1953). The name (derived from ancient Greek ὠκύς *ōkús* and τόκος *tókos* meaning 'quick birth') is based on its uterine-contracting properties (Dale, 1906). Oxytocin has been identified as an important regulator of psychosocial function in humans modulating trust, attachment, emotion recognition, and sexual behavior (Behnia et al., 2014; Kosfeld et al., 2005; Meyer-Lindenberg et al., 2011; Scheele et al., 2012; Shahrestani et al., 2013; Thienel et al., 2014) which earned it the label 'trust, love, or cuddle hormone.' However, oxytocin also stimulates defensive aggression toward group-outsiders (De Dreu et al., 2010) and promotes group-serving dishonesty (Shalvi and De Dreu, 2014) suggesting that oxy-

tocin as well exerts opposing influences on human behavior in different social contexts.

While research in humans in particular brought the social role of oxytocin into prominence, animal experiments moreover revealed a contribution of the hormone to ingestive behavior and metabolism (Arletti et al., 1989; Morton et al., 2012; Olson et al., 1991; Wu et al., 2012). Thus, studies in rodents have shown that oxytocin strongly inhibits food intake and is involved in energy expenditure and glucose homeostasis (Morton et al., 2012). Oxytocin stimulates insulin secretion (Altszuler and Hampshire, 1981; Björkstrand et al., 1996) and is assumed to mediate the food intake–limiting effect of leptin in the PVN (Blevins et al., 2004) and oxytocin mRNA levels were reduced by fasting and restored by leptin (Tung et al., 2008). Moreover, oxytocin neurons in the PVN are a major target of the anorexigenic peptides nesfatin-1 (Maejima et al., 2009) and α-MSH (Sabatier et al., 2003). Central injection of nesfatin-1 and α-MSH stimulated oxytocin release from the dendrites of hypothalamic neurons while the firing rate of oxytocin neurons was inhibited and thus reduced secretion from the neurohypophysis suggesting that oxytocin signaling is mediated by nesfatin-1 through a leptin-independent melanocortin pathway (Maejima et al., 2009; Sabatier et al., 2003).

Oxytocinergic neurons also project to mesolimbic reward-processing centers, such as the caudal part of the VTA, NAcc, and ventral striatum (Stoop, 2012; Succu et al., 2008). Sugar intake, which is partly driven by its hedonic qualities, in parallel increases dopamine levels in the NAcc (Hajnal et al., 2004) and enhances oxytocinergic gene expression in the PVN (Olszewski et al., 2009) and additionally the oxytocin system is activated more strongly by intake of sucrose compared to bland chow (Mitra et al., 2010). Accordingly, exogenously administered oxytocin reduces dopamine turnover in NAcc and striatum (Qi et al., 2008), indicating that oxytocin might control especially the reward-related intake of highly palatable food (Klockars et al., 2015).

Oxytocin as well modulates HPA axis activity both at basal and stressful conditions. In the PVN, oxytocin inhibits the release of CRH which

subsequently reduces downstream ACTH and glucocorticoid release resulting in an overall dampening of HPA axis activity (Engelmann et al., 2004). Suckling-induced increases in plasma oxytocin levels are associated with attenuated baseline levels of plasma ACTH and cortisol postpartum lactating women (Amico et al., 1994; Chiodera et al., 1991). Furthermore, infusion of exogenous oxytocin in men decreased plasma ACTH and cortisol levels in a dose-dependent manner (Legros et al., 1984) suggesting that peripheral oxytocin regulates HPA axis activity at the pituitary or adrenal gland level. Intranasal administration of oxytocin likewise reduces cortisol levels during couple conflict (Ditzen et al., 2009), physical stress (Cardoso et al., 2013), and the Trier Social Stress Test in the presence of social support (Heinrichs et al., 2003). The mechanism underlying the attenuating effect of intranasally administered oxytocin on HPA axis activity seems less clear, possibly involving both central and peripheral domains.

1.4 Intranasal delivery of substances to the central nervous system

The methodological question arises how neuropeptides can be applied to reach the central nervous system in the most effective and least harmful way possible. Animal research allows neuropeptides to be administered directly to the central nervous system (CNS) via stereotactic, e.g., intracerebroventricular (ICV) infusion (Blevins et al., 2016; Haynes et al., 1999; Woods et al., 1979). Since this invasive technique is associated with significant health risk, this route of delivery cannot be routinely employed in a human study context (Cook et al., 2009). So far, the conventional way of increasing central nervous neuropeptidergic concentrations to investigate central effects in the human brain relies on intravenous (IV) infusion. But the experimental and therapeutical use of neuropeptides underlies several limitations when administered systemically. These compounds do not readily pass the blood-brain

barrier (BBB), and evoke potent hormone-like, peripheral side effects when circulating in the blood (Illum, 2003). For instance, systemic insulin infusion results in a drop of glucose levels, i.e., hypoglycemia, triggering the graded activation of endocrine stress axes that can affect brain function (Tesfaye and Seaquist, 2010) and IV administration of oxytocin induces (painful) uterine contractions together with antidiuretic and vascular effects when used in large doses (Shyken and Petrie, 1995).

A solution for bypassing the methodological restrictions of IV administration is provided by the use of intranasal (IN) administration which has been described in several animal paradigms (Dhuria et al., 2009; Parker et al., 2005; Thorne et al., 2004). In humans, neuropeptides have been shown to achieve access to the central nervous system (CNS) within one hour after IN administration (Born et al., 2002). It is assumed that intranasally administered neuropeptides reach the CNS primarily via extracellular transport mechanisms along olfactory sensory neurons (see Figure 1.3). Administered molecules diffuse into the subarachnoid space across the olfactory epithelium and travel alongside intercellular clefts between sustentacular (supporting) cells and olfactory neurons thus crossing the olfactory epithelium (Dhuria et al., 2010). The extracellular pathway is considered as the most important one for the effects of IN administered neuropeptides on brain function (Spetter and Hallschmid, 2015). Small, lipophilic molecules or molecules that are recognized by the membrane, e.g., insulin (Lacroix et al., 2008), may also cross the olfactory epithelium transcellularly via intracellular transport mechanisms involving the uptake into olfactory neurons by passive diffusion or receptor-mediated transcytosis followed by axonal transport to the olfactory bulb. However, this intraneuronal pathway takes several hours to days for IN administered peptides to reach the brain via the olfactory bulb (Baker and Spencer, 1986; Dhuria et al., 2010).

Besides physicochemical properties (lipophilicity, hydrophilicity, etc.), the molecular mass of intranasally administered substances is the most important factor affecting the absorption through the nasal mucosa and their bioavail-

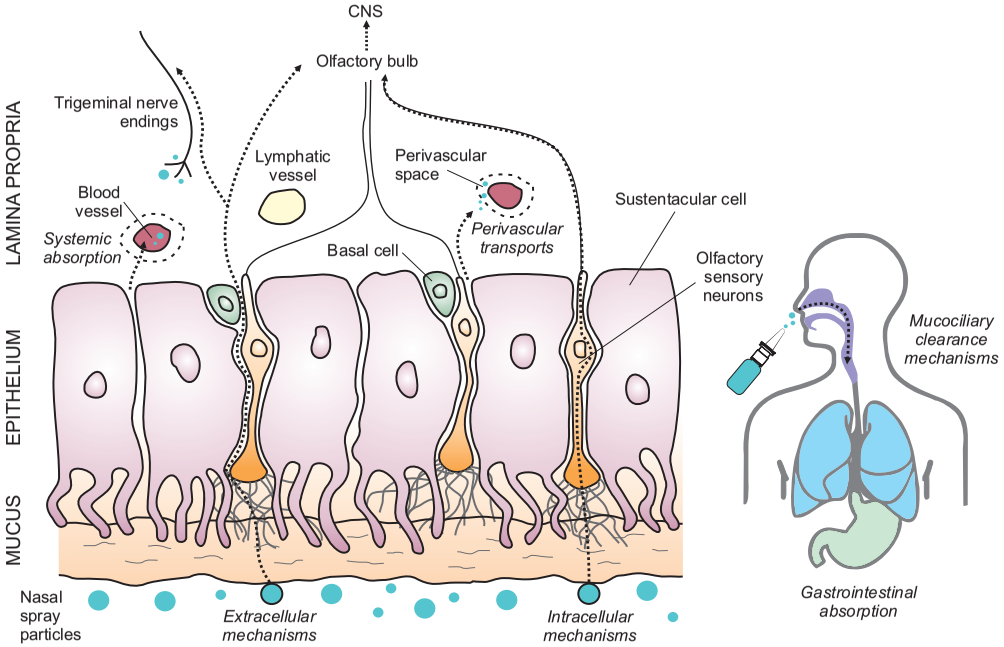

Figure 1.3 Potential neuropeptide absorption routes after intranasal administration in humans. (Caption on next page.)

Figure 1.3 (Previous page.) Peptide molecules (light blue circles) reach the mucus of the nasal cavity, which is innervated by both olfactory neurons and sensory branches of the trigeminal nerve (maxillary nerve). The olfactory epithelium consists of olfactory sensory neurons, sustentacular cells (supporting cells), and basal cells. Peptides can be transported through the nasal mucous membrane and the olfactory bulb to the central nervous system (CNS) intra- and extracellularly by traveling along olfactory neurons and trigeminal nerves as well as by entering perivascular (surrounding) channels of brain's blood vessels in the lamina propria. Due to the nasal mucosa's high degree of vascularisation, intranasally administerd peptides can be absorbed into the network of blood vessels located underneath the basement membrane and into the systemic circulation. Mucociliary clearance mechanisms transport peptide molecules with the mucus towards the nasopharynx for elimination (figure is based on Guastella et al., 2013). Abbreviations: CNS, central nervous system.

ability (Arora et al., 2002). Thus, the intranasal delivery of insulin, orexin A, and oxytocin, which have molecular weights of 5.8 kDa, 3.6 kDa, and 1 kDa, respectively, can be expected to differ due to their specific physicochemical properties (see Table 1.2). Passage of IN administered peptides to the CNS can also be established along the trigeminal nerve (Liu et al., 2012; Thorne et al., 2004), which also innervates the nasal cavity via parasympathetic fibres. Due to the nasal mucosa's high degree of vascularisation, intranasally administered substances can also be delivered to systemic circulation via absorption into the network of capillary blood vessels located underneath the basement membrane, resulting in problems related to drug elimination via hepatic and renal mechanisms, plasma protein binding, proteolysis, and potential peripheral side effects. A subsequent delivery across the BBB to the CNS is possible rather for small, lipophilic compounds than for large, hydrophilic molecules such as peptides and proteins (Dhuria et al., 2010). However, intranasally administered peptides can also reach the brain by entering perivascular compartments surrounding cerebral blood vessels in the nasal cavity and are rapidly distributed throughout the CNS via bulk flow

mechanisms (Lochhead et al., 2015). Finally, mucociliary clearance mechanisms transport a small amount of nasally administered neuropeptides with the mucus to the nasopharynx, where they are swallowed and transported to the lungs and the stomach via the trachea and the esophagus, respectively (Guastella et al., 2013).

1.5 Aims and hypotheses

Insulin sensitivity, body weight, and HPA axis activity are considered significant determinants of metabolic diseases associated with increased mortality and chronic morbidity (Kaur, 2014). As stated above, animal models have shown that insulin, orexin A, and oxytocin directly administered to the brain, play significant roles in the regulation of food intake, glucose metabolism, and HPA axis regulation. In humans, the nasal delivery seems to be a favorable way for direct drug delivery to the central nervous system (Born et al., 2002; Dhuria et al., 2010). While intranasally administered insulin and oxytocin may be considered as the most thoroughly investigated peptides regarding their potential for the enhancement of brain functioning in humans (Chapman et al., 2013; Spetter and Hallschmid, 2015), the current scientific output for IN administration of orexin A in the human setting as yet is relatively sparse. The aim of the present thesis was to evaluate the effects on metabolic control of intranasally administered insulin, orexin A, and oxytocin in healthy normal-weight, young men. Moreover, effects in obese men as well as elderly individuals were obtained to further explore possible therapeutic fields of application. Three studies were performed for this purpose (see Figure 1.4).

Study I aimed at the contribution of central nervous oxytocin administration to the control of food intake in obese individuals. Animal studies (Blevins et al., 2015; Morton et al., 2012) and pilot experiments in humans (Lawson et al., 2015; Ott et al., 2013) indicate that the hypothalamic neuropeptide oxytocin limits food intake. Notably, Ho and Blevins (2013) suggest that the metabolic effects of oxytocin may be even enhanced in diet-induced obese

compared to control animals. These findings raise the question of oxytocin's potential to improve metabolic control in obesity. We therefore investigated the impact of oxytocin via the intranasal route (24 IU) on eating behavior and metabolic function in obese men and compared the results with the effects in normal-weight men obtained in a former study by our group (Ott et al., 2013), where oxytocin exerted an acutely inhibitory impact on palatable snack intake. We applied a validated paradigm including the measurement of ad libitum food intake from a breakfast buffet 45 min after oxytocin administration, followed by the assessment of postprandial, reward-driven snack intake. In this study we formulated the following hypothesis: In men with obesity, intranasal oxytocin administered in the fasted state reduces overall ingestive behavior and this inhibitory effect is even more salient in comparison to normal-weight subjects.

Study II focused on the effect of central insulin on HPA axis activity. Intranasal administration of insulin is known to curb stress axis activity in young (obese) subjects (Böhringer et al., 2008; Hallschmid et al., 2008). Apart from obesity, maladaptive increases in HPA axis activity that predispose to metabolic and cognitive disorders are also strongly associated with aging (Lupien et al., 1999; McEwen, 2000; Seeman and Robbins, 1994). Changes in sleep-associated neuroendocrine secretion patterns that emerge in older age are assumed to be pathophysiological factors in metabolic and cognitive impairments. In this regard, increased activity of the HPA stress axis, which follows a circadian pattern with nadir values during early sleep, may be particularly detrimental. Study II aimed to investigate the effect of intranasal insulin on HPA axis activity of elderly and young subjects in the first night-half, when nadir concentrations of ACTH and cortisol reflect basal HPA axis secretion. Intranasal insulin (160 IU) was administered to elderly (mean age 70.0 years) and young (23.6 years) healthy subjects at 2230 h, 30 min before bedtime. Considering the association between increased age and nocturnal HPA axis hyperactivity (Van Cauter et al., 2000) as well as impaired central nervous insulin signaling (Biessels and Reagan, 2015), it was hypothesized

that intranasal insulin administered before sleep onset has an inhibitory effect on ACTH and cortisol secretion during early sleep particularly in elderly subjects.

The purpose of Study III was to elucidate the influence of orexin A on glucose homeostasis in humans. Orexin A is crucially involved in the control of sleep/wake regulation, feeding behavior, reward processing and energy homeostasis (Sakurai, 2014a,b). Its contribution to glucose homeostasis has not been clarified, although observations in animal studies have led to the assumption that orexin A may improve glucose tolerance and insulin sensitivity (Ducroc et al., 2007; Tsuneki et al., 2012). Using a standardized 2-h oral glucose tolerance test (OGTT) in Study III, we investigated the acute glucoregulatory effect of intranasal administered orexin A (500 nmol) in healthy young subjects with normal glucose tolerance (NGT). It was hypothesized that orexin A has an inhibitory effect on blood glucose excursion and insulin levels in response to glucose challenge.

Hypotheses

1. IN oxytocin reduces homeostatic- and reward-driven food intake in obese men.
2. IN insulin attenuates basal HPA axis activity during early sleep in young and elderly individuals.
3. IN orexin A improves glucose tolerance during an OGTT in healthy young men.

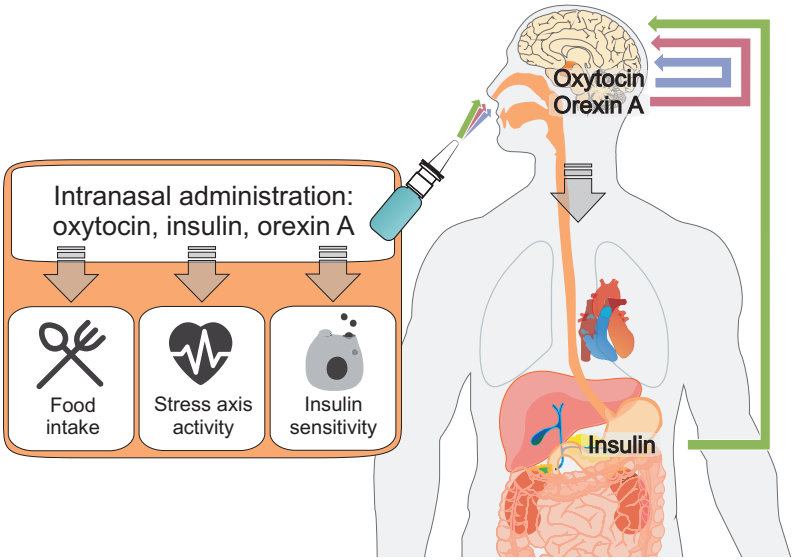

Figure 1.4 Intranasal administration of neuropeptides to assess their contribution to metabolic control. In the present thesis, the effects of the hypothalamic neuropeptides oxytocin and orexin A as well as of the pancreatic peptide insulin, when administered intranasally, on metabolic function were investigated, i.e., on food intake, HPA axis activity, and insulin sensitivity. The intranasal administration route bypass the blood-brain barrier and represents a feasible, non-invasive approach of directly targeting the CNS while systemic side effects are (largely) avoided (figure adapted and modified from Spetter and Hallschmid, 2015).

2 | Study I: Oxytocin and food intake in obese and lean men

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2.1 Introduction

Oxytocin, a hypothalamic nonapeptide which is released into the circulation through the posterior pituitary and also directly acts on central nervous receptors, regulates reproductive functions like mother-infant interaction and lactation and is a potent modulator of social behaviors including attachment and sexual behavior (Meyer-Lindenberg et al., 2011). Studies in animals and pilot experiments in humans have indicated that oxytocin moreover might play a role in the regulation of eating behavior and metabolism. In rodents (Morton et al., 2012) and rhesus monkeys (Blevins et al., 2015) with normal weight, but also with diet-induced obesity, subcutaneous oxytocin administration inhibits food intake, increases energy expenditure and reduces glucose levels. Interestingly, experiments in rodents suggest that the metabolic effects

of oxytocin may be even enhanced in diet-induced obese in comparison to control animals (Ho and Blevins, 2013). In studies in humans, intranasal oxytocin attenuated reward-driven snack intake and decreased postprandial glucose concentrations in normal-weight men (Ott et al., 2013). Against this background, and considering that available weight loss treatments are of modest or temporary efficacy (Halpern and Halpern, 2015; Miras and le Roux, 2014), the intranasal administration of oxytocin to the human brain, which in a broad range of studies has been demonstrated to be associated with absent or minimal side effects (MacDonald et al., 2011), might be a promising pharmacological intervention in obesity (Blevins and Baskin, 2015; Lawson et al., 2015; Zhang et al., 2013).

In the present study, we investigated the impact of oxytocin on eating behavior in obese men and compared the results to the effects obtained in normal-weight men (Ott et al., 2013). Since ingestive behavior is homeostatically regulated in response to energy depletion as well as by brain circuits processing the reward-related, 'hedonic' qualities of food intake (Berthoud et al., 2011; Morton et al., 2014), we applied a validated paradigm that includes a breakfast buffet presented to fasted participants (Hallschmid et al., 2010; Schultes et al., 2005), as well as the delayed assessment of food intake from snacks (Hallschmid et al., 2012; Higgs et al., 2008). In addition, energy expenditure, glucose homeostasis and HPA axis secretory activity were measured before and after oxytocin administration as well as after breakfast intake.

2.2 Methods

Subjects

Eighteen healthy obese men were recruited and their results compared to the findings in 20 normal-weight men, which were obtained with an identical experimental set-up and are described in detail in Ott et al. (2013). Subjects were

recruited via mailing lists and advertisements in local newspapers. Relevant illness was excluded by medical history and clinical examination. Habitual eating behavior of obese subjects was assessed via a lifestyle questionnaire on dietary restraint and tendency toward disinhibition. Dietary restraint, i.e., the conscious effort to restrict calorie intake to control body weight, was assessed using the restraint scale of the Dutch Eating Behavior Questionnaire (DEBQ-R; van Strien et al., 1986). Tendency toward disinhibition was assessed with the disinhibition scale of the Three Factor Eating Questionnaire (TFEQ; Stunkard and Messick, 1985). On average, participants achieved a DEBQ-R score (mean $\pm$ SEM) of 2.55 ± 0.13 and a TFEQ score of 6.53 ± 0.80. The propensity to consume palatable foods was measured at three levels of food proximity (food available, food present, and food tasted) with the Power of Food Scale (PFS; Lowe et al., 2009) at each session (see Table 2.1 for subject characteristics). Subjects were kept unaware of hypothesized treatment effects on food intake and were informed that the experiments concerned the effect of oxytocin on mood, taste preferences and energy expenditure. Participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Experimental procedure

Experiments were carried out in a balanced, double-blind, cross-over, within-subject comparison. Each subject participated in two experimental sessions (oxytocin and placebo) spaced at least 10 days apart. Participants were instructed to abstain from the intake of food and caffeinated and alcoholic beverages after 2000 h on the day preceding each session.

After the subject's arrival at the laboratory at $\sim$0800 h, a venous cannula was inserted into the subject's non-dominant arm to enable drawing of venous blood (see reffig:inoxy1 for the experimental procedure). Thereafter, blood was sampled for baseline assessments of hormonal parameters. At 0942 h each subject was intranasally administered 24 IU oxytocin (0.6 ml Syntocinon®; Defiante Farmacêutica, Funchal/Madeira, Portugal) or placebo

Table 2.1 Subject characteristics.

Variable	Obese men (mean ± SEM)	Normal-weight men (mean ± SEM)	*P* value
Age (years)	27.83 ± 1.38	26.30 ± 0.89	0.35
Body weight (kg)	106.39 ± 2.25	74.81 ± 1.89	<0.001
BMI (kg/m^2)	32.10 ± 0.36	22.66 ± 0.36	<0.001
Lean body mass (kg)	79.96 ± 1.56	61.10 ± 1.40	<0.001
Body fat mass (kg)	26.54 ± 1.28	13.78 ± 0.84	<0.001
Body fat mass (% of total weight)	24.80 ± 0.92	18.19 ± 0.87	<0.001
Body cell mass (% of lean body mass)	56.37 ± 0.54	54.94 ± 0.60	0.09
Body water (liters)	58.53 ± 1.14	44.72 ± 1.02	<0.001
Phase angle (°)	7.00 ± 0.13	6.69 ± 0.13	0.10
PFS (total scale score)	2.71 ± 0.13	2.42 ± 0.14	0.13

Mean ± SEM values are indicated. Body composition was measured by bioelectrical impedance analysis (Nutriguard-M, Data Input, Germany) in a clinical examination taking place shortly before the first experimental session. PFS, total scale score of the Power of Food Scale (PFS), a measure of propensity to consume palatable foods, assessed before substance administration and averaged across conditions. Obese men, $n = 18$; normal-weight men, $n = 20$.

(0.6 ml vehicle containing all Syntocinon ingredients except for the peptide) at 6 individual 0.1 ml-puffs (3 per alternating nostril) with 30-s intervals in-between. Forty-five minutes after administration, subjects were presented with a free-choice ad libitum breakfast buffet from 1030–1100 h. Casual snack intake was assessed 95 min later under the pretext of a snack taste test. Olfactory function was tested at 1200 h. Feelings of hunger, mood, subjective well-being and the perceived experimenter's trustworthiness were repeatedly rated on visual analogue scales (0–100) and energy expenditure was assessed by indirect calorimetry before and after substance administration and after the test breakfast. Heart rate and blood pressure were monitored. At the end of the experiments, subjects were asked to indicate their account of the study purpose and if they thought to have received oxytocin or placebo.

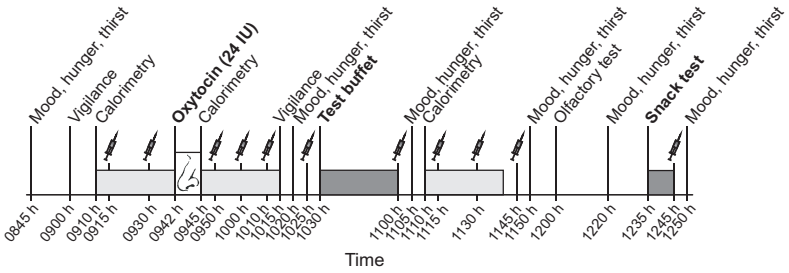

Figure 2.1 Experimental procedure. After baseline assessments of blood parameters, psychological variables, vigilance, and energy expenditure, subjects were intranasally administered oxytocin (24 IU) and placebo, respectively, at 0942 h (nose symbol). At 1030 h, 45 min after substance administration, subjects were allowed to eat ad libitum from a free-choice test buffet for 30 min. About 60 min after termination of the buffet, at 1200 h, olfactory function was assessed, and at 1235 h, 95 min after the end of the buffet, snack intake was measured under the pretext of a taste-rating task. Throughout the sessions, mood, hunger, thirst, and vigilance were assessed, and blood samples were taken (syringe symbols).

Assessments of food intake

The breakfast buffet comprised a collection of bread and rolls, spreads (e.g., jam, honey), sausages, cheese, fruits, puddings, fruits, milk and juice, which totaled 4562 kcal (see Table 2.2 for details). Subjects were left undisturbed during breakfast and were not aware that their food intake was measured by weighing buffet components before and after. This procedure enables the precise assessment of primarily hunger-driven food intake in the fasted state (Hallschmid et al., 2010; Ott et al., 2013; Schultes et al., 2005). Reward-related eating in the relative absence of hunger was assessed under the pretext of a taste test (Hallschmid et al., 2012; Higgs et al., 2008; Ott et al., 2013) that included snacks of three different types but comparable calorie content and macronutrient composition, i.e., 'TUC Cracker Classic' (salty taste; Griesson-de Beukelaer, Polch, Germany; 486 kcal per 100 g; 63 g carbohydrates per 100 g), 'Rice Waffles' (bland taste; Continental Bakeries B.V., Dordrecht, The Netherlands; 390 kcal per 100 g; 63 g carbohydrates per 100 g), and 'Double Chocolate Cookies' (sweet taste; EDEKA, Hamburg, Germany; 500 kcal per 100 g; 57.2 g carbohydrates per 100 g). For each variety, 15 snacks broken into bite size pieces were provided on a separate plate, and labeled snack A, B, and C, respectively, allowing for a considerable amount to be eaten without the plates appearing empty, to ensure that participants would not restrict snack intake based on whether the experimenter could see how much had been consumed. The participant was instructed to taste and rate each snack type on a visual analog scale (VAS) anchored by 0 (not at all) and 100 mm (very palatable/sweet/salty). Subjects were informed that during and after completion of the rating they could eat as many snacks as they liked because remaining food would be discarded, and were left alone for 10 min. Intake was covertly measured by weighing the snacks before and after the test.

Table 2.2 Composition of the test buffet.

Food	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Neutral					
Whole wheat bread	165	360	71	2.3	12
Wheat rolls	240	275	122.4	3.4	6.3
White bread	30	72	14.6	0.4	2.2
Butter	120	928	0.7	99.8	0.8
Whole milk	750	491	36	26.3	24.8
Sweet					
Strawberry jam	50	147	35.8	0.1	0.1
Hazelnut spread	40	218	21.6	12.8	2.8
Honey	40	123	30	0	0.1
Sugar	24	98	24	0	0
Fruit curd	125	140	19.3	3.3	7.7
Vanilla pudding	125	134	20.8	3.8	3.5
Strawberry milk	200	167	18.2	6.8	7.4
Banana	179	168	38.3	0.4	2
Apple	195	104	22.2	1.2	0.6

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Table 2.2 Composition of the test buffet (continued from previous page).

Food	Weight (g)	Energy (kcal)	Carbohydrate (g)	Fat (g)	Protein (g)
Pear	140	78	17.4	0.4	0.7
Orange	180	72	15	0.4	1.8
Tangerine	80	35	8.2	0	0.5
Orange juice	400	173	36	1	4
Savory					
Poultry sausage	40	74	0.1	4.3	8.3
Cervelat sausage	34	120	0.1	10.2	6.1
Sliced cheese	100	374	0	29.2	25.5
Cream cheese (natural)	33	87	0.6	7.8	3
Cream cheese (herbs)	40	124	1	11.6	3.2
Total	3330	4562	553	226	123

All values are rounded to the closest decimal.

Measurement of hunger, thirst, mood, vigilance, and olfactory function

Hunger, thirst, and the perceived trustworthiness of the experimenter were rated on VAS (0–100 mm). Self-reported mood was assessed on 5-point scales covering the categories good/bad mood, alertness/sleepiness and calmness/agitation (MDBF; Steyer et al., 1997). In the 5-min PC-based vigilance task (Diekelmann et al., 2011), a red dot appears at random intervals either on the left or the right side of the screen, and subjects are required to press the respective key as fast as possible, receiving immediate feedback in the form of the reaction time for correct responses or an error message. Mean reaction time was registered and adjusted for mistakes by adding the square root of the product of the mean reaction time and the number of mistakes. Olfactory function was tested 60 min after the test buffet with the validated Sniffin' Sticks commercial test kit (Burghart Elektro- und Feinmechanik GmbH, Wedel, Germany) that covers the three dimensions of olfactory threshold, discrimination, and identification (Hummel et al., 2007).

Measurement of energy expenditure, plasma glucose and hormonal parameters

Resting energy expenditure (expressed as kcal per day) and the respiratory quotient were measured via indirect calorimetry using a DeltaTrac II ventilated-hood system (SensorMedics Vmax 29n; VIASYS® Healthcare, Yorba Linda, CA, USA). Before each use, the device was calibrated with calibration gas to 5 % CO_2 and 95 % O_2. Calorimetric measurements (30 min each) took place at 0910 h (baseline), immediately after intranasal substance administration at 0945 h to assess effects of intranasal oxytocin alone, and again at 1110 h (that is, after the ad-libitum test buffet) to register postprandial energy expenditure. Substrate utilization in the obese participants was calculated based on the following stoichiometric equations: carbohydrate oxidation $(\mathrm{g/min}) = 4.21 \times \mathrm{VCO_2} - 2.962 \times \mathrm{VO_2} - 0.4 \times \mathrm{n}$; fat oxidation $(\mathrm{g/min}) = 1.695 \times \mathrm{VO_2} - 1.701 \times \mathrm{VCO_2} - 1.77 \times \mathrm{n}$ where n represents ni-

trogen excretion from protein oxidation (estimated at $135\,\mu g \times kg \times min^{-1}$; Jeukendrup and Wallis, 2005).

Blood samples obtained in the obese participants to assess serum insulin, C-peptide, cortisol and growth hormone, as well as plasma glucose, lactate, ACTH and non-esterified fatty acids (NEFA) were centrifuged, and samples were stored at $-80\,°C$. Concentrations of glucose and lactate in fluoride plasma were determined with the ADVIA® Chemistry XPT clinical chemistry analyzer according to the hexokinase and colorimetric lactatoxidase method, respectively. Serum insulin, C-peptide and cortisol concentrations were measured with the ADVIA® Centaur XPT immunology analyzer and concentrations of growth hormone and ACTH were measured using the Immulite® 2000 XPi Immunoassay-System. Plasma NEFA were determined according to the ACS-ACOD method (NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany) with the ADVIA® Chemistry XPT clinical chemistry analyzer (all instruments from Siemens Healthcare Diagnostics, Eschborn, Germany).

Statistical analysis

Analyses were generally based on analyses of variance (ANOVA) with the between-subject factor 'group' (obese/normal weight) and the within-subject factors 'treatment' (oxytocin/ placebo), 'time,' 'nutrient' (carbohydrates/protein/fat), 'food type' (savory/sweet/neutral), 'snack type' (salty/sweet/bland) and 'taste' (salty/sweet) as appropriate. Note that comparisons between groups focused on the main parameters of energy intake and expenditure; for detailed results of normal-weight subjects see Ott et al. (2013). Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Areas under the curve (AUC) were calculated according to the trapezoidal rule. Significant ANOVA and AUC effects were specified by pairwise two-sided t-tests. For blood parameters, baseline adjustment was achieved by subtracting individual baseline values from individual post-intervention measurements. All data are presented as means $\pm$ SEM. A P value < 0.05 was considered significant.

2.3 Results

Oxytocin reduces food intake to a greater extent in obese than normal-weight men

In the obese subjects, oxytocin in comparison to placebo reduced overall food consumption from the breakfast buffet by ∼10 % ($F_{(1,17)} = 5.26$, $P < 0.04$ for treatment; Table 2.3 and Figure 2.2a and b). In contrast, the normal-weight subjects did not alter their breakfast intake after oxytocin administration ($P > 0.6$ for all comparisons including treatment × macronutrients and treatment × food types; $F_{(1,36)} = 3.48$, $P = 0.07$ for group × treatment). The difference between obese and normal-weight men in the hypophagic effect of oxytocin especially concerned the intake of carbohydrates ($F_{(1,36)} = 4.44$, $P = 0.042$ for group × treatment). In the obese subjects alone, oxytocin did not exert differential effects on the intake of macronutrients or food types (all $P > 0.3$). In general, obese ate more than normal-weight participants in the respective placebo conditions ($F_{(1,36)} = 4.27$, $P < 0.05$ for group), in particular more carbohydrates and proteins ($F_{(2,72)} = 9.25$, $P < 0.001$ for group × macronutrient). In both groups, hunger ratings were comparable at baseline ($P > 0.2$) and generally unaltered by oxytocin ($P > 0.9$), falling to comparably low values of around 12 % of the maximal score after breakfast ($P > 0.2$; $F_{(1,36)} = 350.54$, $P < 0.001$ for time; $P > 0.12$ for treatment effects). Thirst ratings were likewise unaffected by oxytocin ($P > 0.4$ for all comparisons).

In the snack test during the postprandial period, oxytocin in comparison to placebo induced a reduction in total snack intake of 22 % across all subjects ($F_{(1,36)} = 13.37$, $P < 0.001$ for treatment; Table 2.3 and Figures 2.2c and d). This effect did not differ between the obese and the normal-weight groups ($F_{(1,36)} = 0.01$, $P > 0.9$) although it was of a greater statistical effect size in the obese ($F_{(1,17)} = 9.89$, $P = 0.006$; $\omega_p^2 = 0.31$) compared with the normal-weight participants ($F_{(1,19)} = 5.5$, $P = 0.03$; $\omega_p^2 = 0.18$). In both groups,

it was especially pronounced for chocolate cookie consumption ($F_{(2,56)} = 3.88$, $P < 0.04$ for treatment × snack type; Table 2.3; Figure 2.2d). The oxytocin-induced reductions in total and chocolate snack intake found in the obese subjects remained significant when corrected for total calorie and carbohydrate consumption during the preceding breakfast buffet (both $P \leqslant 0.003$). Although breakfast and snack consumption per se were unrelated in both conditions ($P > 0.4$), the oxytocin-induced reduction in breakfast intake was inversely related to the respective attenuation of snack intake ($r = -0.48$, $P < 0.05$). Intake and rated palatability of chocolate cookies by far exceeded those of the remaining snacks when analyzed across conditions in the obese subjects ($F_{(1,17)} = 70.52$, $P < 0.001$ for snack type). Sweetness and saltiness ratings were highest for chocolate cookies and salt crackers, respectively ($F_{(2,34)} = 221.28$, $P < 0.001$ for snack type × taste). Generally comparable rating patterns were obtained in the normal-weight subjects ($P > 0.15$ for all group comparisons). Oxytocin did not affect ratings for chocolate cookies and salt crackers (all $P > 0.15$) in the obese but increased the rated palatability (placebo, 2.89 ± 0.50; oxytocin, 3.82 ± 0.47; $P < 0.03$) and, to a lesser extent, perceived sweetness of rice waffles (placebo, 1.74 ± 0.40; oxytocin, 2.63 ± 0.61; $P < 0.06$).

Energy expenditure is not acutely affected by oxytocin administration

Oxytocin did not alter resting energy expenditure assessed by indirect calorimetry during the entire experimental period in the obese ($F_{(1,17)} = 0.03$, $P > 0.87$ for treatment × time; $F_{(1,17)} = 0.32$, $P > 0.58$ for treatment; Figure 2.2f) and the normal-weight subjects (all $P > 0.12$; $F_{(1,36)} = 0.47$, $P > 0.49$ for group × treatment × time). The rise in energy expenditure by ∼22 % found in the obese subjects between the fasting state (baseline) and the postprandial state reflects diet-induced thermogenesis (that is, the energy emitted as heat during metabolizing of food; $F_{(1,17)} = 200.91$, $P < 0.001$ for time). In the postprandial period, oxytocin compared with placebo appeared to decrease respiratory quotient (placebo, 0.90 ± 0.02; oxytocin, 0.83 ± 0.02) and carbo-

Table 2.3 Calorie intake during the test buffet and snack test.

	Food type	Obese men			Normal-weight men			
		Placebo	Oxytocin	*P* value	Placebo	Oxytocin	*P* value	*P* value (group × treatment)
Eating assessment								
Breakfast (kcal)	Total	1421 ± 99	1274 ± 92	0.035	1180 ± 103	1190 ± 105	0.84	0.07
	Carbohydrates	736 ± 51	659 ± 57	0.07	517 ± 41	540 ± 41	0.43	0.042
	Fat	478 ± 54	430 ± 44	0.15	517 ± 54	509 ± 57	0.84	0.43
	Protein	207 ± 15	187 ± 13	0.08	145 ± 16	142 ± 14	0.82	0.26
	Neutral foods	726 ± 68	681 ± 74	0.26	519 ± 62	544 ± 68	0.42	0.16
	Sweet foods	409 ± 53	375 ± 48	0.46	346 ± 40	336 ± 42	0.64	0.64
	Savory foods	287 ± 45	219 ± 31	0.040	314 ± 38	309 ± 29	0.91	0.19
Snack test (kcal)	Total	216 ± 24	162 ± 27	0.006	283 ± 44	227 ± 44	0.03	0.95
	Chocolate cookies	144 ± 25	116 ± 24	0.030	185 ± 41	138 ± 38	0.007	0.34
	Rice waffles	22 ± 5	16 ± 2	0.26	18 ± 3	13 ± 2	0.15	0.78
	Salt crackers	51 ± 11	30 ± 6	0.07	81 ± 19	75 ± 16	0.75	0.49

Mean ± SEM values are indicated. *P* values are derived from paired, two-tailed *t*-tests and group × treatment ANOVA interactions, respectively. Obese men, $n = 18$; normal-weight men, $n = 20$.

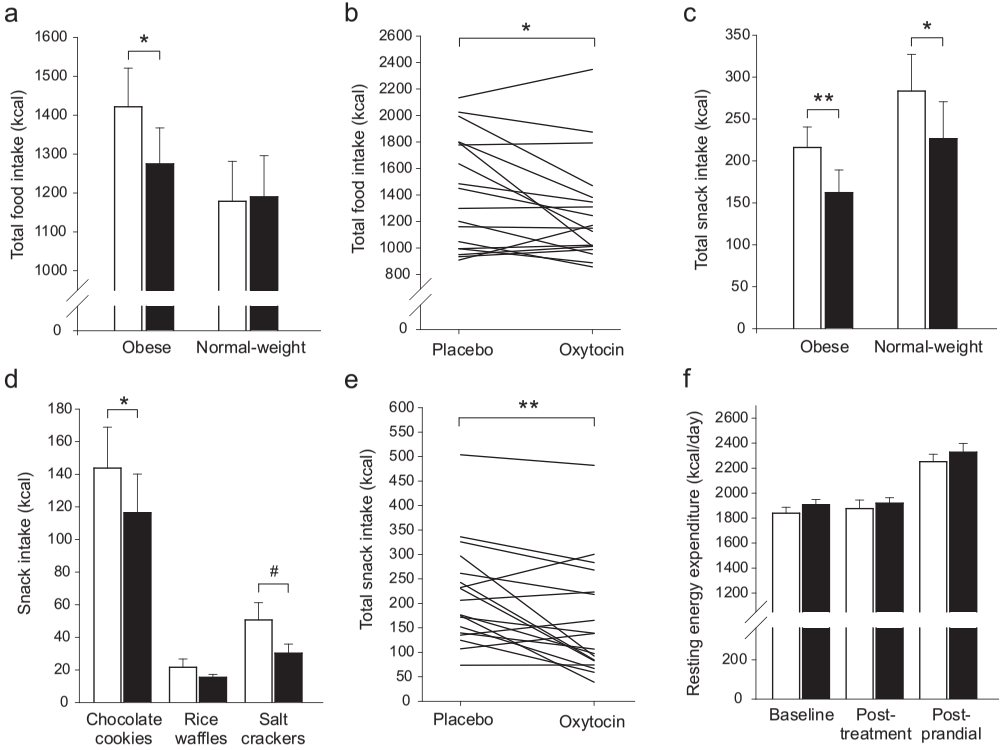

Figure 2.2 Food intake results. (Caption on next page.)

Figure 2.2 (Previous page.) Upper panels indicate mean ± SEM total intake (kcal) from the test breakfast after placebo (white bars) or oxytocin (black bars) administration in obese ($n = 18$) and normal-weight men ($n = 20$) (**a**) and individual total food intake with values of both sessions connected by lines in obese men (**b**), as well as total snack intake in obese and normal-weight men (**c**). Lower panels depict, in the group of obese men, snack intake (kcal) according to snack categories (**d**), individual total snack intake with individual values of both sessions connected by lines (**e**), as well as resting energy expenditure during the baseline (0910–0940 h), post-treatment (0945–1015 h), and postprandial periods (1110–1140 h) (**f**). $^{\#}P < 0.08$, $^{*}P < 0.05$, $^{**}P < 0.01$ for comparisons between conditions (pairwise two-sided t-tests).

hydrate utilization (placebo, 0.26 ± 0.03 g/min; oxytocin, 0.17 ± 0.02 g/min; both $P = 0.02$) whereas increasing fat utilization (placebo, 0.05 ± 0.01 g/min; oxytocin, 0.10 ± 0.01 g/min; $P = 0.01$) in the obese group. However, these effects were no longer significant when the analysis of respiratory quotient was corrected for total breakfast calorie intake and analyses of carbohydrate oxidation and fat utilization were corrected for carbohydrate and fat intake, respectively (all $P > 0.17$).

Oxytocin reduces HPA axis activity and the glucose response to food intake in obese and normal-weight subjects

During baseline, none of the blood parameters differed between conditions (all $P > 0.18$). Oxytocin exerted a suppressive effect on fasting HPA axis activity in the obese men, reducing plasma ACTH and serum cortisol concentrations between administration and breakfast intake ($F_{(1,17)} = 6.20$, $P = 0.02$; $F_{(1,17)} = 5.81$, $P < 0.03$, respectively, for treatment effects, and $t_{(17)} = 2.65$, $P < 0.02$; $t_{(17)} = 2.30$, $P = 0.03$, respectively, for the difference in $AUC_{0930–1025\,h}$; Figures 2.3a and b), whereas concentrations were comparable between conditions after breakfast (all $P \geqslant 0.12$ for respective comparisons). In the normal-weight individuals respective effects extended to the postpran-

dial period (Ott et al., 2013) but still were largely comparable to the pattern found in obese subjects ($P > 0.14$ for respective group × treatment interactions). Supplementary analyses in the group of obese subjects indicated that the oxytocin-induced decreases in preprandial cortisol concentrations ($AUC_{0930–1025\,h}$) were not significantly correlated with measures of breakfast intake (all $P > 0.72$). Likewise, decreases in postprandial cortisol levels ($AUC_{1100–1145\,h}$) were not significantly correlated with cookie intake (all $P > 0.12$, Pearson's coefficients).

Circulating concentrations of glucose, lactate, insulin, and C-peptide showed the expected meal-related increases across conditions in the obese participants (all $P < 0.001$ for time; Figures 2.3c–e). Whereas levels of lactate, insulin, and C-peptide were not affected by oxytocin administration (all $P > 0.19$), oxytocin exerted a sustained suppressive effect on glucose levels during the postprandial period ($F_{(3,50)} = 3.77$, $P < 0.02$ for treatment × time; Figure 2.3c). This effect was still evident when adjusted for preceding total and carbohydrate-specific breakfast intake (both $P < 0.05$), but statistically unrelated to the oxytocin-induced decrease in cortisol concentrations ($P > 0.43$). An oxytocin-triggered reduction in postprandial glucose levels was likewise found in normal-weight individuals ($F_{(1,36)} = 0.15$, $P = 0.7$ for group × treatment). In the obese subjects, total plasma NEFA concentrations were suppressed after breakfast intake ($F_{(1,19)} = 131.42$, $P < 0.001$ for time) without significant treatment effects ($P > 0.67$; Figure 2.3f); circulating concentrations of growth hormone were comparable between conditions (all $P > 0.24$).

Olfactory performance, mood, vigilance, perceived trustworthiness, and treatment awareness

In the olfactory task, no treatment effects on perceptual thresholds, olfactory discrimination and olfactory identification emerged in the obese participants (all $P > 0.17$). Self-rated mood, reaction times in the vigilance task and the perceived trustworthiness of the experimenter were likewise unaffected

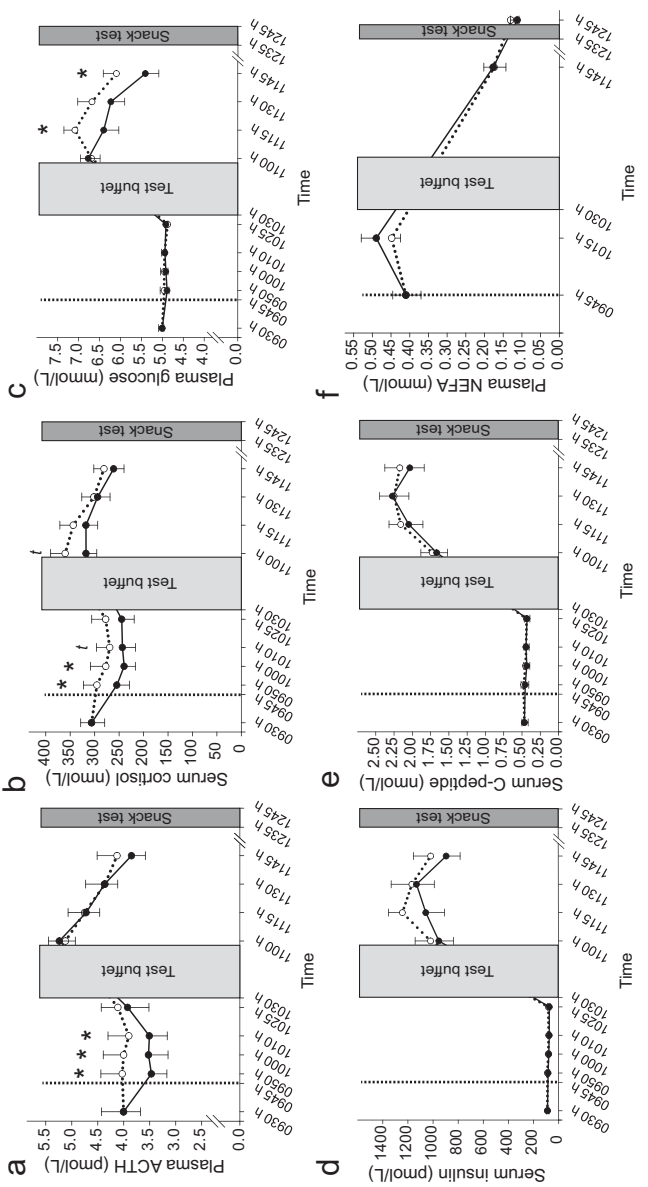

Figure 2.3 Blood parameters. (Caption on next page.)

Figure 2.3 (Previous page.) Mean ± SEM concentrations of plasma adrenocorticotropic hormone (**a**), serum cortisol (**b**), plasma glucose (**c**), serum insulin (**d**), serum C-peptide (**e**) and plasma NEFA (**f**) assessed before (averaged across the 0915 h and 0930 h baseline values) and after intranasal administration (vertical dotted line) of oxytocin (24 IU; black circles and solid lines) and placebo (vehicle; white circles and dotted lines) in obese men ($n = 18$). Subjects ate a test breakfast from 1030–1100 h and ingested snacks under the pretext of a taste test from 1235–1245 h. Mean baseline values of both conditions are averaged to a common baseline. $^{*}P < 0.05$, $^{t}P < 0.1$ for comparisons between conditions (pairwise two-sided t-tests).

(all $P > 0.27$). Eight of the 18 participants (44 %) correctly guessed their respective treatment conditions ($\chi^2_{(3;N=18)} = 2.68$, $P > 0.6$), indicating that the participants overall gained no treatment awareness. All of these results were well comparable with the findings in the group of normal-weight subjects (Ott et al., 2013).

2.4 Discussion

We demonstrate that the acute intranasal administration of oxytocin inhibits reward- but also hunger-driven food intake in obese men and that this effect is not compensated by changes in energy expenditure. In normal-weight men, the oxytocin-induced reduction in calorie consumption was restricted to snacking, indicating that the inhibitory effect of oxytocin on food intake is generally larger in obese than normal-weight subjects. Oxytocin moreover attenuated secretory activity of the HPA axis and curbed the postprandial excursion of glucose levels in obese and normal-weight men, suggesting an insulin-sensitizing action of the hormone.

Oxytocin has been shown in a number of experiments in rodents to inhibit feeding after intracerebroventricular injection (Arletti et al., 1989; Olson et al., 1991) as well as after peripheral administration, which both supposedly

trigger hypothalamic oxytocin release in a feedforward fashion (Maejima et al., 2011; Morton et al., 2012). Recently, comparable effects have been obtained after intranasal administration (Maejima et al., 2015). Oxytocin's restraining effect on snacking in the postprandial period may be mediated via oxytocinergic projections to the brain reward circuit (Melis et al., 2009), an assumption supported by signs of oxytocin-induced enhancements in the perceived palatability and sweetness of the moderately appealing snacks (rice waffles) offered to our obese subjects. The fact that the attenuating effect of oxytocin on snack intake focused on chocolate cookies, which were preferentially eaten and rated most palatable, further suggests a strong rewardrelated component of the observed oxytocin action.

In the obese in contrast to normal-weight subjects, the hypophagic effect of oxytocin clearly pertained to meal intake in the fasted state, decreasing calorie consumption from the breakfast buffet by 10 %. The conclusion that oxytocin elicits stronger effects in obese than normal-weight humans is supported by findings of more pronounced weight-lowering oxytocin effects in diet-induced obese compared to control rats, which were associated with stronger oxytocin-triggered increases in c-Fos expression in the area postrema and the nucleus of the solitary tract of the hindbrain (Morton et al., 2012). Moreover, prolonged anorexigenic effects of oxytocin have been reported in mice kept on a high-fat diet in comparison to control animals (Maejima et al., 2011). Enhanced oxytocin sensitivity in the obese state has been assumed to be mediated via improved high-affinity receptor binding of oxytocin due to elevated cholesterol levels (Ho and Blevins, 2013), and would be in line with reports that circulating oxytocin concentrations, which were not measured in the present study, are inversely related to BMI and waist circumference (Qian et al., 2014). In recent experiments by Lawson et al. (2015) that focused on oxytocin effects in the fasted state, ad-libitum energy intake was reduced in both normal- and overweight men. The contrast to our results might stem from differences in study design including the more pronounced induction of foodanticipatory processes in the former study (Lawson et al., 2015). Taking

into account potential rebound effects, our study indicates that the impact of oxytocin on breakfast intake in obese men is not compensated for by increases in postprandial thermogenesis and is still evident when subjects are allowed to snack. In conjunction with our observation that stronger anorexigenic effects of oxytocin during breakfast are associated with relatively smaller respective reductions in snack intake, this pattern points to a global, albeit tightly regulated enhancement of satiety signaling by oxytocin. This effect is not readily reflected in subjective hunger ratings which accords with the general treatment unawareness of our subjects. In line with our results, pilot data suggest that long-term oxytocin administration may support weight loss in obese patients (Zhang et al., 2013).

The finding of reduced breakfast intake after oxytocin administration in the fasted state suggests that the peptide restrains hunger-driven, primarily homeostatically regulated eating, although under normal circumstances ingestive behavior triggered by food deprivation also involves a strong hedonic component. Our analyses did not yield indicators that the inhibitory effect of oxytocin on breakfast intake in the obese men focused on such hedonic aspects of food consumption or could be considered a mere consequence of oxytocin acting on reward processing. Still, in obese compared with normal-weight individuals, brain reward circuits show greater activation in response to palatable versus bland foods (Nummenmaa et al., 2012) whereas fronto-cortical inhibitory control of food intake appears to be reduced (Ziauddeen et al., 2012). Therefore, the enhanced hypophagic effect of oxytocin in obese compared with normal-weight subjects may also be interpreted against the background of a stronger reinforcing quality of food intake and the tendency to overeat pleasurable food in obesity (Gibson, 2006). In line with this, our obese subjects consumed $\sim$240 kcal more than the normal-weight subjects in the respective placebo conditions. In animal experiments, oxytocin receptor antagonists acutely attenuate the anorexigenic impact of hormones like corticotropin-releasing hormone (Olson et al., 1991), whereas alpha-melanocyte stimulating hormone, a catabolic messenger, triggers oxytocin

release from supraoptic neurons (Sabatier et al., 2003). Oxytocin might furthermore restrain food intake by acting on downstream mediators of the leptin signal (Blevins et al., 2004) and enhancing cholecystokinin signaling (Lawson et al., 2015). Tests of olfactory function in our experiments indicated that the decrease in food intake most likely was not mediated by direct effects on sensory processing. Biasing effects on ingestive behavior related to demand characteristics and social desirability were excluded by respective interviews.

Oxytocin administration did not induce significant alterations in fasting and postprandial resting energy expenditure. Signs of decreases in the respiratory quotient and carbohydrate utilization and respective increases in fat utilization emerging in the obese subjects during the postprandial period disappeared after adjustment for preceding calorie intake, suggesting that they might rather be attributed to oxytocin-induced decreases in macronutrient consumption. In diet-induced obese rats losing weight during systemic chronic oxytocin administration, the decrease in energy expenditure normally associated with weight loss was prevented by oxytocin, probably via effects on hypothalamic thermoregulation (Morton et al., 2012). In obese nonhuman primates, chronic subcutaneous administration of oxytocin increased energy expenditure in the dark cycle by 14 % (Blevins et al., 2015). Thus, our finding suggests that rather than exerting acute effects on energy expenditure, oxytocin contributes to its regulation on a long-term basis, which might turn out to be a critical factor in future clinical applications. Oxytocin blunted plasma glucose excursions during the postprandial period in obese as well as normal-weight subjects. Notably, this effect was still evident after correcting the data for differences in calorie and carbohydrate intake from the breakfast buffet, which suggests an oxytocin-induced improvement in insulin sensitivity. Accordingly, oxytocin enhanced insulin sensitivity and glucose tolerance in rodent models of diet-induced obesity independent of its effects on body weight (Morton et al., 2012) and also in rhesus monkeys with diet-induced obesity (Blevins et al., 2015). In the study by Lawson et al. (2015) intranasal oxytocin reduced fasting insulin secretion without affecting glucose levels,

which also implies insulin-sensitizing properties of oxytocin and underlines that the role of oxytocin in glucose homeostasis in humans is in need of further investigation. The reduction in HPA axis activity by oxytocin extends previous findings of an attenuating impact of intranasal oxytocin on cortisol secretion in response to stress (Cardoso et al., 2013). Acute and chronic activation of endocrine stress axes facilitates the intake of 'comfort food,' that is, highly palatable food (Dallman et al., 2003). Vice versa, consuming sucrose reduces HPA axis activity in a negative feedback loop by activation of central nervous reward circuits (Ulrich-Lai et al., 2010). With regard to the link between emotional regulation and food intake (Gibson, 2006), a mediation of oxytocin's attenuating impact on food intake via reductions in HPA axis activity might be expected to be of particular relevance in obese subjects. However, in our studies the two phenomena were statistically related only in normal-weight participants (Ott et al., 2013).

In summary, our study indicates a restraining effect of oxytocin on hunger- and reward-driven eating behavior in obese humans that goes along with a suppression of HPA axis activity and signs of enhanced peripheral insulin sensitivity. In contrast to messengers like insulin and leptin, whose anorexigenic impact is blunted when body weight is increased (Begg and Woods, 2013), oxytocin exerts a potent acute inhibition of food intake in obese subjects which even surpasses the effect found in normal-weight humans. These results clearly warrant further investigations on long-term oxytocin effects in metabolic disorders.

3 | Study II: Insulin and sleep-related HPA axis activity

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3.1 Introduction

The efficient regulation of neuroendocrine stress systems including the HPA axis is relevant not only with regard to their activation in response to environmental challenges, but also to endogenous circadian rhythms (Buckley and Schatzberg, 2005). Release of ACTH and cortisol is triggered by CRH and regulated via glucocorticoid feedback at the hippocampal and hypothalamic levels. In the first night-half, ACTH and cortisol concentrations reach nadir values which indicate basal secretory HPA axis activity in the absence of external stimulation (Kern et al., 1996). Elderly humans display changes in sleep-related neuroendocrine patterns, in particular a decrease in growth hormone release and an increase in HPA axis activity, which are paralleled by a reduction in the amount of time spent in rapid eye-movement (REM) and slow-wave sleep (Ohayon et al., 2004; Van Cauter et al., 2000). Since

nocturnal hypercortisolism in aging individuals as a result of weakened central nervous control of HPA axis activity is assumed to contribute to disorders such as obesity, depression, and cognitive impairments (McEwen, 2000; Popp et al., 2015), identifying non-glucocorticoid factors that inhibit HPA axis activity may contribute to new approaches in the prevention and treatment of these ailments. Intranasal insulin, which is known to reach the brain compartment within one hour after administration (Born et al., 2002), has been repeatedly shown to attenuate HPA axis secretion (Benedict et al., 2004; Böhringer et al., 2008; Hallschmid et al., 2008). We investigated the effect of intranasal insulin on HPA axis secretion during early sleep in healthy elderly compared to young subjects. Considering the association between increased age and nocturnal HPA axis hyperactivity (Van Cauter et al., 2000) as well as impaired central nervous insulin signaling (Biessels and Reagan, 2015), we expected an inhibitory effect of insulin on ACTH and cortisol secretion particularly in elderly subjects.

3.2 Methods

Subjects

Fourteen healthy elderly volunteers (8 men, 6 women; age, mean $\pm$ SEM, 70.00 ± 0.63 years; BMI, $24.83 \pm 0.66\,\mathrm{kg/m^2}$) and 30 healthy young individuals (16 men, 14 women; age, 23.63 ± 0.45 years; BMI, $22.93 \pm 0.33\,\mathrm{kg/m^2}$) participated in, respectively, Experiments I and II. In the young participants, sleep-related electroencephalographic power spectra and memory performance were analyzed in addition and have been reported elsewhere (Feld et al., 2016). Relevant illness of our subjects was excluded by medical history and clinical examination. All subjects reported having a regular sleep-wake cycle and were not on medication except for estrogen-dominant oral contraceptives taken by all young women. All subjects spent one night in the sleep laboratory to adapt to the experimental procedure; visual inspection

of respective polysomnographical results ensured that none of the subjects displayed abnormal sleep characteristics. They gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Study design and procedure

Experiments followed a balanced, placebo-controlled, double-blind, within-subject, crossover design. All participants took part in two sessions which were identical except for the administration of insulin or placebo. Sessions were scheduled to be apart as close to 28 days as possible and the young women did not participate during their menstruation phases. Subjects were told to get up around 0700 h and to abstain from naps or caffeine intake on experimental days, and to follow their usual dinner routines around 1800–1900 h. Experiments started around 2000 h. Electrodes were attached for standard polysomnographic recordings including electroencephalogram (at sites C3 and C4) that were scored offline according to standard criteria as wake, sleep stages N1, N2, N3, and REM sleep. At 2230 h, subjects were intranasally administered a total dose of 1.6 ml insulin (160 IU; Insulin Actrapid®; Novo Nordisk, Mainz, Germany) or vehicle (carrier solution) via sixteen 0.1 ml puffs (8 per each nostril) in 1-min intervals. Subjects were allowed to sleep between 2300 h (lights off) and 0700 h (awakening), which corresponded to the period of polysomnographical recordings.

Blood sampling and control measures

Peripheral blood for the assessment of serum cortisol, C-peptide, insulin, as well as glucose and plasma ACTH was sampled during a pre-sleep baseline and at 20- to 40-min intervals during the first night-half until 0320 h (see Figure 3.1). For the group of elderly subjects, slight adjustments in the blood sampling schedule were introduced in order to increase the feasibility of repeated blood sampling and to restrict the burden of experimental participa-

tion, resulting in minor respective differences to the group of young subjects outside the main time period of interest (2300–0020 h). Blood was drawn via long thin tubes enabling blood collection from an adjacent room while minimizing disruptive effects on the subject's sleep. Routine assays were used to determine concentrations of ACTH, cortisol, C-peptide (all Immulite, DPC, Los Angeles, CA), insulin (Insulin ELISA Kit, Dako, Glostrup, Denmark), and glucose (HemoCue® Glucose 201 Analyzer, HemoCue AB, Ångelholm, Sweden).

Appetite, thirst, and sleepiness were self-reported on visual analogue scales (0–100 mm) in both experiments. Mood, well-being and subjective sleep quality were assessed via established rating scales, and heart rate and blood pressure were monitored before and after sleep.

Statistical analyses

For analysis of sleep stages, one female and one male participant of Experiment II were excluded because of data loss. Analyses relied on Greenhouse-Geisser-corrected analyses of covariance (ANCOVA) for repeated measurements with baseline values as covariates and the between subject-factor 'sex' (male/female) and the within-subject factors 'treatment' (insulin/placebo) and 'time.' Areas under the curve calculated according to the trapezoidal rule and single time points were compared by *t*-tests. For comparisons between elderly and young subjects, linear mixed models were used with the between-subject factor 'age' (elderly/young). In addition, individual slope coefficients were obtained in the form of beta weights of linear regression lines fitted to ACTH and cortisol values between 2300–0320 h, and were compared between groups by two-tailed unpaired *t*-tests. A P value < 0.05 was considered significant; data are presented as mean $\pm$ SEM.

3.3 Results

Increased HPA axis activity during early sleep in elderly compared to young subjects

Cortisol $AUC_{2300–0320\,h}$ values were higher in elderly compared to young subjects ($13,472 \pm 584$ vs. $11,034 \pm 972\,nmol/L \times min$, $t_{(41)} = -2.22$, $P = 0.032$; $t_{(42)} = -0.74$, $P = 0.463$ for respective ACTH values). Accordingly, the increases in ACTH and cortisol concentrations emerging across the first night-halves of the respective placebo conditions were stronger in elderly than young subjects (beta weight means, ACTH, 0.15 ± 0.02 vs. 0.06 ± 0.01, $t_{(19)} = -3.48$, $P = 0.003$; cortisol, 6.81 ± 1.66 vs. 0.64 ± 1.21, $t_{(41)} = -2.96$, $P = 0.005$). Nadir values of ACTH and cortisol did not differ between groups regarding levels (all $P > 0.20$) and timing ($P > 0.24$). Cortisol $AUC_{2300–0320\,h}$ values of the respective placebo conditions were moderately correlated with BMI in the elderly ($r = 0.54$, $P = 0.048$), but not in the young subjects ($r = -0.15, P = 0.43$).

Intranasal insulin dampens early-sleep cortisol concentrations in elderly subjects

Blood parameters did not differ between conditions during baseline (all $P \geqslant 0.15$). In the elderly subjects, insulin compared to placebo administration decreased cortisol concentrations during the first night-half (2300–0320 h; $F_{(1,10)} = 5.83$, $P = 0.036$ for treatment; $t_{(13)} = 2.40$, $P = 0.03$ for the difference in $AUC_{2300–0020\,h}$), whereas this effect was absent in young participants (all $P > 0.44$; $F_{(22,129)} = 2.23$, $P = 0.003$ for treatment $\times$ time $\times$ age; Figure 3.1a). In the elderly, the insulin-induced decrease in cortisol concentrations emerged irrespective of the subjects' sex ($P > 0.32$). Its extent was proportional to the respective cortisol nadir level in the placebo condition ($r = 0.60$, $P = 0.03$, Pearson's coefficient), but was statistically unrelated to changes in nocturnal levels of insulin, C-peptide, and glucose (all $P > 0.38$;

$P > 0.46$ for the group of young subjects). Plasma ACTH levels were comparable between groups ($P = 0.13$) and were not influenced by treatment (both $P \geqslant 0.56$ for treatment; all $P \geqslant 0.10$ for single time point comparisons; Figure 3.1b).

Serum insulin and blood glucose concentrations

Serum insulin concentrations were not affected by insulin administration in the elderly subjects (all $P \geqslant 0.58$). In the young participants they rose shortly after substance administration but were comparable between conditions thereafter ($P \geqslant 0.73$ for treatment $\times$ time; Figure 3.1c, upper lines), with no statistical differences to the group of elderly subjects ($P = 0.24$ for age). In both groups, serum C-peptide concentrations slightly decreased after intranasal insulin administration (both $P < 0.1$ for differences at 2320 h), but did not differ between conditions thereafter ($P = 0.68$ and $P = 0.85$, respectively, for treatment $\times$ time; $P > 0.62$ for age). In accordance with the ephemeral increase in peripheral insulin concentrations, in the group of young subjects blood glucose levels were acutely decreased after peptide administration at 2300 h, but subsequently returned to placebo condition values ($P = 0.65$ for treatment $\times$ time). Across conditions, blood glucose levels were lower in elderly than young individuals ($P < 0.001$ for age; Figure 3.1c, lower lines).

Sleep parameters and control measures

Independent of the treatment, elderly in comparison to younger subjects had longer wake and light sleep (N1) periods at the expense of slow wave (N3) and REM sleep ($F_{(2,92)} = 29.78$, $P < 0.001$ for sleep stage $\times$ age; Table 3.1) assessed across the whole night. Sleepiness ratings and subjective estimations of sleep onset and sleep quality did not differ between groups (all $P \geqslant 0.20$). Intranasal insulin compared to placebo generally did not alter sleep latency, whole-night sleep architecture and total sleep time (all $P > 0.29$). Early sleep (2300–0320 h) likewise was unaffected by insulin in the elderly (all

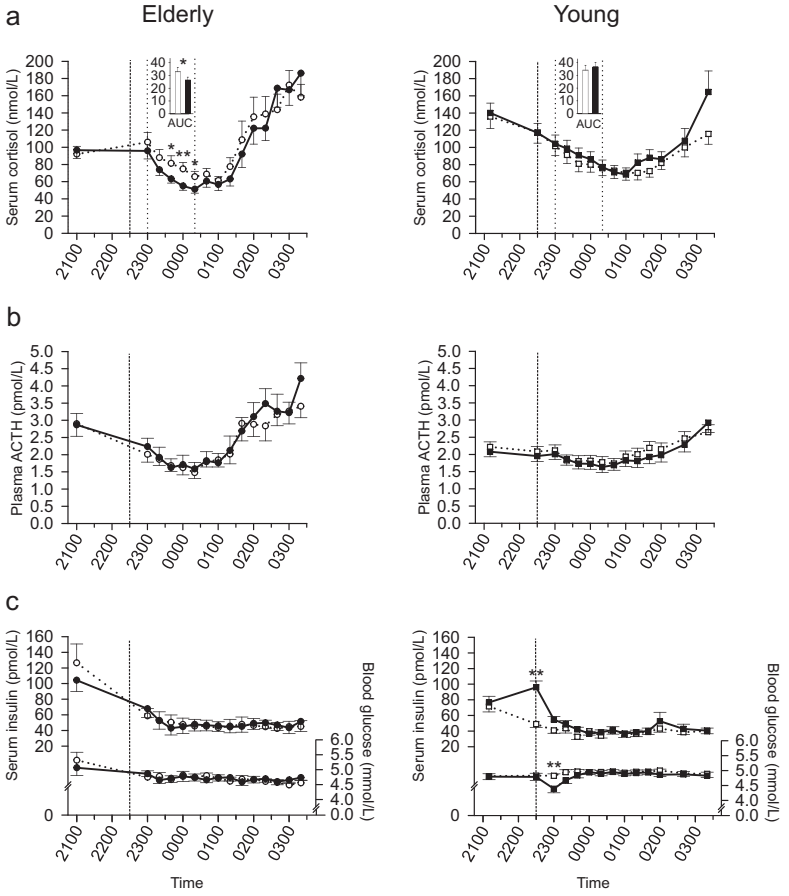

Figure 3.1 Blood parameters. Mean ± SEM concentrations of serum cortisol (**a**; inserts depict $AUC_{2300–0020\,h}$ values in $(nmol/L \times min)/100$), plasma ACTH (**b**), serum insulin and blood glucose (**c**; upper and lower lines, respectively) measured in elderly ($n = 14$; left panels) and young participants ($n = 30$; right panels) who were intranasally administered insulin (160 IU; black dots/squares/bars and solid lines) or placebo (white dots/squares/bars and dotted lines) at 2230 h (dotted line). $^{*}P < 0.05$, $^{**}P < 0.01$ for comparisons between groups (t-tests).

$P > 0.13$) and young subjects ($P > 0.20$), as was subjective sleep quality ($P > 0.53$; Table 3.1). Self-rated mood as well as hunger, thirst, and sleepiness ratings were not affected by insulin (all $P \geqslant 0.15$; see Table 3.1 for these and cardiovascular measures). Elderly compared to young subjects showed a trend towards elevated systolic blood pressure values ($P = 0.07$ for age). Heart rate and blood pressure were generally not affected by intranasal insulin (all $P \geqslant 0.18$).

3.4 Discussion

We demonstrate that intranasal insulin, which has been shown to reach the brain compartment (Born et al., 2002) and elicit functional brain effects (Hallschmid et al., 2004b) within one hour after administration, reduces circulating concentrations of cortisol during the first night-half in elderly subjects. This result is in accordance with reports by our and other groups that intranasal insulin attenuates HPA axis activity in young men after long-term treatment (Benedict et al., 2004) and when administered before a social stress test (Böhringer et al., 2008). Physiologically, circulating insulin reaches the CNS via a receptor-mediated saturable transport across the blood-brain barrier and might dampen HPA axis secretion by acting on hypothalamic nuclei and limbic structures like the hippocampal formation, which express large numbers of insulin receptors (Plum et al., 2005). In contrast, euglycemic hyperinsulinemic clamps rather increase HPA axis secretion (Fruehwald-Schultes et al., 1999), probably by boosting hormone synthesis in the adrenal cortex that is not effectively reached by intranasal insulin. In comparison to their young controls, our elderly participants displayed increased secretory HPA axis activity as well as an increased amount of time spent awake and in sleep stage 1, but a decreased duration of REM sleep and sleep stage 3, indicators of decaying sleep quantity and quality generally associated with aging (Van Cauter et al., 2000). Intranasal insulin did not induce significant changes in sleep architecture, which is in line with the lack of respective

Table 3.1 Sleep characteristics and control parameters.

	Elderly (mean ± SEM)			Young (mean ± SEM)			
	Insulin	Placebo	*P* value	Insulin	Placebo	*P* value	*P* value (group)
Total sleep (min)	456.57 ± 11.12	458.39 ± 5.68	0.89	461.59 ± 3.37	460.70 ± 4.57	0.84	0.57
Wake (%)	17.68 ± 2.36	15.36 ± 2.21	0.46	1.29 ± 0.31	1.33 ± 0.34	0.92	<0.001
N1 (%)	12.36 ± 1.37	11.34 ± 1.11	0.28	6.94 ± 0.73	6.86 ± 0.93	0.91	<0.001
N2 (%)	51.64 ± 2.97	53.61 ± 2.16	0.34	54.44 ± 1.36	55.01 ± 1.60	0.64	0.41
N3 (%)	5.40 ± 1.27	6.15 ± 1.42	0.27	15.89 ± 0.98	15.72 ± 0.97	0.83	<0.001
REM (%)	12.87 ± 1.48	13.49 ± 0.88	0.74	20.47 ± 0.83	19.98 ± 1.00	0.65	<0.001
Sleep latency (min)	28.71 ± 10.74	24.29 ± 5.59	0.70	21.40 ± 3.12	20.52 ± 4.59	0.81	0.29
Sleep quality	2.61 ± 0.32	2.50 ± 0.29	0.69	2.45 ± 0.12	2.42 ± 0.15	0.84	0.19
Sleepiness (evening)	48.57 ± 3.76	42.86 ± 5.07	0.39	50.76 ± 3.54	51.44 ± 3.52	0.87	0.18
Sleepiness (morning)	31.43 ± 7.33	33.33 ± 6.44	0.92	32.65 ± 4.15	38.95 ± 4.93	0.23	0.56
Hunger (evening)	19.29 ± 3.99	27.86 ± 3.95	0.008	32.99 ± 4.24	33.29 ± 4.88	0.95	0.10
Hunger (morning)	31.67 ± 3.45	31.67 ± 4.05	>0.99	67.87 ± 4.79	65.91 ± 4.99	0.63	<0.001
Thirst (evening)	30.00 ± 4.69	34.29 ± 4.88	0.31	49.24 ± 4.66	50.21 ± 3.01	0.81	0.003
Thirst (morning)	50.83 ± 3.99	46.67 ± 6.07	0.38	66.22 ± 4.15	64.34 ± 4.87	0.63	0.037
Mood (evening)	4.14 ± 0.23	3.71 ± 0.19	0.14	3.83 ± 0.16	4.07 ± 0.10	0.11	0.83
Mood (morning)	3.71 ± 0.32	3.36 ± 0.26	0.14	3.90 ± 0.14	3.73 ± 0.15	0.17	0.20
Well-being (evening)	4.77 ± 0.12	4.38 ± 0.29	0.24	4.47 ± 0.13	4.60 ± 0.11	0.26	0.56
Well-being (morning)	4.77 ± 0.17	4.62 ± 0.14	0.34	4.60 ± 0.12	4.60 ± 0.13	>0.99	0.52
Heart rate (evening)	62.46 ± 3.14	64.85 ± 2.83	0.35	60.30 ± 1.52	60.27 ± 1.63	0.98	0.19
Heart rate (morning)	62.75 ± 3.09	62.75 ± 2.73	>0.99	62.36 ± 1.56	61.29 ± 2.05	0.56	0.54
Systolic BP (evening)	129.14 ± 3.69	131.43 ± 3.90	0.58	123.43 ± 2.93	123.90 ± 2.74	0.79	0.05
Systolic BP (morning)	129.77 ± 4.23	124.15 ± 4.47	0.10	119.61 ± 2.30	122.61 ± 2.67	0.18	0.04
Diastolic BP (evening)	79.14 ± 1.97	81.79 ± 2.80	0.44	81.17 ± 2.27	81.83 ± 2.03	0.72	0.83
Diastolic BP (morning)	79.15 ± 1.57	77.54 ± 1.69	0.40	74.82 ± 1.80	74.46 ± 1.86	0.86	0.07

(Caption on next page.)

Table 3.1 (Previous page.) Mean ± SEM total sleep time and time spent in different sleep stages (relative to total sleep period) are indicated for both experimental nights (2300–0700 h). Psychological and physiological control variables were obtained before and after sleep. Subjective sleep quality was assessed by 4-point scale checklists including seven adjectives (e.g., 'calm,' 'relaxed'), and the average score was calculated. Appetite, thirst, and sleepiness were rated on visual analogue scales (0–100 mm) anchored at 'not at all' and 'extreme.' Mood and well-being were assessed on 5-point scales. BP, blood pressure (mmHg). *P* values derive from comparisons between conditions (*t*-tests) and group ANOVA main effects; elderly subjects, $n = 14$; young subjects, $n = 28$.

effects of peripheral hyperinsulinemia (Sturis et al., 1995). Although these observations suggest that insulin per se does not induce robust changes in sleep architecture, it cannot be excluded that higher doses of insulin or longer administration periods are necessary to modulate sleep quantity and quality in humans (Hallschmid et al., 2008; see Feld et al., 2016 for effects of intranasal insulin on electroencephalographic power spectra).

Intranasal insulin delivery reduced early-sleep cortisol concentrations in elderly but not in young participants. Importantly, sleepiness and subjectively experienced sleep onset and quality were comparable between groups, ruling out acute differences in psychological stress levels as mediators of the observed age-specific insulin effect. It is also unlikely that the small amount of intranasal insulin reaching the blood stream via spillover and causing a short drop in plasma glucose levels masked a centrally inhibiting insulin effect in the younger subjects by stimulating cortisol release. Spillover-induced increases in circulating insulin concentrations are negligible (Ott et al., 2015) compared to elevations needed to stimulate HPA axis activity under euglycemic conditions (Fruehwald-Schultes et al., 1999). Moreover, blood glucose levels remained clearly above the hypoglycemic threshold of 3.6–3.8 mmol/L where hormonal counter-regulation sets in. The fact that changes in cortisol were generally unrelated to parameters of peripheral glucose homeostasis points to

a central nervous mediation of insulin's suppressive effect on sleep-related cortisol concentrations in elderly subjects. We were not able to detect treatment effects on ACTH concentrations in the present study, so that an ACTH independent mechanism of adrenal regulation may also be involved (Bornstein et al., 2008). This question clearly is in need of further clarification.

Our finding of an insulin effect in the elderly but not young subjects fits with previous studies indicating that in young, healthy men, attenuating effects of intranasal insulin on basal HPA axis activity only emerge after long-term administration (Benedict et al., 2004). Acutely attenuating effects of intranasal insulin on basal cortisol concentrations during wakefulness were found in obese (Hallschmid et al., 2008), but not in normal-weight men (Benedict et al., 2004). Obesity is associated with and also appears to be promoted by excessive HPA axis secretion, e.g., due to chronic stress (Incollingo Rodriguez et al., 2015). Normal aging likewise goes along with alterations in the regulation of the HPA system, in particular increased nocturnal cortisol secretion (Van Cauter et al., 2000). In our sample of elderly subjects, BMI was positively related to serum cortisol concentrations during the first night-half. In general, however, the elderly participants of the present study displayed a high level of physical health and only moderate signs of nocturnal HPA axis up-regulation, which might explain the relative subtlety of the observed insulin effect. Still, the extent of insulin-induced cortisol reductions was associated with the height of respective cortisol nadir levels. Therefore, inhibitory effects of insulin on HPA axis secretory activity are expected to be stronger in aging subjects who show weaker nocturnal stress axis inhibition. In these subjects, intranasal insulin may be a helpful means to normalize nocturnal HPA axis activity and improve sleep-associated endocrine regulation. It remains to be seen if intensified or prolonged insulin administration paradigms may moreover induce associated changes in sleep parameters and, in this way, potentiate the beneficial metabolic and cognitive impact of central nervous insulin administration (Spetter and Hallschmid, 2015).

4 | Study III: Orexin A and glucose homeostasis

Thienel, M., Elsässer, T., Lamprinou, A., Klement, J., Peter, A., and Hallschmid, M. (2017). Intranasal orexin A acutely improves glucose tolerance in healthy men. *in preparation*

4.1 Introduction

Orexin A (hypocretin-1) is an excitatory hypothalamic neuropeptide that exerts multifaceted effects in the brain and the body periphery, which up to now have been primarily investigated in animal experiments. Orexin A neurons are bilaterally distributed within the lateral hypothalamic area and adjacent regions and project widely to brain regions regulating autonomic functions, feeding, and sleep (e.g., cerebral cortex, hippocampus, amygdala, locus coeruleus, and raphe nuclei; Kukkonen et al. (2002); Nambu et al. (1999)). Patients with narcolepsy with cataplexy, a neurological disorder characterized by transient episodes of excessive daytime sleepiness and muscle weakness, show a selective loss of hypothalamic orexin A-containing neurons (Thannickal et al., 2000) and blunted orexin A levels in the CSF (Nishino et al., 2000). In animal studies, orexin A has been shown to increase food

intake and body weight (Edwards et al., 1999) probably through inhibition of anorexigenic POMC neurons and simultaneous stimulation of NPY neurons in the nucleus arcuatus of the hypothalamus (Muroya et al., 2004). This pattern suggests that orexin A is essential not only for maintaining a normal sleep-wake cycle but also affects energy homeostasis and metabolism (Sutcliffe and de Lecea, 2000).

The expression of orexin A receptors in the pancreas and the intestinal mucosa (Ehrström et al., 2005) has led to the assumption that orexin A might contribute to the regulation of intestinal glucose uptake and insulin-dependent glucose utilization (Ducroc et al., 2007). In rats, intraperitoneal administration of orexin A reduces plasma glucose levels during an OGTT probably by influencing intestinal glucose uptake (Ducroc et al., 2007). Moreover, it was demonstrated in rats that insulin-dependent muscle glucose uptake is increased by hypothalamic orexin A administration through activation of the sympathetic nervous system and β_2-adrenergic signaling pathways (Shiuchi et al., 2009). In the present study, we investigated the effect of orexin A on glucoregulation assessed by means of an OGTT in healthy young men with NGT. Orexin A was administered via the intranasal pathway that is known to enable substances like neuropeptides to travel to the brain compartment in animals (Dhuria et al., 2009) and humans (Born et al., 2002). Assuming an improving effect of orexin A on insulin sensitivity, we expected an inhibitory effect of orexin A administration on blood glucose and insulin concentrations.

4.2 Methods

Subjects

Nineteen healthy normal-weight men were recruited on site via the university's mailing lists. All relevant illness was excluded by medical history and clinical examination taking place before the first experimental session. Body composition assessed by bioelectrical impedance analyses (Nutriguard-M,

Data Input, Germany) was performed at the start of each experimental session (see Table 4.1 for subject characteristics). Participants gave written informed consent to the study that conformed to the Declaration of Helsinki and was approved by the local ethics committee.

Table 4.1 Subject characteristics.

Variable	Mean ± SEM
Age (years)	22.89 ± 0.63
Body weight (kg)	71.43 ± 1.24
BMI (kg/m^2)	22.47 ± 0.33
HbA_{1c} (mmol/mol)	33.11 ± 0.76
HbA_{1c} (%)	5.17 ± 0.07
Lean body mass (kg)	61.36 ± 1.28
Body fat mass (kg)	10.35 ± 0.62
Body fat mass (% of total weight)	14.43 ± 0.84
Body cell mass (% of lean body mass)	57.17 ± 0.63
Body water (liters)	44.92 ± 0.93
Phase angle (°)	7.21 ± 0.15
PFS (total scale score)	2.80 ± 0.14

Mean ± SEM values are indicated. Body composition was measured by bioelectrical impedance analysis (Nutriguard-M, Data Input, Germany) in a clinical examination taking place shortly before each experimental session. PFS, total scale score of the Power of Food Scale (PFS), a measure of propensity to consume palatable foods, assessed before substance administration and averaged across conditions; $n = 19$.

Experimental procedure

Experiments were carried out in a balanced, double-blind, cross-over, within-subject comparison. Each subject participated in two experimental sessions (orexin A and placebo) spaced at least 14 days apart. Participants were in-

structed to abstain from the intake of food and caffeinated and alcoholic beverages after 2000 h on the day preceding each session.

After the subject's arrival at the laboratory at ∼0800 h, baseline vigilance was tested (see Figure 4.1 for the experimental procedure). A venous cannula was inserted into the subject's nondominant arm to enable drawing of venous blood. Thereafter, blood was sampled for baseline assessments of hormonal parameters. At 0915 h each subject was intranasally administered 500 nmol orexin A (1.781 mg of $C_{152}H_{243}N_{47}O_{44}S_4$ acetate salt; Bachem, Bubendorf, Switzerland; soluted in 2.3 ml water for injection) or placebo (water for injection) at 20 individual 0.1 ml-puffs (10 per alternating nostril) with 1-min intervals in-between. After intranasal administration, neuropeptides reach the central nervous compartment within ∼45 min (Born et al., 2002). Timing and dosing were further based on previous experiments on intranasal orexin A in humans (Baier et al., 2011, 2008; Weinhold et al., 2014) and animals (Deadwyler et al., 2007). At 1000 h an OGTT (300 ml Accu-Check Dextrose O.G.T. solution containing 75 g anhydrous glucose) was performed with venous blood samples taken at 0, 10, 20, 30, 60, 90, and 120 minutes for the measurement of plasma glucose, serum insulin, and serum C-peptide concentrations. After the OGTT, from 1305–1330 h, participants were presented with a lunch consisting of a conventional pizza margherita (300 g; energy value: 217 kcal per 100 g; 32.3 g carbohydrates per 100 g; 8.7 g proteins per 100 g; 5.4 g fat per 100 g). Casual snack intake was assessed 5 min thereafter under the pretext of a snack taste test. Throughout the experiment, feelings of hunger, mood, and subjective well-being were repeatedly rated on visual analogue scales (0–100 mm) and energy expenditure was assessed by indirect calorimetry before and after substance administration. Vigilance as well as heart rate and blood pressure were monitored throughout the experiment. At the end of experiments, subjects were asked to indicate their account of the study purpose and if they thought to have received orexin A or placebo.

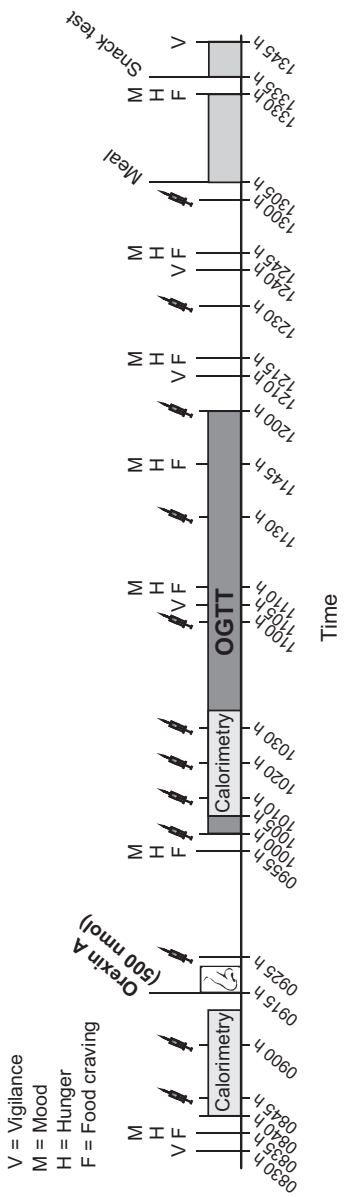

Figure 4.1 Experimental procedure. After baseline assessments of blood parameters, psychological variables, vigilance, and energy expenditure, subjects were intranasally administered orexin A (500 nmol) and placebo, respectively, at 0915 h (nose symbol). At 1000 h, 45 min after substance administration, subjects underwent a 2 h oral glucose tolerance test (OGTT) with 75 g glucose (dark grey bar) followed by a further measurement of energy expenditure. About 60 min after termination of the OGTT, at 1305 h, subjects were given a standard meal and at 1335 h, snack intake was measured under the pretext of a taste-rating task. Throughout the sessions, mood, hunger, food craving, vigilance, and heart rate as well as blood pressure were assessed, and blood samples were taken (syringe symbols).

Measurement of plasma glucose, hormonal parameters, and energy expenditure

Blood samples to assess serum insulin, C-peptide, cortisol, and growth hormone, as well as plasma glucose, lactate, ACTH, and NEFA were centrifuged, and samples were stored at −80 °C. Concentrations of glucose and lactate in fluoride plasma were determined with the ADVIA® Chemistry XPT clinical chemistry analyzer according to the hexokinase and colorimetric lactatoxidase method, respectively. Serum insulin, C-peptide, and cortisol concentrations were measured with the ADVIA® Centaur XPT immunology analyzer and concentrations of growth hormone and ACTH were measured using the Immulite® 2000 XPi Immunoassay-System. Plasma NEFA were determined according to the ACS-ACOD method (NEFA-HR(2), Wako Chemicals GmbH, Neuss, Germany) with the ADVIA® Chemistry XPT clinical chemistry analyzer (all instruments form Siemens Healthcare Diagnostics, Eschborn, Germany).

Resting energy expenditure (expressed as kcal/day) and the respiratory quotient were measured via indirect calorimetry using a DeltaTrac II ventilated-hood system (SensorMedics Vmax 29n; VIASYS® Healthcare, Yorba Linda, CA, USA). Before each use, the device was calibrated with calibration gas to 5 % CO_2 and 95 % O_2. Calorimetric measurements (30 min each) took place at 0840 h (baseline) and immediately after onset of OGTT at 1005 h.

Assessments of hunger, thirst, food craving, mood, and vigilance

Hunger, thirst, and food craving were rated on visual analog scale (0–100 mm). Self-reported mood was assessed on 5-point scales covering the categories good/bad mood, alertness/sleepiness and calmness/agitation (MDBF; Steyer et al., 1997). For the examination of vigilance, subjects performed a simple 5-min PC-based task (Diekelmann et al., 2011) before and after substance administration. In this 5-min task, a red dot appeared at random intervals either on the left or the right side of the screen, and subjects were required to

press the respective key as fast as possible, receiving immediate feedback in the form of the reaction time for correct responses or an error message. Mean reaction time was registered and adjusted for mistakes by adding the square root of the product of the mean reaction time and the number of mistakes.

Reward-driven food intake

Reward-related eating in the relative absence of hunger was assessed under the pretext of a taste test (Hallschmid et al., 2012; Higgs et al., 2008; Ott et al., 2013) that included snacks of three different types, i.e., salt crackers, rice waffles, and chocolate cookies, respectively, but comparable calorie content and macronutrient composition (Ott et al., 2013). Participants were instructed to taste and rate each snack type on a VAS anchored by 0 (not at all) and 100 mm (very palatable/sweet/salty/sour). Subjects were informed that they could eat as many snacks as they liked, and were left alone for 10 min. Intake was covertly measured by weighing the snacks before and after the test.

Statistical analyses

For the analyses of blood parameters and vigilance, respectively, one participant was excluded because of data loss. Analyses were generally based on ANOVA with the within-subject factors 'treatment' (orexin A/placebo), 'time', 'snack type' (salty/sweet/bland), and 'taste' (salty/sweet/sour) as appropriate. Degrees of freedom were corrected using the Greenhouse-Geisser procedure. Areas under the curve were calculated according to the trapezoidal rule covering the relevant time periods. Significant ANOVA and AUC effects were specified by two-sided pairwise t-tests. For blood parameters, baseline adjustment was achieved by subtracting individual baseline values from individual post-intervention measurements. Insulin sensitivity indexes were calculated according to the respective formulas given in Otten et al. (2014). All data are presented as means $\pm$ SEM. A P value < 0.05 was considered significant.

4.3 Results

Orexin A improves glucose homeostasis

Baseline concentrations of glucose, insulin and C-peptide were comparable between conditions ($P > 0.2$ for all comparisons). Subjects did not differ in their fasting insulin sensitivity across conditions reflected in HOMA-IR indices (placebo, 1.29 ± 0.15; orexin A, 1.13 ± 0.13; $P = 0.39$). During the 2 h OGTT, plasma glucose excursions were blunted by orexin A compared to placebo administration ($F_{(1,16)} = 6.22$; $P = 0.02$ for treatment; Figure 4.2a), especially in the first 60 min ($P = 0.007$ for $AUC_{1000–1100\,h}$). Serum insulin levels were moderately increased after orexin A compared to placebo administration 5 min after the glucose load ($P = 0.063$), with a subsequent slight attenuation at 10 min ($P = 0.073$) and 30 min thereafter ($P = 0.049$); Figure 4.2b). Accordingly, Cederholm insulin sensitivity index values calculated from glucose and insulin excursions were significantly increased (placebo, 65.43 ± 3.95; orexin A, 71.16 ± 3.98; $P = 0.03$). C-peptide excursions in general matched insulin concentrations, with an immediate attenuation ($P = 0.04$ and $P = 0.048$, respectively) after glucose load in the orexin A compared to placebo condition (Figure 4.2c). The orexin A-induced changes in insulin concentrations between the first 60 min of the OGTT were statistically unrelated to the respective decrease in plasma glucose $AUC_{1000–1100\,h}$ values ($r = 0.35$; $P = 0.16$, Pearson's coefficient).

ACTH, cortisol, lactate, growth hormone, and NEFA

Baseline concentrations of ACTH, cortisol, lactate, growth hormone, and NEFA did not differ between conditions (all $P > 0.4$). Orexin A treatment increased ACTH concentrations acutely after peptide administration at 0925 h ($P = 0.009$; Figure 4.2e) and increased cortisol secretion thereafter at 1000 h ($P = 0.009$) and 1130 h ($P = 0.088$; Figure 4.2f). These effects however were not substantiated by analyses covering the whole experimental period (both

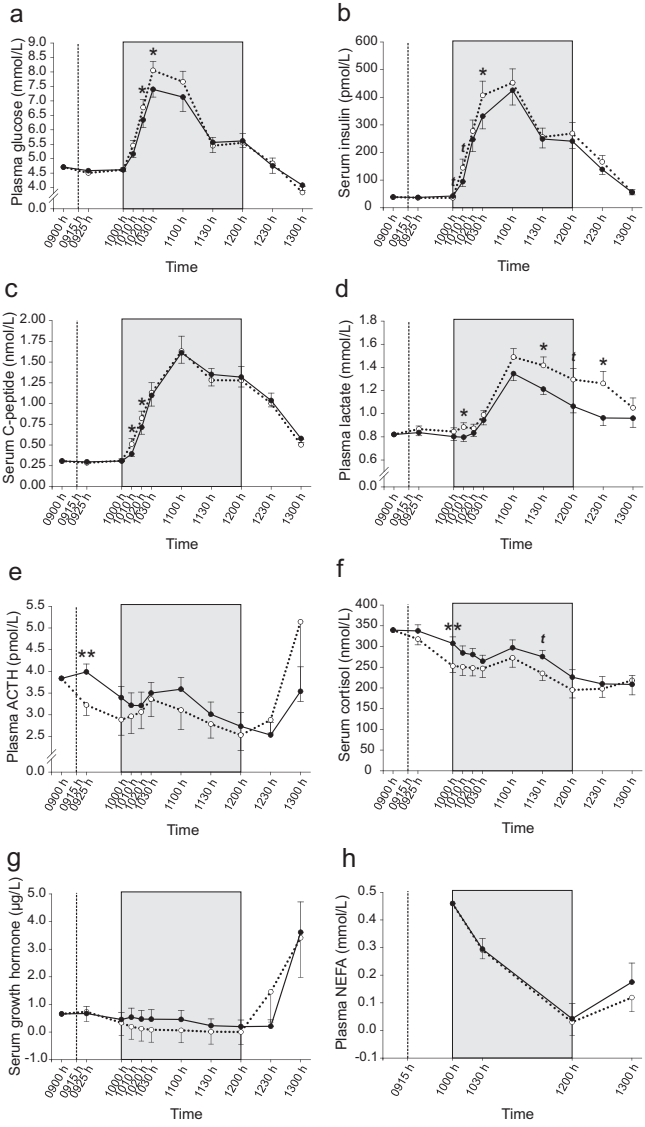

Figure 4.2 Blood parameters. (Caption on next page.)

Figure 4.2 (Previous page.) Mean ± SEM concentrations of plasma glucose (**a**), serum insulin (**b**), serum C-peptide (**c**), plasma lactate (**d**), plasma ACTH (**e**), serum cortisol (**f**), serum growth hormone (**g**), and plasma NEFA (**h**) assessed before (averaged across the 0845 h and 0900 h baseline values) and after intranasal administration (vertical dotted line) of orexin A (500 nmol; black circles and solid lines) and placebo (aqua; white circles and dotted lines) in healthy men ($n = 18$). Subjects underwent an oral glucose tolerance test (OGTT solution containing 75 g glucose) from 1000–1200 h (grey boxes). Mean baseline values of both conditions are averaged to a common baseline. $^{**}P < 0.01$, $^{*}P < 0.05$, $^{t}P < 0.1$ for comparisons between conditions (pairwise t-tests).

$P > 0.27$ for treatment × time) and by ANOVA and AUC analyses focusing on the time period during the OGTT (all $P > 0.16$). The differences in ACTH and cortisol concentrations between conditions after peptide administration were statistically unrelated to the orexin A-induced blunting of the glucose response ($AUC_{1000–1100\,h}$; $r = 0.21$, $P = 0.41$; $r = 0.4$, $P = 0.12$, respectively; Pearson's coefficients).

Besides a general reduction in lactate concentrations in the orexin A compared to the placebo condition during the whole experimental time after glucose load ($AUC_{1000–1300\,h}$, $t_{(17)} = -2.38$, $P = 0.03$; $F_{(1,17)} = 4.57$, $P = 0.047$ for treatment), participants' individual peaks in lactate concentrations were significantly reduced after peptide administration (1.61 ± 0.08 vs. 1.41 ± 0.05; $t_{(17)} = -2.25$, $P = 0.038$; Figure 4.2d). The orexin A-induced reductions in lactate peak levels were significantly correlated to the respective decrease in glucose response ($AUC_{1000–1100\,h}$; $r = 0.55$, $P = 0.019$).

Growth hormone and NEFA concentrations did not indicate effects of orexin A during the experimental period ($P = 0.57$ and $P = 0.49$, respectively, for treatment × time; Figure 4.2g-h).

Heart rate, and blood pressure, and energy expenditure

Heart rate was unaffected by substance administration throughout the experiment (all $P > 0.4$; Figure 4.3a). Systolic blood pressure was slightly elevated in the orexin A compared to the placebo condition acutely after peptide administration at 0925 h (placebo, 122 ± 2 mmHg; orexin A, 127 ± 2 mmHg, $P = 0.062$), but comparable apart from that (all $P > 0.35$ for treatment $\times$ time and single time-point comparisons; Figure 4.3b). Diastolic blood pressure was blunted in the orexin A condition during OGTT at 1030 h (placebo, 73 ± 1 mmHg; orexin A, 69 ± 1 mmHg, $P = 0.022$) and slightly thereafter at 1230 h (placebo, 75 ± 1 mmHg; orexin A, 72 ± 2 mmHg, $P = 0.058$; Figure 4.3c). Energy expenditure assessed by indirect calorimetry was comparable between conditions both at baseline (placebo, 1709 ± 51 kcal/day; orexin A, 1667 ± 40 kcal/day, $P > 0.45$) and after glucose intake (placebo, 1773 ± 48 kcal/day; orexin A, 1800 ± 50 kcal/day, $P > 0.58$; Figure 4.3d).

Orexin A increases food craving but does not influence reward-driven food intake

Across conditions, hunger and thirst ratings were comparable at baseline (both $P > 0.9$) and generally unaltered by orexin A (all $P > 0.35$ for respective interactions). However, ratings of food craving were significantly increased in the orexin A compared to the placebo condition ($F_{(1,18)} = 7.28$, $P = 0.015$ for treatment), falling to comparable low values after meal intake at 1330 h ($P = 0.49$ for single time point comparison; Figure 4.3f). Snack intake during the postprandial period was not affected by orexin A ($P < 0.43$ for treatment; Figure 4.3e). Orexin A increased the perceived saltiness of salt crackers (placebo, 7.58 ± 0.18; orexin A, 8.17 ± 0.21; $P < 0.05$).

Psychological parameters and vigilance

Mood and subjective well-being according to the self-rated categories good/-bad mood ($P > 0.6$) and calmness/agitation ($P > 0.2$) were not affected by

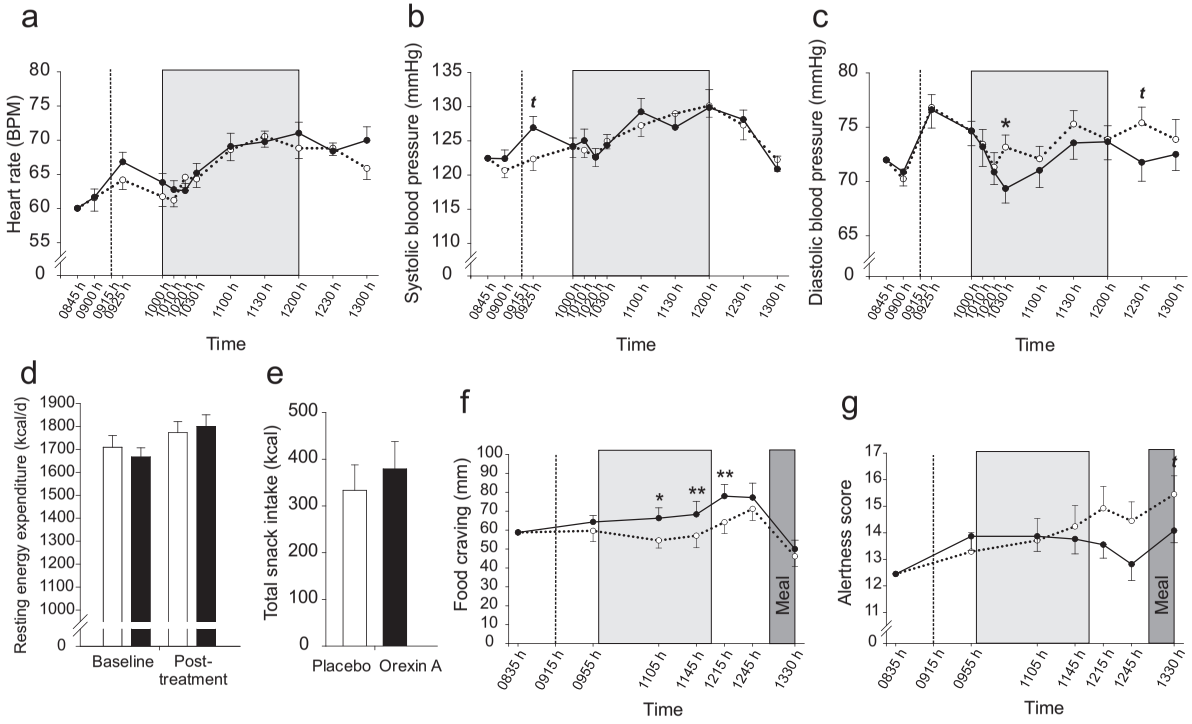

Figure 4.3 Physiological and psychological control measurements, resting energy expenditure, and total snack intake. (Caption on next page.)

Figure 4.3 (Previous page.) Upper panels indicate mean ± SEM heart rate (**a**), systolic (**b**) and diastolic blood pressure (**c**) assessed before and after intranasal administration (vertical dotted line) of orexin A (500 nmol; black circles and solid lines) and placebo (aqua; white circles and dotted lines). Lower panels depict resting energy expenditure during the baseline (0840–0910 h) and post-treatment (1005–1035 h; **d**), and cumulative snack intake (**e**) in the placebo (white bars) and the orexin A condition (black bars). Mean ± SEM food craving according to visual analogue scales (**f**) and alertness according to the self-rated mood questionnaire (**g**) assessed during the whole experimental period is indicated. $^{**}P < 0.01$, $^{*}P < 0.05$, $^{t}P < 0.1$ for comparisons between conditions (pairwise t-tests, $n = 19$).

orexin A, while subjects were more alert in the placebo condition compared to orexin A ($F_{(3,60)} = 3.92$; $P = 0.01$ for treatment × time; Figure 4.3g). Reaction time was not affected by orexin A (all $P > 0.22$ for respective comparisons).

4.4 Discussion

We show an acutely enhancing effect of orexin A administration on glucose tolerance in healthy men. In fasted subjects undergoing an oral glucose tolerance test after intranasal orexin A administration, we found that orexin A attenuates the glucose peak response to a glucose challenge. This effect was accompanied by an initial trendwise increase in insulin concentrations, but a decreased response of insulin and C-peptide during the first thirty minutes after glucose load. Orexin A also attenuated lactate excursions throughout the whole experimental period and acutely increased ACTH and cortisol secretion, but did not affect levels of growth hormone and NEFA. Enhanced glucose tolerance was reflected by an orexin A-induced increase in the Cederholm index, an indicator for peripheral insulin sensitivity and glucose uptake into skeletal muscle (Cederholm and Wibell, 1990). Intranasal orexin A administration also increased perceived craving for food and slightly

elevated systolic blood pressure, while diastolic blood pressure and alertness were attenuated.

Our results of an acute stimulatory effect of orexin A on glucose tolerance extend previous experiments in animals to the human setting (Tsuneki et al., 2012). The methodological approach of OGTT served as a standardized measure of insulin resistance and revealed the contribution of orexin A to glucose homeostasis in humans. In rats exposed to an OGTT following an overnight fast, blood glucose levels were decreased by pre-treatment with 55 µg/kg intraperitoneal orexin A, effects mediated via an inhibition of intestinal glucose absorption that was dependent on the glucose transporter protein SGLT1 (Ducroc et al., 2007). Injection of 5 pmol orexin A into the ventromedial nucleus of the hypothalamus in rodents led to enhanced glucose uptake and glycogen synthesis in skeletal muscle by activation of the sympathetic nervous system and β_2-adrenergic signaling (Shiuchi et al., 2009). Hypothalamic orexin expression in normal and obese mice negatively correlates with changes in blood glucose (Yamanaka et al., 2003). Accordingly, hypoglycemia upregulates prepro-orexin (Sakurai et al., 1998) and orexin mRNA (Griffond et al., 1999) and leads to an activation of orexin-containing neurons in the endocrine pancreas at a peripheral level (Ouedraogo et al., 2003).

Lactate concentrations were reduced by orexin A throughout the experiment. Lactate, as a critical energy substrate, is supposed to regulate the orexin system depending on its extracellular concentration (Parsons and Hirasawa, 2010). Due to relatively high blood glucose concentrations during OGTT, intranasal orexin A administration may have improved the conversion of lactate to glucose via hepatic gluconeogenesis. Notably, orexin receptor type 1 (OX1R)-deficient mice on high-fat diet show improved glycemic regulation and insulin sensitivity (Funato et al., 2009) and intraperitoneal administration of OX1R antagonist reduces food intake, body weight gain, fasting blood glucose levels and plasma insulin levels, while normalizing the increased metabolic rate in genetically obese mice (Haynes et al., 2002). Therefore,

improved hyperglycemia and hyperinsulinemia in mice with diet-induced obesity following a continuous ICV administration of orexin A (0.5 nmol/day; Ramadori et al., 2011) maybe attributed to an OX2R-dependent mechanism (Haynes et al., 2002).

In our study, insulin concentrations first showed signs of a swifter increase after orexin A administration directly followed by an attenuation, which is partially in line with reports of a stimulatory impact of orexin A on insulin secretion in animals under non-fasting conditions (Nowak et al., 2000) and after an intraperitoneal glucose tolerance test (Park et al., 2015). In vitro, conversely, orexin A significantly decreases glucose-stimulated insulin secretion in isolated rat islets (Ouedraogo et al., 2003). However, the observed acute insulin response and blunted glucose excursion in the present study were statistically unrelated. In consideration of differential experimental conditions and the lack of evidence in a human study context, the influence of orexin A on insulin secretion is in need of further investigation.

Orexin A acutely increased plasma ACTH and serum cortisol levels, which accords with previous experiments in rodents showing that ICV administration of orexin A activates the HPA axis (Al-Barazanji et al., 2001; Sakamoto et al., 2004). Orexin A increases cortisol secretion from human adrenocortical cells in vitro (Mazzocchi et al., 2001) and orexin neuron activity is conversely increased by psychological stressors (Chang et al., 2007; Furlong et al., 2009). Accordingly, orexin A is involved in the regulation of cardiovascular functions by increasing sympathetic nerve activity in terms of a defence response concomitant with high vigilance (Sakurai, 2014b; Samson et al., 1999; Shirasaka et al., 1999), which is also reflected in our results of slightly elevated systolic blood pressure levels right after peptide administration. In contrast, our subjects self-reported less alertness and displayed decreased diastolic blood pressure in the orexin A condition compared to placebo. Studies in rodents show that microinjections of low doses of orexin A and orexin B in the nucleus of the solitary tract can also stimulate parasympathetic activity (Ciriello et al., 2013; de Oliveira et al., 2003; Shih and Chuang, 2007). Against this

background, our results may be interpreted to indicate a time-dependent effect of intranasal orexin A on sympathetic activity and vigilance, i.e., initially increasing HPA axis activity and blood pressure with a subsequent drop in alertness.

In our study, intranasal administration of orexin A significantly increased the craving for food, which is in line with results from previous studies showing an orexigenic effect of ICV administration of orexin A and orexin B in several species (Sakurai, 2007). Although orexin neurons send excitatory projections to the VTA and therefore are tightly involved in the modulation of reward-related processes (Harris et al., 2005; Sakurai, 2014b), orexin A administration did not significantly increase reward-driven food intake in the snack test. The uptake of glucose and the standardized meal beforehand could have masked the expected effect on hedonic food intake. Orexin A also did not alter resting energy expenditure in our sample of healthy men. Central infusion of orexin A in rodents repeatedly resulted in increased body temperature and energy expenditure (Mavanji et al., 2015; Messina et al., 2014) clearly indicating a link between the peptide and thermoregulation. Taking into account the role of orexin A in basal sympathetic activation (Messina et al., 2014), the postprandial rise in energy expenditure following the glucose load may have blurred the impact of orexin A via ceiling effects.

In sum, our data indicate an improvement in glucose tolerance in healthy men after intranasal orexin A administration, buttressing the assumption that orexin A plays a role in blood glucose homeostasis. Considering that hyperglycemia and insulin resistance are core symptoms of type 2 diabetes and that no severe side effects of intranasal orexin A administration have been reported as yet—albeit in a very limited number of studies in humans (Baier et al., 2011, 2008; Weinhold et al., 2014)—, our preclinical data in healthy men may bode well for future pharmacological approaches that target the orexin system. Still, the mechanisms underlying our observations should be investigated in further studies in humans, which may help delineate the potential of orexin A in the treatment of metabolic ailments.

5 | Conclusions and general discussion

The aim of the studies presented in this thesis was to scrutinize specific roles of central insulin, orexin A, and oxytocin in metabolic control in healthy humans. Metabolic disorders and obesity are on the rise and represent a worldwide health concern tied to enormous medical costs (Cawley and Meyerhoefer, 2012; Wang et al., 2011). The growing number of molecules known to be implicated in energy homeostasis raises nearly limitless possibilities for an auxiliary medical approach in the treatment of obesity and associated metabolic disorders. Biologically occurring peptides, such as insulin, orexin A, and oxytocin may hold some potential as future therapeutics due to good efficacy, safety, tolerability as well as high selectivity and potency (Fosgerau and Hoffmann, 2015).

Together, the findings in the studies conducted in this thesis indicate that the intranasally administered neuropeptides oxytocin, insulin, and orexin A target pivotal determinants in the development of metabolic disorders, such as body weight, HPA axis activity, and insulin sensitivity. Oxytocin exerted an acutely inhibitory impact on food intake that was enhanced rather than decreased in obese compared with normal-weight men, dampened HPA axis activity, and blunted postprandial glucose levels in both groups of subjects, suggesting an oxytocin-induced improvement in insulin sensitivity (Study I).

In Study II it was found that intranasally administered insulin compared to placebo reduced serum cortisol levels during early sleep (between 2300 h and 0020 h) in elderly, but not in young participants indicating that central insulin acts as an inhibitory signal in the regulation of basal HPA axis activity and suggesting that improving brain insulin signaling could normalize nadir stress axis activity in older age. Finally, orexin A attenuated plasma glucose and lactate peak levels and blunted the early rises in insulin and C-peptide concentrations during an oral glucose tolerance test in healthy normal-weight humans, which indicates an essential contribution of orexin A to human glucose metabolism (Study III).

5.1 Interplay of oxytocin, orexin A, and insulin in glucose metabolism

Considering the results shown in the present thesis, the effects of IN oxytocin and orexin A seem to converge in their role in glycemic control and their insulin-sensitizing properties, respectively. Maintaining glucose homeostasis is essential for daily functioning and thus for survival. Perturbation in glucose homeoastasis and insulin action may lead to severe medical conditions, including type 2 diabetes and obesity. The mechanisms behind the effects of orexin A and oxytocin in glycemic control and on pancreatic insulin secretion, respectively, are still in need of investigation. Both orexin neurons in the LHA and magnocellular oxytocin neurons in the SON have been found to serve as glucose sensors (Moriguchi et al., 1999; Song et al., 2014). Orexin neurons are glucose-inhibited relative to intracelluar energy levels (Venner et al., 2011), whereas oxytocin neurons are glucose-excited (Song et al., 2014) according to their respective anabolic/orexigenic and catabolic/anorexigenic properties. Furthermore, both orexin neurons in the LHA and oxytocin neurons in the PVN may be important targets for the SCN control of glucose metabolism (Foppen et al., 2016).

As evidenced by immunohistochemical methods, OX1R as well as OXTR are localized in rat pancreatic α- and β-cells (Kirchgessner and Liu, 1999; Ouedraogo et al., 2003; Suzuki et al., 2013) suggesting that both orexin A and oxytocin are involved in the release of insulin as well as glucagon. However, the question whether central or peripheral mechanisms are more or less involved when it comes to the role of orexin A and oxytocin in glucose homeostasis remains unclear (Björkstrand et al., 1996; Nowak et al., 2000).

5.1.1 Opposing effects on glucose homeostasis

In vitro, orexin A significantly decreases glucose-stimulated insulin secretion and stimulates glucagon release in low glucose concentration in isolated rat islets (Ouedraogo et al., 2003). Conversely, Nowak et al. (2005) have shown that orexin A dose-dependently stimulates insulin secretion at basal (6.66 mmol/L) and high glucose (26.4 mmol/L) concentrations in the isolated perfused pancreas. In vivo, subcutaneous (SC) and ICV injection of orexin A significantly increases circulating insulin and glucose concentrations rats under non-fasting conditions (Nowak et al., 2000). Continuous ICV infusion into rats fasted for 5 h results in an increase in plasma glucose levels and prevents the daytime decrease of endogenous glucose production in the liver (Yi et al., 2009). On the contrary, IV and ICV administration of orexin A causes a reduction of fasting blood glucose levels in normal mice and streptozotocin-induced diabetic mice without affecting serum insulin concentrations, whereas these effects can not be observed under fed conditions (Tsuneki et al., 2002). Similarly, pre-treatment with intraperitoneal injection of orexin A decreases blood glucose levels in OGTT using rats fasted overnight (Ducroc et al., 2007) which is in line with the results presented in Study III. Against this background, orexin A may affect glucose homeostasis by regulating pancreatic hormone secretion, although a direct peripheral effect of IN orexin A seems questionable, not least because after IN administration, peripheral orexin A concentrations are not significantly increased (Dhuria et al., 2009). The ob-

served discrepancies in the results suggest that counterregulatory response might be modulated by glucose availability and metabolic state on the one hand and some still unknown factors on the other. Thus, no clear role for orexin A in the regulation of glucose metabolism can be established yet.

Concerning the effect of oxytocin on glucose homeostasis, a similar pattern can be observed. There is evidence of both acute stimulatory and inhibitory impacts of oxytocin on insulin secretion and glucose utilization, respectively, in animals and humans. Intravenous administration of oxytocin increases insulin secretion in response to intravenously administered glucose in men (Chiodera et al., 1984). Oxytocin given intranasally in dogs (Altszuler and Hampshire, 1981) as well as subcutaneously and intracerebroventricularly in rats (Björkstrand et al., 1996) causes a significant rise not only of insulin but also of glucagon and glucose levels. However, ICV oxytocin administration in prediabetic mice on high-fat diet reduces fasting insulin as well as glucose levels during a glucose tolerance test, whereas streptozotocin-induced diabetic mice show an increase in fasting insulin secretion (Zhang et al., 2013). However, Lawson et al. (2015) report a decrease in fasting insulin serum levels 60 min after IN oxytocin administration without affecting glucose levels. In contrast but in accordance with the results of Study I, IN oxytocin administered before a meal reduces postprandial peaks in plasma glucose excursion in healthy normal-weight men, while insulin secretion is not significantly altered (Ott et al., 2013). Similarly, IN oxytocin blunts plasma glucose peak response and increases the early response in insulin concentrations during an OGTT in healthy men (Klement et al., 2017). In nonhuman primates, chronic SC oxytocin administration twice a day does not change fasting plasma insulin levels but significantly lowers glucose levels after 4 weeks of continuous administration (Blevins et al., 2015). Taken together, the short- and long-term effects of oxytocin on glucose homeostasis seem to depend on different factors, including route of administration and subject's metabolic state. Considering the rapid and sustained increase in plasma oxytocin concentrations following its intranasal delivery (Klement et al., 2017; Striepens et al., 2013), it can be

assumed that IN oxytocin might exert effects on glucose levels, as shown in Study I, at least partly via direct, peripheral action. Possibly preautonomic oxytocin neurons projecting to the liver are most likely primarily involved, instead of those projecting to the pancreas (Buijs et al., 2001; O'Hare and Zsombok, 2016).

5.1.2 Effects of orexin A on oxytocin release

Interestingly, among the magnocellular neurons of the PVN and SON, OX1R immunoreactivity has been reported in oxytocin neurons (Bäckberg et al., 2002). Aminergic neurotransmitters, including adrenaline, serotonin, and histamine, increase oxytocin release in vitro (Gálfi et al., 2005; Kapoor and Sladek, 2000; Radács et al., 2006) and preincubation with orexin A or orexin B reduce these monoamine-induced increases in oxytocin levels in rat neurohypophyseal tissue cultures (Ocskó et al., 2012). However, if the administration of the monoaminergic compound precedes the orexin application, neither orexin A nor orexin B induce changes in oxytocin release (Ocskó et al., 2012). These findings suggest that the orexin system is intricately involved with oxytocin secretion in the neurohypophysis and might further play a role in the pathogenetic development of metabolic diseases by reducing the effects of increased, monoamine-mediated oxytocin secretion (Ocskó et al., 2012; Vacher et al., 2002). Interactions of orexins and oxytocin on hypothalamic and neurohypophyseal as well as peripheral levels are in need of further investigation, particularly in the in vivo situation.

5.2 Therapeutic considerations

Conventional treatment of obesity focuses on tackling abdominal fat, hypertension, hyperglycemia, and dyslipidemia to reduce the risk of subsequent diseases. Obesity prevention strategies so far include a behavioral therapeutic approach fostering lifestyle and dietary changes. The supplementary use of

pharmaceutical compounds with appetite suppressant activity (anorectics) and antinutritive substances additional to lifestyle change may be further useful for patients who have been unsuccessful with diet and exercise alone (Apovian et al., 2015; Kaur, 2014). The US Food and Drug Administration (FDA) has approved five weight loss drugs (orlistat, lorcaserin, naltrexone-bupropion, phentermine-topiramate, and liraglutide) for long-term use in obese or overweight individuals (Khera et al., 2016; Yanovski and Yanovski, 2014), achieving only modest efficacy of 5 to 10 % weight loss at 52 weeks (Hainer, 2011; Halpern and Halpern, 2015; Khera et al., 2016; Miras and le Roux, 2014). After achieved weight loss in obese patients, moreover, multiple compensatory hormonal mechanisms encouraging weight gain, such as decreased concentrations of leptin and insulin as well as increased ghrelin levels, persist for at least one year and must be overcome in order to maintain weight loss (Sumithran et al., 2011).

Beyond this background, enhancing central nervous neuropeptidergic signaling via intranasal administration might be a promising and advantageous strategy to improve metabolic function in the clinical setting (Chapman et al., 2013; Spetter and Hallschmid, 2015). Key advantages are the relative safety of peptide delivery via IN route, the high efficacy in directly targeting the brain and patient's higher compliance compared to conventional methods of parenteral administration. Although there are still unanswered questions regarding the mechanisms of intranasal neuropeptide administration, the administration of IN insulin, orexin A, and oxytocin does not provoke any severe treatment side effects, if any are reported at all (Baier et al., 2011; MacDonald et al., 2011; Shemesh et al., 2012).

5.2.1 Potential of oxytocin in different clinical contexts

Due to oxytocin's significant involvement in the regulation of social cognition and behavior, an increasing body of evidence suggests that altered oxytocin signaling seems to play a pathophysiological role in mental and developmen-

tal disorders characterized by social dysfunction, such as autism spectrum disorders (ASD), borderline personality disorder (BPD), schizophrenia, and social anxiety disorder (SAD) (Hofmann et al., 2015; Meyer-Lindenberg et al., 2011; Romano et al., 2016). Initial clinical studies revealed promising results—IN oxytocin promoted emotion recognition, face processing, and eye contact in youth with ASD (Andari et al., 2010; Auyeung et al., 2015; Domes et al., 2013; Guastella et al., 2010), although evidence is missing for a sustainable effect on clinical measures. An abundance of experiments examining oxytocin's therapeutic potential in BPD, SAD, and schizophrenia led to partially diverging results (Hofmann et al., 2015; Kirsch, 2015), suggesting that IN oxytocin administration shows clinical potential in psychiatric disorders, however, more studies are needed to determine the treatment indication and specific symptom targets (Hofmann et al., 2015). In contrast, oxytocin's contribution to the regulation of food intake received much less attention than its role in psychosocial behavior (Spetter and Hallschmid, 2017). Animal experiments have shown that oxytocin administration inhibits ingestive behavior in normal-weight rodents (Iwasaki et al., 2015) as well as in genetically obese (Altirriba et al., 2014; Iwasaki et al., 2015) and DIO rodents (Deblon et al., 2011; Maejima et al., 2011; Zhang et al., 2013), suggesting that oxytocin treatment could bypass insulin and leptin resistance, which may occur in association with obesity (Schwartz et al., 2000). A clinical pilot study revealed that 8-week long-term administration of IN oxytocin reduced BMI levels by $3.26 \pm 1.9\,\mathrm{kg/m^2}$ and decreased waist and hip circumference in a small sample of obese participants (Zhang et al., 2013). Although the results of the latter study should be interpreted with caution due to different initial conditions in the treatment and the control group regarding BMI ($36\,\mathrm{kg/m^2}$ in the oxytocin group vs. $30\,\mathrm{kg/m^2}$ in the placebo group) and age (41.3 vs. 29.3 years), increased body weight seems to be a promising target of clinical interventions. Assuming that the acute oxytocin-induced reduction in food intake observed in Study I is equivalent over three meals per day and sustained over time, obese patients might lose more than 21 kg body weight within one

year, which supports the possibility using IN oxytocin as a future adjunctive anti-obesity medication combined with behavioral therapeutic programmes.

5.2.2 Insulin as an enhancer of cognition and metabolism

Intranasal insulin has been shown to improve memory function in patients with amnestic mild cognitive impairments and early Alzheimer's disease (Craft et al., 2012; Reger et al., 2008a,b) and older adults with type 2 diabetes, which is also associated with cognitive decline (Novak et al., 2014). These results suggest that intranasal insulin might be a promising therapeutic intervention in the treatment as well as in the prevention of Alzheimer's disease. Regarding to metabolic function, IN insulin has been considered as a potential therapeutic agent in the treatment of obesity and metabolic syndrome. Acute and chronic intranasal insulin administration exerts anorexigenic effects (Benedict et al., 2008; Hallschmid et al., 2004a, 2012), and increases postprandial thermogenesis and energy expenditure (Benedict et al., 2011). The dampening effect of chronic IN insulin on HPA axis activity in obese (Hallschmid et al., 2008), as well as the beneficial effect of acute IN insulin administration during the response to social challenges like the Trier Social Stress Test (Böhringer et al., 2008), could further normalize stress-induced chronic HPA axis overactivation associated with the pathophysiology of obesity and cognitive decline. Our findings from Study II extend the existing literature and may augur well for therapeutic application in patients with deteriorations in endocrine functions such as hypercortisolism.

5.2.3 Orexin A: Therapeutic implications beyond narcolepsy

To date, experiments with IN orexin A administration in humans have exclusively focused on narcolepsy, a neurodegenerative disease caused by loss of CNS orexin A signaling (De la Herrán-Arita et al., 2011) leading to intermittent, uncontrollable episodes of falling asleep during the daytime. Orexin A

shows a stabilizing effect on REM sleep and reduces direct wake-to-REM transitions in narcoleptic patients when IN administered before night sleep (Baier et al., 2011; Weinhold et al., 2014) and also improves olfactory dysfunction, another well-known aspect of narcolepsy (Baier et al., 2008). It is noteworthy to mention that the orexin receptor antagonist suvorexant (Belsomra®) is applied in the treatment of chronic insomnia (Norman and Anderson, 2016). The beneficial effects of orexin A on glucose homeostasis has been shown in several animal studies (Ducroc et al., 2007; Sakurai, 2014a; Shiuchi et al., 2009; Tsuneki et al., 2012) but this remains to be further investigated in humans. However, our results from Study III suggest the orexin system as another potential target of diabetes therapy.

5.3 Limitations and concluding remarks

Considering the broad possible therapeutic applications, the future for intranasal administration of naturally occuring peptides looks bright. The intranasal pathway for drugs to reach the CNS offers a feasible, non-invasive, and favorable approach for achieving a good therapeutic effect with reduced or even without systemic side effects. However, future studies need to finally answer new questions that emerge on the background of the presented data and have to overcome a few (methodological) obstacles concerning IN administration and some of these are discussed below.

Intranasal drug delivery is influenced by the compound's physicochemical properties, i.e., stability (Lai and Topp, 1999), lipophilicity (Waterhouse, 2003), and molecular weight (Pardridge, 2002) which essentially determines absorption rate and bioavailability. There seems to be less control over dosing with intranasal delivery of high-molecular-weight drugs, e.g., neuropeptides, compared with parenteral and oral routes due to differing absorption rates and delivery pathways within the nasal epithelium (see section 1.4; Dhuria et al., 2010). Moreover, the amount of absorbed peptide molecules in relative contribution to the net uptake may differ interindividually due to anatomic

variabilities of the nasal cavity in size and volume (Samoliński et al., 2007). Suitable strategies in order to enhance the delivery of macromolecules from the nasal cavity to CNS should be developed and implemented in future studies. For instance, animal studies indicated that the brain specificity of the IN route for insulin may be improved via coadministration of cell-penetrating peptides that facilitate cellular uptake of the peptide and may reduce the level of systemic insulin exposure (Kamei and Takeda-Morishita, 2015). Other emerging peptide technologies like multifunctional peptides (Fosgerau and Hoffmann, 2015) or the use of long-lasting peptide analogs (Benedict et al., 2007; Mizuno et al., 2015) might also help increasing efficacy and broadening the therapeutic applicability. To further reduce variability in the response to nasal sprays between experiments and individuals, a standardization of IN administration and guidelines for its reporting in human research is strongly recommended (Guastella et al., 2013).

Another aspect to be addressed in the future are long-term effects and side effects of intranasal administration of neuropeptides. While a single dose of oxytocin, insulin, and orexin A is undoubtedly safe, there is sparse empirical evidence about repeated administration over a longer time period. Subchronic IN administration of insulin (Benedict et al., 2005, 2007) and oxytocin over 8 weeks (Muin et al., 2015; Zhang et al., 2013) and even a 4-month daily treatment with IN oxytocin in schizophrenic patients (Busnelli et al., 2016) does not exert severe side effects or other medical complications. However, studies in vitro showed that sustained exposure of hypothalamic cells to high concentrations of insulin diminishes central insulin signaling via inactivation and degradation of the insulin receptor and insulin receptor substrate-1 (Mayer and Belsham, 2010). Thus, dosage and duration of IN insulin administration in a clinical setting has to be considered very critically. Regarding oxytocin, some studies in humans revealed a ‘dark side’ of the peptide by increasing in-group coherence at the expense of intergroup affiliation (De Dreu et al., 2011; Shalvi and De Dreu, 2014) or hindering cooperative behavior in BPD after IN administration of oxytocin (Bartz et al., 2011). Moreover, experiments

on chronic IN oxytocin administration in rodents point to possible aversive effects on social and partner preference behavior (Bales et al., 2013; Huang et al., 2014). Such psychosocial outcomes should be carefully addressed in therapeutic contexts. It is also noteworthy that SC oxytocin treatment of obese diabetic mice for 2 weeks dose-dependently prevented further body weight gain which was surprisingly accompanied by worsened basal glycemia and glucose tolerance (Altirriba et al., 2014). Results like these call for the careful selection of the experimental or therapeutic conditions in which oxytocin treatment can be expected to act beneficially on obesity and its comorbidities.

In sum, the results in the present thesis seem promising for IN administration of insulin, oxytocin, and orexin A in a clinical setting, where it may support conventional treatment approaches in obesity and metabolic disorders. Considering that data on long-term therapeutic and side effects of IN administration of these as well as other peptides in humans are still limited, much more empirical work is necessary to fully grasp the potential of IN peptide delivery in patients. With over 700 registered clinical trials worldwide investigating intranasal administration (clinicaltrials.gov), this rapidly growing method of administration is on the way up.

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A | Curriculum Vitæ

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Education

2006–2012	Diploma in psychology (‘with distinction’), University of Innsbruck
2006–2011	Intermediate diploma in musicology, University of Innsbruck

Professional and Research experience

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03-11/2011	Research assistant, Institute of Neuroendocrinology, University of Lübeck

Teaching

2016	Lecture 'Insomnia / Therapy of sleep disorders,' Graduate Training Centre of Neuroscience, University of Tübingen
2015	Seminar 'Social competence training,' University of Tübingen
since 2013	Courses and seminars in Medical Psychology, University of Tübingen

Awards

2016	Ernst-and-Berta-Scharrer-Prize (German Society of Endocrinology; DGE e.V.) for the work *Oxytocin's inhibitory effect on food intake is stronger in obese than normal-weight men*
2014	DZD Award (German Center for Diabetes Research; DZD e.V.) for the work *Oxytocin's contribution to the control of food intake in humans*

B | List of Publications

Thienel, M., Elsässer, T., Lamprinou, A., Klement, J., Peter, A., and Hallschmid, M. (2017). Intranasal orexin A acutely improves glucose tolerance in healthy men. *in preparation*

Spetter, M. S., Feld, G. B., **Thienel, M.**, Preissl, H., Hege, M. A., and Hallschmid, M. (2017). Oxytocin curbs calorie intake via food-specific increases in the activity of brain areas that process reward value and establish cognitive control. *Neuropsychopharmacology. submitted*

Thienel, M., Wilhelm, I., Benedict, C., Born, J., and Hallschmid, M. (2017). Intranasal insulin decreases circulating cortisol concentrations during early sleep in elderly humans. *Neurobiology of Aging*, 54:170–4.

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