UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

Zentrum für Experimentelle Medizin

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Metabolic-hedonic regulation of food processing in the human brain

Dissertation

zur Erlangung des Doktorgrades Dr. rer. biol. hum.

an der Medizinischen Fakultät der Universität Hamburg

vorgelegt von:

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Hamburg 2019

Angenommen von der

Medizinischen Fakultät am: 08.03.2020

Veröffentlicht mit Genehmigung der Medizinischen Fakultät der Universität Hamburg.

Prüfungsausschuss, der/die Vorsitzende: PD Dr. Stefanie Brassen

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1. Introduction

1.1. Outline

Understanding eating-related behaviours, such as food valuation and choice, has become of particular relevance in the face of alarmingly high rates of obesity, which worldwide have almost tripled since 1975 ('WHO | Noncommunicable diseases country profiles 2018', 2018). Obesity constitutes a major risk factor for type 2 diabetes (T2D), cardiovascular diseases, musculoskeletal disorders, and cancer (Calle, Rodriguez, Walker-Thurmond, & Thun, 2003; H. Kim et al., 2015), and is associated with an increased overall risk of death compared to normal body weight (Adams et al., 2006). It can be attributed to an increasingly sedentary lifestyle, accelerating urbanization, and changing modes of transportation, but predominantly it is caused by the over-consumption of very palatable, high-caloric foods. These have become generally affordable, are nearly always on offer, and are often energy-dense with high amounts of fat and sugar. Yet many aspects of how the brain processes palatability and controls feeding behaviour have not been fully unravelled to date. This thesis investigates neural processes accompanying feeding behaviour particularly in reward-related networks, with a specific focus on how these are affected by the hormone insulin, and how reward expectations are integrated in food choice.

Generally, the central processing of food cues and the formation and implementation of eating decisions involves metabolic (see paragraph 1.2.1.) and reward (see paragraph 1.2.2.) mechanisms, processed in specific homeostatic and non-homeostatic circuits in the brain (Berthoud, 2011), as well as higher level cognitive functions such as learning, memory, and self-control (Higgs, 2015). The present work focuses on a hormonal modulator of ingestive behaviour, insulin, and on valence processing in food choice, in healthy individuals, but also in humans on the verge of developing metabolic disorders. More precisely, the hypothesis is tested that insulin not only affects homeostatic brain regions, but also modulates hedonic aspects of food through its effects on the mesolimbic path. In a second study, it is investigated how such hedonic aspects are encoded in the amygdala, and how they are integrated in consumption decisions.

In the first study "Central insulin modulates food valuation via mesolimbic pathways", a pharmacological functional magnetic resonance imaging (fMRI) approach is used to study the effects of insulin, a key hormone in the regulation of energy balance, on the neural processes accompanying the hedonic valuation of food in reward-related brain regions. Further, it is investigated how these processes differ between individuals depending on their peripheral insulin functioning. Understanding how insulin affects the neural control of food processing in reward-related pathways may fundamentally enhance our insights on the neural cross-talk between homeostatic and reward-related feeding systems and on how dysfunctions in this cross-talk contribute to pathological eating behaviour.

The second study "Valence signatures and food choice modulation in the human amygdala" focusses on the role of hedonic values in consumption decisions. Here, data are provided underlining the role of topographically distinct amygdala subregions for palatability integration during eating decisions in lean humans. Implicit food liking processes, encoded through spatially distributed activation patterns in the amygdala, are identified, which are directly linked to the impact of food pleasantness on consumption decisions. Additionally, network dynamics are described unravelling how valence information is integrated into food choice. These data shed light on how hedonic aspects can mediate appetitive eating decisions in humans, and on the role of the amygdala herein.

1.2. Central nervous system control of feeding

Alongside with processes in the gastrointestinal tract (Holtmann & Talley, 2014; J. T. McLaughlin & McKie, 2016), the feeding network in the central nervous system (CNS) (Fig. 1) regulates consumption behaviour and metabolism. Here, a limited number of hypothalamic and brainstem nuclei, in particular the arcuate nucleus of the ventromedial hypothalamus (Arc), constitute the brain's command centre for controlling energy balance. Through integration of hormonal and nutrient signals from the periphery, such as hormonal signals arising from the stomach, as well as information from other regions of the brain, these nuclei coordinate energy intake and expenditure (Cone, 2005; Cone et al., 2001; Varela & Horvath, 2012).

Arc neurons send projections to, and receive inputs from other parts of the hypothalamus such as the lateral hypothalamic area (LHA) (Chronwall, 1985; Elias et al., 1998; Everitt et al., 1986). Amongst others, the LHA is connected to the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the amygdala (Fig. 1). These regions contribute to the rewarding aspects of consuming palatable foods and are critical for learning about environmental cues used to predict motivationally relevant outcomes (Clark, Hollon, & Phillips, 2012). The amygdala is involved in the selection of food on the basis of previous experience (Berthoud & Morrison, 2008; Rolls & Rolls, 1973) and in integrating valence and emotional information (Burdakov, Gerasimenko, & Verkhratsky, 2005; Markowitsch, 1999); it thus contributes to the encoding of incentive values in the guidance of actions (Arana et al., 2003; Balleine & Killcross, 2006; Montague & Berns, 2002). The NAc is considered an interface for various reward-related processes and action, based on its sub-regional dopaminergic and opioidergic hotspots encoding motivation and hedonic impact, respectively (Castro & Berridge, 2014; Mitchell, Berridge, & Mahler, 2018; Smith, Berridge, & Aldridge, 2011), and on its connections with the amygdala, the prefrontal cortex (PFC), and motor cortices (Roitman, Wheeler, & Carelli, 2005). In the context of ingestive behaviour, the NAc is thought to act at the interface between metabolic circuits and predominantly dopaminergic

pathways involved in incentive salience processing triggering appetitive behaviour (Berridge, 2007; Berridge & Kringelbach, 2015; Stuber & Wise, 2016). The amygdala and the NAc in turn have bi-directional connections with higher cortical structures, which orchestrate the top-down control of food intake (Fig. 1).

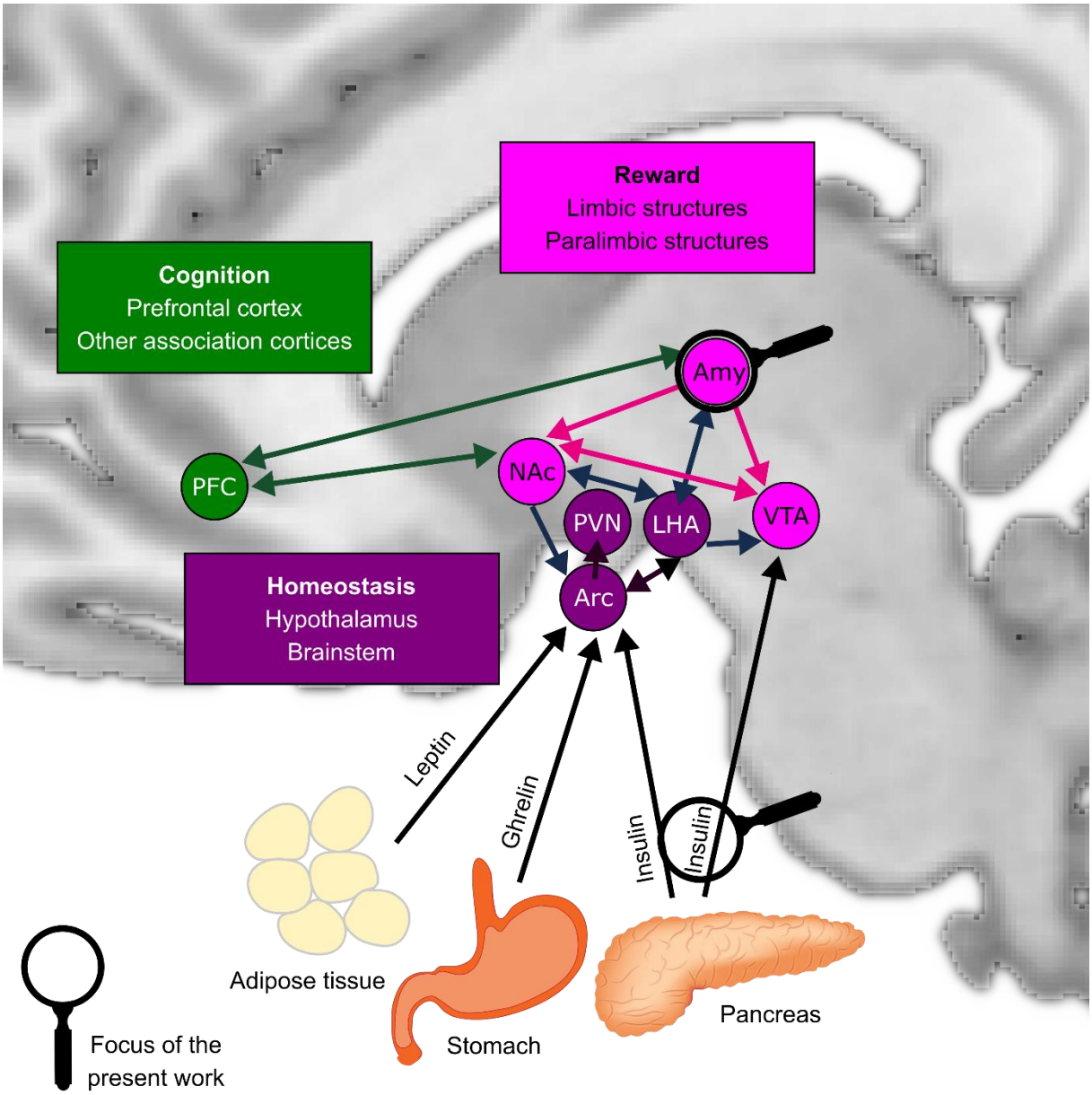

Figure 1 | CNS feeding network

Schematic representation of selected elements of the CNS feeding network, following (Alonso-Alonso & Pascual-Leone, 2007; Berthoud & Morrison, 2008; Fischer & O'Connell, 2017; Stuber & Wise, 2016).

Amy, amygdala; Arc, arcuate nucleus; LHA, lateral hypothalamic area; NAc, nucleus accumbens; PFC, prefrontal cortex; PVN, paraventricular nucleus; VTA, ventral tegmental area

Importantly, recent evidence challenges the traditional view that distinct CNS networks regulate the homeostatic versus non-homeostatic control of feeding behaviour (Ferrario et al., 2016; C. M. Liu & Kanoski, 2018; M. A. Rossi & Stuber, 2018). In fact, both direct and indirect projections from the NAc (Kampe, Tschöp, Hollis, & Oldfield, 2009; O'Connor et al., 2015) and the amygdala (DeFalco et al., 2001) to the hypothalamus could explain the ability for mesolimbic processes, activated by salient environmental cues and incentives, to impact on homeostatic regulatory circuits and to ultimately drive up food intake. Hormones such as insulin act not only on homeostatic structures, e.g. the hypothalamus (Niswender, Baskin, & Schwartz, 2004; Obici, Feng, Karkanias, Baskin, & Rossetti, 2002), but also on structures commonly implicated with reward-processing, such as the NAc and the VTA (Ferrario & Reagan, 2018; Labouèbe et al., 2013; Stouffer et al., 2015). Additionally, cognitive feeding-relevant processes can in turn overwrite the metabolic urge for high caloric food (Hare, Camerer, & Rangel, 2009; Hare, Malmaud, & Rangel, 2011; Hollmann et al., 2012), and expectations of reward can contribute to food consumption decisions independently of the energy supply provided by the food (Coccurello & Maccarrone, 2018; Yeomans, Blundell, & Leshem, 2004). The present thesis investigates mechanisms of how insulin affects mesolimbic regions in humans and how it thus modulates the hedonic value of food cues, and how such hedonic aspects are represented and integrated into food choice.

To lay the basis for the two studies central to this dissertation and to single out research gaps, in the next sections two of the main components of the central nervous system feeding network, i.e. the homeostatic system and the hedonic system, will be depicted in more detail.

1.2.1. Homeostatic control of feeding

When we are hungry and body energy levels are low, we eat to satisfy nutritional needs. Restoration of energy levels through food intake is primarily regulated through metabolic signals, integrated in homeostatic neurocircuits including the hypothalamus and its subnuclei (Berthoud & Münzberg, 2011; Murphy & Bloom, 2006; Schwartz, Woods, Porte, Seeley, & Baskin, 2000). The term "homeostasis" describing the maintenance of a stable internal environment independent of the external environment was popularized by Walter Cannon in the 1930s (Cannon, 1935). It is well suited to express the observation that under normal circumstances all living beings will seek out food sources and will increase food consumption to avoid critically low energy levels.

1.2.1.1. Hypothalamic control of energy homeostasis

Neurons in the Arc are understood as the ‘‘entry point’’ to the homeostatic feeding circuit as they are strongly influenced by peripheral signals and their perturbation robustly influences food intake (Chen, Lin, Kuo, & Knight, 2015; Cone et al., 2001; Coppari et al., 2005). Information on body energy levels reaches the Arc in the form of gastrointestinal satiation signals mediated via the vagal nerve, such as cholecystokinin, peptide YY (PYY3–36), glucagon-like peptide (GLP-1), and ghrelin, alterations in glucose and fatty acid levels in the blood stream, and other feeding-related peripherally-derived hormones, such as insulin (Chong, Vogt, Hill, Brüning, & Zeltser, 2015; Cone, 2005; Cone et al., 2001; He et al., 2019; Schwartz, Seeley, Campfield, Burn, & Baskin, 1996).

The Arc contains two antagonistic cell groups, which bi-directionally control feeding behaviour: orexigenic agouti-related protein (AgRP)/neuropeptide y (NPY)-coexpressing neurons and anorexigenic propiomelanocortin (POMC)-expressing neurons. AgRP/NPY neurons stimulate feeding via melanocortin receptors and intracellular calcium regulation (Gropp et al., 2005; Zagmutt, Mera, Soler-Vázquez, Herrero, & Serra, 2018). Exposure to a (moderate) high fat diet results in increased activation of AgRP neurons, and, in the same vein, administration of AgRP into the CNS increases food intake (Hagan et al., 2000; M. Rossi et al., 1998), emphasizing the role of AgRP in the motivation for food (Figlewicz et al., 2013). In contrast, POMC decreases food intake and increases energy expenditure by binding to melanocortin-4 receptors in the paraventricular nucleus (PVN) in the hypothalamus and by regulating glutamate signalling. Photostimulation of POMC neurons reduces food intake (Aponte, Atasoy, & Sternson, 2011), whereas mutations in the gene encoding POMC in humans result in obesity (van der Klaauw & Farooqi, 2015). Both AgRP and POMC neurons in the Arc express receptors for insulin. Animal studies found infusion of insulin into the Arc to decrease lever presses for food (Figlewicz, Bennett, Aliakbari, Zavosh, & Sipols, 2008) and to reduce food intake for a prolonged period of time (Bruijnzeel, Corrie, Rogers, & Yamada, 2011).

The Arc send efferents to the LHA, which in addition receives inputs from the thalamus and hindbrain (Grill & Hayes, 2012; Zheng, Patterson, & Berthoud, 2005). Here, neurons expressing the orexigenic peptides orexin and melanin-concentrating hormone (MCH) integrate endocrine signals with reward-seeking behaviours (Borgland et al., 2009; Cason et al., 2010; Harris, Wimmer, & Aston-Jones, 2005; Sharf et al., 2010). LHA neurons modulate their activity in response to hormonal signals such as insulin (Berthoud & Münzberg, 2011), leptin (Jo, Chen, Chua, Talmage, & Role, 2005; Leinninger et al., 2009), glucose (Burdakov et al., 2005; Kong et al., 2010; Marston, Hurst, Evans, Burdakov, & Heisler, 2011), and the sensory properties of food (Bernardis & Bellinger, 1996). Via direct connections, LHA orexin neurons can modulate the activity of VTA

dopaminergic neurons (Borgland et al., 2009; Borgland, Taha, Sarti, Fields, & Bonci, 2006; Godfrey & Borgland, 2019; Stuber & Wise, 2016). At the same time, LHA MCH neurons alter dopamine release in the NAc (Pissios et al., 2008) and increase food intake and hedonic taste responses in an opioid-dependent manner, as findings regarding the blockage of dopaminergic effects through naltrexone, an opioid antagonist, suggest (Lopez et al., 2011; MacDonald, Billington, & Levine, 2004; Zheng, Patterson, & Berthoud, 2007). With its connections to the VTA and the NAc (Coccurello & Maccarrone, 2018), the LHA represents an important link between hypothalamic and brainstem pathways on the one hand and mesolimbic motivational processes on the other hand. This is further supported by studies employing electrical stimulation in the LHA, which elicits voracious feeding and self-stimulating behaviour (Simon, Zafra, & Puerto, 2019; Stuber & Wise, 2016). Optogenetic stimulation studies also have confirmed the possibility of feeding- and reinforcement-induction by selective stimulation of gamma-Aminobutyric acid (GABA) fibres originating in the LHA and projecting to the VTA (Jennings et al., 2015; Nieh et al., 2015).

1.2.1.2. Dysfunctions in homeostatic feeding and the role of insulin resistance

Via the above described circuits and mechanisms, under normal circumstances information on energy levels is translated into appropriate behavioural (e.g. termination of food consumption), autonomic (e.g. gastric emptying) and endocrine (e.g. insulin secretion) responses. Yet how easily some individuals gain weight in an obesogenic environment, i.e. an environment which facilitates access to palatable, high caloric food and which promotes cue-induced feeding, seems inconsistent with the idea of a robust homeostatic regulatory system that controls food intake and body weight. One possible explanation is that the system controlling energy homeostasis is more capable of protecting from weight loss than effectively preventing from weight gain (Ahima et al., 1996; Schwartz et al., 2003). Additionally, this observation points towards the existence of major dysregulations of the CNS feeding network as a cause and/or consequence of overnutrition and obesity. One possible contributor to such dysfunctions, namely aberrant insulin functioning, will be closer examined in the following.

Overconsumption of food creates a persistent supply of energy that initially leads to perpetually elevated circulating levels of insulin (Clegg et al., 2011; Davis, Choi, & Benoit, 2010; Morton, Meek, & Schwartz, 2014; Vogt & Brüning, 2013). Even in the absence of excessive weight gain, high fat diets induce peripheral resistance to insulin (Samuel & Shulman, 2012; Shulman, 2000). While this peripheral insulin resistance is relatively well understood, the causes and consequences of central insulin resistance are far less studied (Alves, Oliveira, & Moreira, 2012; Felice, 2013). First animal (Hennige et al., 2009) and human (Guthoff et al., 2011) studies provide evidence for central

insulin resistance, at the level of the blood-brain barrier (BBB) and within the brain (Tschritter et al., 2009).

The BBB is dynamic and adaptable single layer of cells, which includes endothelial, ependymal and tanycytic cells, and which plays an important role in the protection, nutrition and homeostasis of the CNS (Chow & Gu, 2015; Daneman & Prat, 2015). Through transport and secretion, the BBB relays information between the periphery and the CNS. Normally, insulin is transported from the periphery to the brain, as studies making use of radioactively labelled insulin in rodents have shown (Banks, Jaspan, Huang, & Kastin, 1997). However, obese individuals display decreased cerebrospinal fluid (CSF)-to-plasma insulin ratios, pointing towards reduced transport of insulin across the BBB (Kern et al., 2006; Rhea & Banks, 2019; Woods, Seeley, Baskin, & Schwartz, 2003). This is supported by studies in rodents which demonstrate that the transport of insulin across the BBB of obese mice is significantly lower than in thin mice (Urayama & Banks, 2008).

On the neural level, studies have identified central insulin resistance to impair neuronal plasticity via detrimental effects on glutamatergic and cholinergic pathways (Trudeau, Gagnon, & Massicotte, 2004). In rats, dietary manipulations leading to increased body adiposity also resulted in impaired hypothalamic insulin signalling (Dornellas et al., 2015). In obese Zucker rats, a genetic rodent model that exhibits hyperphagia, hyperlipidemia, and hyperinsulinemia, intraventricular insulin infusion did not result in the same reduction of food intake observed in lean rats (Ikeda et al., 1986).

Yet the number of studies investigating the implications of central insulin resistance in humans is rather small, and direct links to feeding-relevant behaviour are still lacking. Additionally, as previously mentioned, insulin receptors are not only present in homeostatic brain regions, but also in reward-related structures, as will be described in the next chapter.

1.2.2. Non-homeostatic control of feeding

Most individuals do not only eat for metabolic needs, but also for pleasure. Especially in humans, the initiation of food consumption often starts as a purely cognitive decision, even in the absence of any depletion signal. Such non-homeostatic, “need-free” processes involve feeding driven by environmental, cognitive, appetitive and rewarding factors, processed mainly in corticolimbic regions such as the NAc, the amygdala, and the PFC.

1.2.2.1. The mesolimbic dopamine system

The mesolimbic dopamine system, connecting the VTA with the NAc, is thought to critically mediate the rewarding aspects of food (Berridge & Kringelbach, 2015; Haber & Knutson, 2010). Generally, reward evaluation relies on the release of dopamine from neurons that originate in the VTA and project to the NAc (Kelley, Baldo, Pratt, & Will, 2005; Kelley & Berridge, 2002). Through its effect on these mid- and forebrain structures, dopamine boosts goal-directed behaviour aiming at the obtainment of various rewards (Berridge, 2007; Sesack & Grace, 2010). These rewards range from natural rewards, such as sex (Cummings & Becker, 2012), to substances of abuse, such as alcohol (Engel & Jerlhag, 2014; Melchior & Jones, 2017), methamphetamine (Hedges et al., 2018) and cocaine (Gerth, Alhadeff, Grill, & Roitman, 2017; Volkow & Morales, 2015).

In this mesolimbic system, food-related cues activate rapid dopamine release to reinforce food intake (Day, Roitman, Wightman, & Carelli, 2007; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004). Consumption of palatable food leads to changes in reward neurotransmitters (de Araujo et al., 2008; Tuulari et al., 2017), e.g. to increased dopamine release (Bassareo & Chiara, 1999; Hernandez & Hoebel, 1988), and to changes in synaptic density (S. Liu et al., 2016) and efficacy (Stuber et al., 2008). In turn, drug-induced increases in dopamine levels in the NAc boost food-motivated behaviour, while specific dopamine ablation in the NAc using 6-hydroxydopamine has the opposite effect (Baldo & Kelley, 2007). Similar to the effects of dopamine, local injection of the mu-opioid agonist Tyr-D-Ala-Gly-(me) Phe-Gly-ol (DAMGO) into the NAc elicits strong food intake, particularly of palatable sweet and high-fat foods (Kelley et al., 2002; Will, Franzblau, & Kelley, 2003). This underlines the contribution of dopamine and opioid neurotransmitters to palatable food motivation (Barbano & Cador, 2007; Cooper, 2007; Zhang, Balmadrid, & Kelley, 2003).

Imaging studies in humans have confirmed that food stimuli and food–related visual or olfactory cues activate mesocorticolimbic brain circuits, amongst those the orbitofrontal cortex (OFC), insula, amygdala, NAc, and VTA (Bragulat et al., 2010; Pelchat, Johnson, Chan, Valdez, & Ragland, 2004; Schur et al., 2009; Simmons, Martin, & Barsalou, 2005). This is consistent with a role of these structures in general hedonic representation (insula, amygdala, NAc, VTA, (Sescousse, Redouté, & Dreher, 2010)) or in representations related to the value of specific types of rewards, such as palatable foods (OFC, (Man, Clarke, & Roberts, 2009; Rolls, 2008; Sescousse et al., 2010)).

1.2.2.2. Insulin and dopamine

Insulin exerts indirect influences on dopamine functioning via receptors expressed on neurons within the Arc, but also exerts direct influences via extrahypothalamic receptors (Carter & Swardfager, 2016). Precisely, insulin receptors are found throughout the mesolimbic brain circuit

(Davis et al., 2010; Figlewicz, Evans, Murphy, Hoen, & Baskin, 2003; Kleinridders, Ferris, Cai, & Kahn, 2014; S. Murray, Tulloch, Gold, & Avena, 2014; Werther et al., 1987) (Fig. 2), thus, besides signalling in hypothalamic neurocircuits, affecting feeding for pleasure.

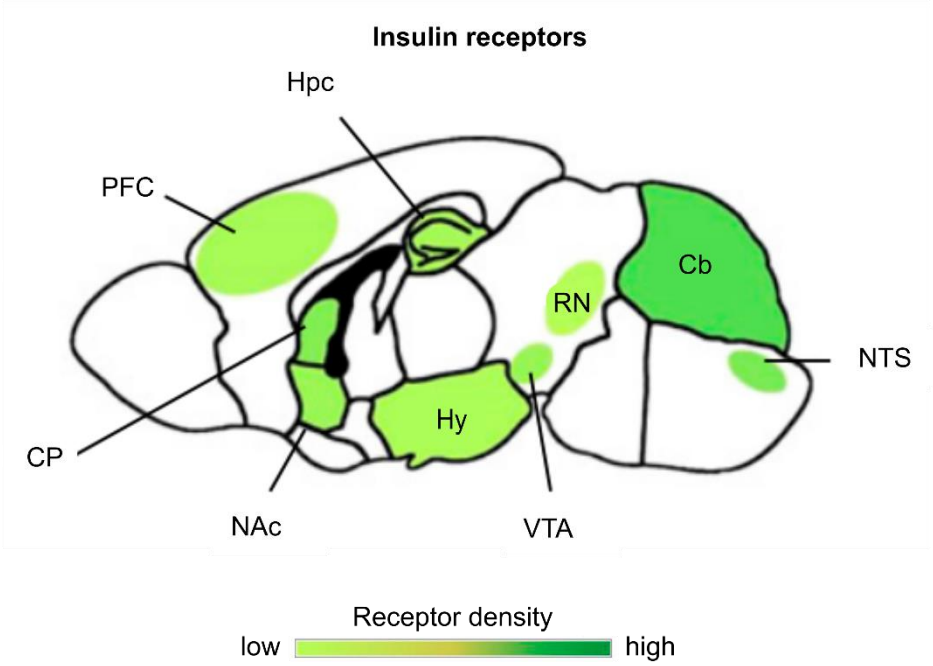

Figure 2 | Insulin receptors in the rodent CNS

Expression of insulin receptors in the rodent brain, as determined via quantitative real-time polymerase chain reaction of samples dissected from mice, adapted from (Kleinridders et al., 2014). With this technique, insulin receptors were detected not only in the hypothalamus, but also in reward-related regions such as the NAc.

Cb, cerebellum; CP, caudate putamen; Hpc, hippocampus; Hy, hypothalamus; NAc, nucleus accumbens; NTS, nucleus tractus solitarii; PFC, prefrontal cortex; RN, raphe nucleus; VTA, ventral tegmental area

Through its direct effects, insulin regulates the dopaminergic system in various ways: (i) It affects the uptake of released dopamine by induction of dopamine reuptake transporter (DAT) expression (Figlewicz, Szot, Chavez, Woods, & Veith, 1994), (ii) it alters dopamine half-life by regulating the expression of the dopamine-degrading enzymes monoamine oxidase (MAO) (Kleinridders et al., 2015), (iii) it influences the spike frequency of cholinergic interneurons and dopaminergic neurons (Cai et al., 2018; Dunn, Abumrad, Patterson, Kessler, & Tamboli, 2019; Könner et al., 2011; Stouffer et al., 2015), and (iv) it induces long-term depression of excitatory

synapses onto dopamine neurons through endocannabinoid-mediated presynaptic depression of glutamate release (Labouèbe et al., 2013; S. Liu, Labouèbe, Karunakaran, Clee, & Borgland, 2013).

Accordingly, direct injections of insulin into the NAc (Stouffer et al., 2015) and the VTA (Labouèbe et al., 2013; Mebel, Wong, Dong, & Borgland, 2012) impact dopamine release in these structures. When administered into the VTA, insulin depresses somatodendritic dopamine through the up-regulation of the number and/or function of DAT and thus reduces feeding of sweetened high-fat food under sated conditions (Mebel et al., 2012). Insulin injection in the VTA also decreases glutamatergic synaptic transmission onto dopaminergic neurons, which in turn reduces dopaminergic activity and subsequent dopamine release in target regions of the mesolimbic system (Naef, Seabrook, Hsiao, Li, & Borgland, 2018). As a consequence, central insulin action in animals has been connected to suppression of conditioned place preference and to reduction of anticipatory activity for food rewards (Labouèbe et al., 2013), to reduced hedonic feeding (Mebel et al., 2012), to lower preference for food cues (Könner et al., 2011), and to decreased sucrose self-administration (Figlewicz, Bennett, Naleid, Davis, & Grimm, 2006). In hyperinsulinemic mouse models, the capacity of insulin to cause a synaptic depression of VTA dopamine neurons was found to be reduced (S. Liu et al., 2013).

It should be noted, though, that these findings are restricted to animals. Specifically studies in humans are scarce (Farr, Li, & Mantzoros, 2016), and selective effects of aberrant central insulin action are still under debate (Vogt & Brüning, 2013) or even unstudied when it comes to reward-related networks. The first study of the present thesis specifically aims at filling these research gaps.

1.2.2.3. Dysfunctions of the reward network

In obese individuals, neuroimaging studies have reported conflicting findings regarding neural responses to food stimuli, ranging from hypo- to hyperactive reactions (Burger & Berner, 2014; Hendrikse et al., 2015; Makaronidis & Batterham, 2018; Sayer et al., 2016). Amongst others, this apparent controversy might be caused by the uncontrolled impact of insulin on food processing. One frequently discussed theory in obesity is the reward deficiency theory, a theory that can be applied to a wide range of substance abuses and which is based on observations of decreased striatal signals in obese individuals (Kenny, 2011; Volkow, Wang, & Baler, 2011; G.-J. Wang et al., 2001). This hypofunction in the NAc has been attributed to a pre-existing neural vulnerability, as well as to adaptive neuroplasticity following perpetual overeating (Stice & Yokum, 2016; Volkow et al., 2011). How changes in central insulin functioning contribute to the regulation of food

processing in hyperinsulinemic humans, however, has not yet been studied, but will be one central topic of the first study.

1.2.2.4. Palatability coding in the amygdala

In addition to the NAc and the VTA, another key structure in hedonic food processing is the amygdala. Although not formally considered a part of the gustatory system in the stricter sense, the amygdala has bi-directional connections with nearly every level of the gustatory pathway (Carmichael & Price, 1995; Mufson, Mesulam, & Pandya, 1981; Norgren, 1976) and also contains taste-responsive neurons (Grossman, 2009; Scott et al., 1993). With these reciprocal connections to the primary gustatory cortex and taste-related areas in the brainstem (McDonald, 1998; Sah, Faber, Lopez De Armentia, & Power, 2003; Veening, Swanson, & Sawchenko, 1984), it is an important region in processing the emotional valence of food stimuli (i.e. palatability) (Fadok, Markovic, Tovote, & Lüthi, 2018; Fontanini, Grossman, Figueroa, & Katz, 2009; Piette, Baez-Santiago, Reid, Katz, & Moran, 2012; Sadacca, Rothwax, & Katz, 2012; Schiff et al., 2018; Stone, Maffei, & Fontanini, 2011). Furthermore, it plays a crucial role in engaging valence-specific behavioural responses (O'Neill, Gore, & Salzman, 2018). When investigating hedonic feeding, and specifically how hedonic food values are encoded and integrated in food choice, the amygdala constitutes a prime candidate, yet direct evidence for its contribution herein in humans are still lacking. To fill this research gap, the second study of this thesis investigates neural activation patterns in the amygdala accompanying hedonic valuation and links those to feeding-relevant decisions.

In animals, variable palatability of food is encoded through specific signals in the amygdala, with food items which are similarly liked also evoking comparable response patterns (Fontanini et al., 2009; Sadacca et al., 2012). Moreover, temporary inactivation of the amygdala through muscimol, a $GABA_A$-receptor agonist, reduces palatability-specificity in the gustatory cortex (Piette et al., 2012). Functional lesions in the amygdala through deep brain stimulation block liking reactions (Ross et al., 2016), and excitotoxic lesions in the amygdala of monkeys abolish previously learned flavour preferences (Agustín-Pavón, Parkinson, Man, & Roberts, 2011).

Conventional univariate analyses of human amygdala engagement in food valence have produced mixed findings, with only some studies validating the role of the amygdala for palatability processing in humans. In these studies, the amygdala responded to pleasantness and unpleasantness, during both the anticipation as well as the consumption of food (O'Doherty, Deichmann, Critchley, & Dolan, 2002; O'Doherty, Rolls, Francis, Bowtell, & McGlone, 2001) and the perception of odors (Patin & Pause, 2015; Sorokowska et al., 2016; Winston, Gottfried, Kilner, &

Dolan, 2005). Viewing palatable versus bland foods was also found to activate the amygdala (Nummenmaa et al., 2018). Other studies did not report amygdala contribution to hedonic processing (Jin, Zelano, Gottfried, & Mohanty, 2015; Small et al., 2003). This is possibly due to functional heterogeneity of amygdala neurons and ignorance of univariate methods towards information carried through brain activity patterns that are distributed across neurons or cortical regions. If regional representations of opposing valences were interspersed within the amygdala, as animal studies suggest (Beyeler et al., 2018; J. Kim, Pignatelli, Xu, Itohara, & Tonegawa, 2016), univariate analyses would be unsuited to tease apart these unique populations and patterns.

In order to avoid such shortcomings, multivariate pattern-based analyses (MVPA) (Kriegeskorte, Mur, & Bandettini, 2008; Nili et al., 2014) have been used to investigate relationships between multivoxel measures of amygdala activity during odour and taste perception (Fournel, Ferdenzi, Sezille, Rouby, & Bensafi, 2016; Jin et al., 2015). Rather than proceeding on a voxel-by-voxel basis, these MVPA evaluate the correlation of activation across brain regions, so that their results can more easily be interpreted as a signature of neural networks. Findings imply that multivoxel amygdala patterns encode the whole dimension of valence, ranging from pleasantness to unpleasantness (Jin et al., 2015).

In this context, studies in animals have shown that amygdala subregions contain distinct neuron populations, which are distinctly activated by negative or positive stimuli (Beyeler et al., 2018, 2016; J. Kim et al., 2016; O'Neill et al., 2018; Paton, Belova, Morrison, & Salzman, 2006). Very recent rodent data extend these findings also to food valence: Topographically distinct areas of the amygdala were innervated by sweet and bitter taste representing cortical fields and silencing these specific amygdala neurons diminished valence assignment (L. Wang et al., 2018).

1.2.2.5. Amygdala contribution to goal-directed behaviour

In addition to encoding palatability via the above described mechanisms, the amygdala and its projections are crucial for the use of reward expectations in the guidance of goal-directed behaviour (Holland & Gallagher, 2004; O'Neill et al., 2018). A study using microinjections of naloxone into the basolateral amygdala (BLA) showed that this structure is required to integrate increased hedonic value of a reward into subsequent instrumental responses for this reward (Wassum, Ostlund, Maidment, & Balleine, 2009). Also neurons in the central amygdala (CeA) drive up feeding behaviour by enhancing the rewarding aspects of food after the initial consumption (Douglass et al., 2017).

Studies in humans build upon this by providing evidence that at the time of decision-making, activity in the amygdala encodes important decision variables, which the authors interpreted as

active amygdala participation in food choice (Grabenhorst, Schulte, Maderwald, & Brand, 2013). MVPA, as used in the second study of this thesis, now offers a promising approach to not only detect spatial segregation of appetitive versus aversive patterns, but to also address the question of whether hedonic valuation signals in the amygdala are incorporated into value computations which subsequently trigger food choice behaviour.

1.2.2.6. Overall value computation in the prefrontal cortex

While recent research has started to unravel the neurocircuits underlying hedonic valuation (Dalenberg, Weitkamp, Renken, Nanetti, & Ter Horst, 2017; Grabenhorst, D'Souza, Parris, Rolls, & Passingham, 2010; Small et al., 2003; Stice, Burger, & Yokum, 2013) and those underlying of food decisions (Ferrario et al., 2016; Hare et al., 2011; Leng et al., 2017; Rangel, 2013; Rihm et al., 2019), how valence signals are integrated into food choices is less understood.

The ventromedial PFC (vmPFC) contributes to the computation of overall values of choice options (Hare et al., 2009; Kable & Glimcher, 2009; Paulus & Frank, 2003) and the guidance of reward-related behaviours (FitzGerald, Friston, & Dolan, 2012; Gläscher, Hampton, & O'Doherty, 2009). Recent optogenetic findings in rodents suggest GABA-ergic bottom-up effects from the amygdala to the vmPFC on the modulation of reward-related behaviours (Seo et al., 2016). This agrees with lesion data in animals (Floresco & Ghods-Sharifi, 2007; Rudebeck, Mitz, Chacko, & Murray, 2013) and humans (Hampton, Adolphs, Tyszka, & O'Doherty, 2007) indicating a critical role for the amygdala in establishing reward expectation values in the vmPFC.

In addition, there is large evidence for the integration of signals from the NAc with vmPFC value computation for both monetary and primary rewards, during both the decision and the consumption phase (Bartra, McGuire, & Kable, 2013; Clithero & Rangel, 2014; Kable & Glimcher, 2007; Jan Peters & Büchel, 2010). With these reciprocal connections to the amygdala (Baxter & Murray, 2002; Cho, Deisseroth, & Bolshakov, 2013; Freese & Amaral, 2009; Jamie Peters, Kalivas, & Quirk, 2009) and to the NAc (Haber & Knutson, 2009), the vmPFC is in a position to integrate hedonic values into food choices. The present thesis examines whether and how dopaminergic motivation signals act in concert with liking-specific amygdala signals to modulate actual food decisions mediated by the vmPFC.

1.3. Study aims

1.3.1. Study 1: Central insulin modulates food valuation via mesolimbic pathways

In order to investigate the effect of insulin on central hedonic food processing in humans, particularly in the mesolimbic pathway, we combined fMRI with a pharmacological challenge, specifically insulin application, and an affective validation paradigm of food and non-food cues, in healthy young participants who had fasted overnight. Participants were selected so that they comprised individuals with normal peripheral insulin sensitivity as well as non-diabetic individuals with increased levels of peripheral insulin resistance. This enabled us to examine the effects of central insulin under normal and pathological circumstances. When applied intranasally, as was done in this study, insulin has been shown to bypass the BBB in humans, and to reach the CNS within a short period of time (approximately 30 minutes after application) without relevant systemic absorption (Benedict, Kern, Schultes, Born, & Hallschmid, 2008; Born et al., 2002; Spetter & Hallschmid, 2015). Through intranasal application, it can be ruled out that present findings in individuals with increased levels of peripheral insulin resistance are confounded by a potentially attenuated transport of insulin across the BBB (Heni, Kullmann, Preissl, Fritsche, & Häring, 2015). In the data acquired during this study, we investigated the effect of insulin on behavioural performances in response to different food cues and non-food cues, serving as controls. Further, we investigated insulinergic effects on neural responses and on effective network dynamics in reward-related brain regions. On the basis of these data, we tested several hypotheses: (i) In individuals with normal insulin sensitivity, intranasal insulin (INI) reduces the behavioural preference for food, but not for non-food stimuli, (ii) accompanied by reduced food value signals in the mesolimbic path. (iii) Participants with abnormal peripheral insulin sensitivity exhibit aberrant central insulin functioning.

1.3.2. Study 2: Valence signatures and food choice modulation in the human amygdala

The expectation of tastiness is a particularly strong driver in day-to-day food consumption decisions. The amygdala plays an important role on both hedonic valuation processes and valence-related behaviour, yet the relationship between both processes is less understood. To elucidate the role of valence assignment in the amygdala during eating decisions we applied pattern-based representational similarity and effective connectivity analyses to two separate fMRI data sets acquired while overnight fasted volunteers with normal body status (measured via weight circumference) performed an explicit liking task on a wide range of food stimuli on one day and a consumption decision task on the same food items on another day. Building upon

previous studies in humans (Jin et al., 2015) and particularly in animals (Beyeler et al., 2018; Namburi et al., 2015; L. Wang et al., 2018), we tested several predictions: (i) Food liking values are differentially encoded across the entire palatability spectrum in the amygdala with topographically segregated activity patterns signalling pleasantness and unpleasantness. (ii) Food-specific single-trial liking-patterns in the amygdala predict the impact of palatability on subsequent food choices. (iii) The amygdala contributes liking values into food choices by modulating the vmPFC-NAc network.

2. Study 1: Central insulin modulates food valuation via mesolimbic pathways

2.1. Materials and methods

2.1.1. Participants

All participants analysed in the present thesis were recruited via (online) announcements and existing databases. Upon the participant's declaration of interest to participate in the present studies, an extensive interview was conducted via telephone. During this interview, we assessed whether participants were eligible for MR studies, queried their eating habits, and confirmed their willingness to fasten overnight, to receive insulin as a nasal spray, and to have blood samples taken. Exclusion criteria comprised current or previous psychiatric or neurological disorders, acute and chronic physical illness including diabetes, current psychopharmacological medication as well as MR-specific exclusion criteria. To exclude systematic confounds during affective food valuation, severe food allergies or adherence to specific eating habits, e.g. a vegan or caloric restriction diet, constituted further exclusion criteria. No participant had deliberately tried to change his/her eating behaviour or body status in the six months preceding the experiment, nor had there been any unintended changes in body weight. All participants had normal or corrected-to-normal visual acuity.

Following the telephone interview, sixty-seven suited volunteers were invited for an initial screening day (Fig. 3a). On this day, blood samples were taken from which haemoglobin A1c (HbA1c) levels were analysed to exclude cases of diabetes. In addition, participants were again interviewed for their eating habits and health history, specifically if they had any chronical diseases affecting their gastrointestinal tract. During this screening day and in waiting periods during the experimental days, participants filled out a range of questionnaires. These assessed participants' depression scores (Beck Depression Inventory, BDI (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961)), their external, emotional and restrained eating behaviour (Dutch Eating Behaviour Questionnaire, DEBQ (Strien, Frijters, Bergers, & Defares, 1986)), food addiction symptoms (Yale Food Addiction Score, YFAS (Gearhardt, Corbin, & Brownell, 2009)), different markers of personality (Eysenck Personality Questionnaire, EPQ (Ruch, 1999)), reward and punishment sensitivity (Sensitivity to Punishment and Sensitivity to Reward Questionnaire, SPSR (Torrubia, Avila, Moltó, & Caseras, 2001)) and their level of physical activity (International Physical Activity Questionnaire, IPAQ (Hagströmer, Oja, & Sjöström, 2006)).

After the screening day, ten participants were excluded due to abnormally high HbA1c levels or depression scores in the BDI, and six participants voluntarily ended their participation in the experiment because of personal reasons.

Fifty-one volunteers participated on all experimental days of the present study. However, since fasting blood glucose levels (6.55, 6.27 and 6.77 $mmol*l^{-1}$) and eating protocols of three individuals revealed that they did not follow the 10 hour fasting instruction, these individuals were excluded, resulting in forty-eight participants included in the first study of this thesis (20–34 years, mean = 25.83, standard deviation (SD) = 3.30; 25 female).

Insulin resistance is strongly associated with overeating, weight gain, obesity, and body mass index (BMI) (Danielsson et al., 2009; Erdmann et al., 2008; Schindler et al., 2006). Since we were interested in participants with normal and aberrant insulin functioning, 50% of our sample comprised lean adults (BMI 18.5–25 $kg*m^{-2}$, n = 24), whereas the other age- and sex-matched half of our sample consisted of overweight/obese participants (BMI 25.1–38 $kg*m^{-2}$, n = 24).

The local ethics committee approved the study and all participants gave written informed consent and were financially compensated for participation.

2.1.2. Experimental design

After successful screening, participants attended two experimental days (Fig. 3b). These days were separated by at least one week to rule out sustained effects of insulin. On each experimental day, participants arrived in the morning between 7:30 and 10:30 o'clock after having fasted overnight for at least 10 hours. First, participants rated their current feeling of hunger on a scale from 0 ('not hungry at all') to 10 ('extremely hungry') (Fig. 3b, Assessment I). After this, anthropometric measurements were taken – specifically, participants' weight, height, body fat percentage, and waist circumference was assessed. For measurements of waist circumference, a measuring tape was positioned between the iliac crest and the lowest rip, with participants standing upright in a relaxed position. Blood samples were collected, from which blood glucose, insulin, leptin and C-peptide were analysed. After these assessments, participants received either 160 international units (IU) of insulin (Insuman Rapid, 100 IU/ml) or vehicle (0.27% m-Kresol, 1.6% glycerol, 98.13% water). The dose of insulin was chosen based on earlier studies showing effects of INI on neural activity in homeostatic, reward-related (Heni et al., 2012), and (working) memory-related brain regions (Guthoff et al., 2010; Krug, Benedict, Born, & Hallschmid, 2010), on postprandial energy expenditure (Benedict et al., 2011), and on food intake (Benedict et al., 2008). As compared to lower doses of INI (e.g. 40 IU or 80 IU), administration of 160 IU shows the most reliable effects (Kullmann et al., 2017; Shemesh, Rudich, Harman-Boehm, & Cukierman-Yaffe, 2012; Thanarajah, Hoffstall, et al., 2019; Thanarajah, Iglesias, et al., 2019), without causing severe adverse effects (Schmid et al., 2018). Participants received eight puffs per nostril, each puff consisting of 0.1 ml solution containing 10 IU human insulin or 0.1 ml placebo. The order of

application of insulin and placebo was randomized and balanced; the application was double-blind.

Before scanning, during a training session, participants were familiarized with the validation task and the set-up. Thirty minutes after nasal spray application, participants began the paradigm in the MR scanner.

During the paradigm, participants were asked to rate their overall preference for food and non-food items with yes ('I like this') or no ('I do not like this') via button press, which was followed by a four-point rating scale where they were asked to provide a detailed rating, indicating how much they liked or disliked each item (Fig. 3c). Parametric values were derived by transferring the binary and the four-point rating onto a single scale ranging from 1 to 8:

Binary response	No ('I do not like this')				Yes ('I like this')			
Four-point rating	- - - -	- - -	- -	-	+	+ +	+ + +	+ + + +
Parametric value	1	2	3	4	5	6	7	8

Food and non-food stimuli were pseudo-randomly presented during three runs: No more than three pictures from one category were presented in a row. Each run began with the instruction ('We will soon start with the question: Do you like the presented item or not?') and lasted up to 12 minutes. Single runs were separated by a one minute break to relax.

After completion of the scans, outside the scanner, participants again rated their feeling of hunger and a second set of blood samples was collected to control for spill-over effects of insulin into the peripheral blood circulation, measured via serum insulin concentration (Kullmann et al., 2018) (Fig. 3b, Assessment II).

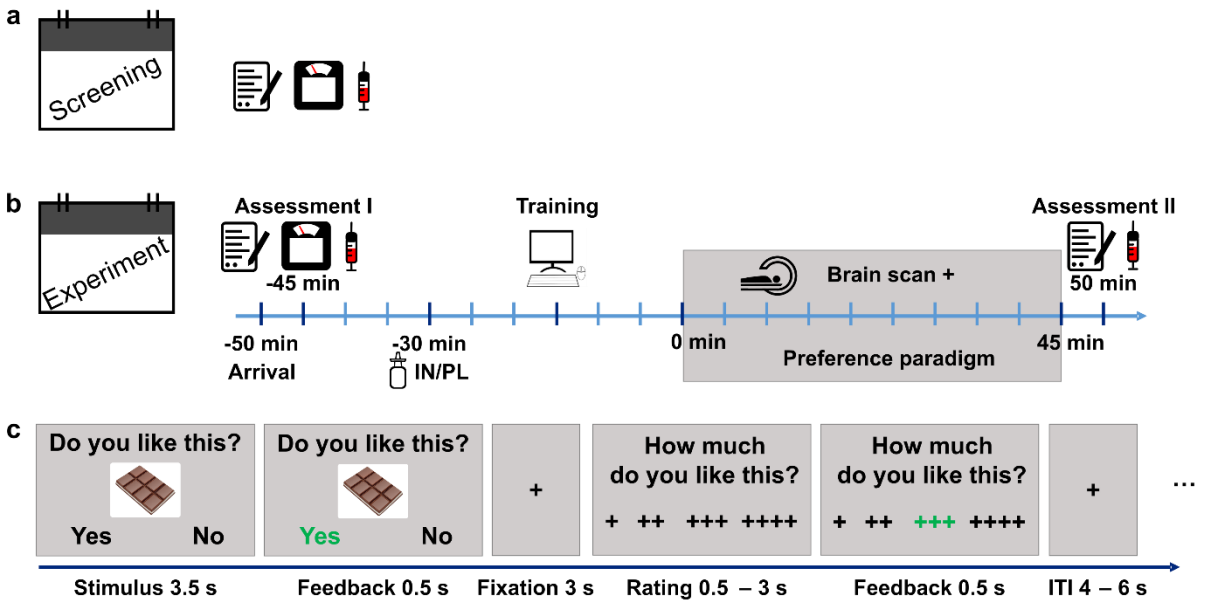

Figure 3 | Study 1: Design and experimental task

(a) During an initial screening day, blood samples were taken from which HbA1c was analysed. In addition, participants filled out questionnaires, were interviewed for their health history and eating habits, and body measures were taken. (b) Time course of the two experimental days: Upon their arrival, participants rated their feeling of hunger, filled out different questionnaires, and a first set of blood samples was taken. After this, participants received the nasal spray (either insulin (IN) or placebo (PL)). In the 30 minutes waiting period, participants practiced the preference paradigm on a laptop. Afterwards, they completed the paradigm inside the MR scanner. After completion of the scans, another set of blood samples was taken. (c) Schematic representation and timing of the experimental paradigm: During a single trial, one food or non-food picture was presented for 4 seconds. During the first 3.5 seconds, participants had to indicate their general liking of that item, by pressing one of two buttons (yes/no). Feedback of the chosen answer was provided for 0.5 seconds. After a fixation period of 3 seconds and during a maximum duration of 3 seconds, participants detailed their preference rating using a four-point rating scale, by pressing one of four buttons. After another feedback, the trial ended with an inter-trial interval (ITI) of 4 to 6 seconds.

2.1.3. Stimulus sets

Prior to the experiments described in this thesis, stimulus batteries had been created and had been validated in an independent sample of participants (n = 16). The present studies made use of two parallel sets of stimuli, each consisting of 70 food and 70 non-food colour images (Tbl. 1,

Fig. 4). All images were selected from the internet, had a size of 400x400 pixels and depicted food and non-food items centrally positioned on a white background. Food pictures did not contain brand names or packaging and featured both sweet and savoury items (Fig. 4 left column). Depicted food items covered common high- and low-palatable foods within a wide range of macronutrient composition and caloric content (sugar: 0 – 78g/100g, complex carbohydrates: 0 – 66.2g/100g, protein: 0.2 – 25.4g/100g, fat: 0 – 62g/100g, absolute calories: 16 – 666 kcal/100g). To ensure that participants would be able to draw on relevant past experiences with the same or very similar foods, foods were selected which are widely available to buy (e.g. chocolate, fries, grapes, apples, ice cream, oranges, sweets, nuts).

Accessories and trinkets were chosen as non-food items (Fig. 4 right column), which were intended to elicit comparable levels of liking or disliking.

Validation of the two sets revealed that the two sets did not differ significantly regarding the mean preference ratings of the stimuli or average picture salience, nor did the food items of the two sets differ regarding macronutrient composition (Tbl. 1, all $T(68) < 1.730$, all $p > 0.085$).

The two stimulus sets were presented in a randomized order across scanning days and participants.

Table 1 | Stimuli characteristics

In a validation study conducted prior to the here reported studies, an independent sample of 16 participants rated the preference of food and non-food items on scales from 1 (~ "I do not like this at all") to 4 (~ "I like this very much").

For every image, a saliency index was calculated based on the Image Signature algorithm, as described by Hou et al. (Hou, Harel, & Koch, 2012). This approach calculates saliency maps by the identification of visually conspicuous image locations based on a discrete cosine transform (DCT), which transforms spatial signals to frequency signals.

Information on macronutrient content was taken from https://fddb.info/ and is given in grams per 100 gram of product, irrespective of the amount of food shown in the image.

The two stimulus sets did not differ in regard to preference scores, image salience, macronutrient or total caloric content. Values indicate means and SD.

	Set 1	Set 2	*T*	*p*
All items				
Picture saliency	0.18 ± 0.07	0.20 ± 0.08	1.730	0.085
Preference score, validation study	2.67 ± 0.54	2.72 ± 0.58	0.767	0.444
Food items				
Picture saliency	0.19 ± 0.06	0.20 ± 0.06	0.700	0.485
Preference score, validation study	2.86 ± 0.56	2.98 ± 0.52	1.361	0.176
Sugar (g/100g)	16.09 ± 19.85	16.87 ± 18.96	0.236	0.813
Complex carbohydrates (g/100g)	11.93 ± 15.66	13.82 ± 17.6	0.673	0.502
Fat (g/100g)	12.80 ± 13.78	12.53 ± 13.85	0.116	0.908
Protein (g/100g)	7.08 ± 6.76	5.89 ± 5.22	1.168	0.245
Total calories (kcal)	257.43 ± 182.95	263.43 ± 189.97	0.190	0.849
Non-food items				
Picture saliency	0.17 ± 0.08	0.19 ± 0.09	1.637	0.104
Preference score, validation study	2.49 ± 0.47	2.47 ± 0.52	0.218	0.828

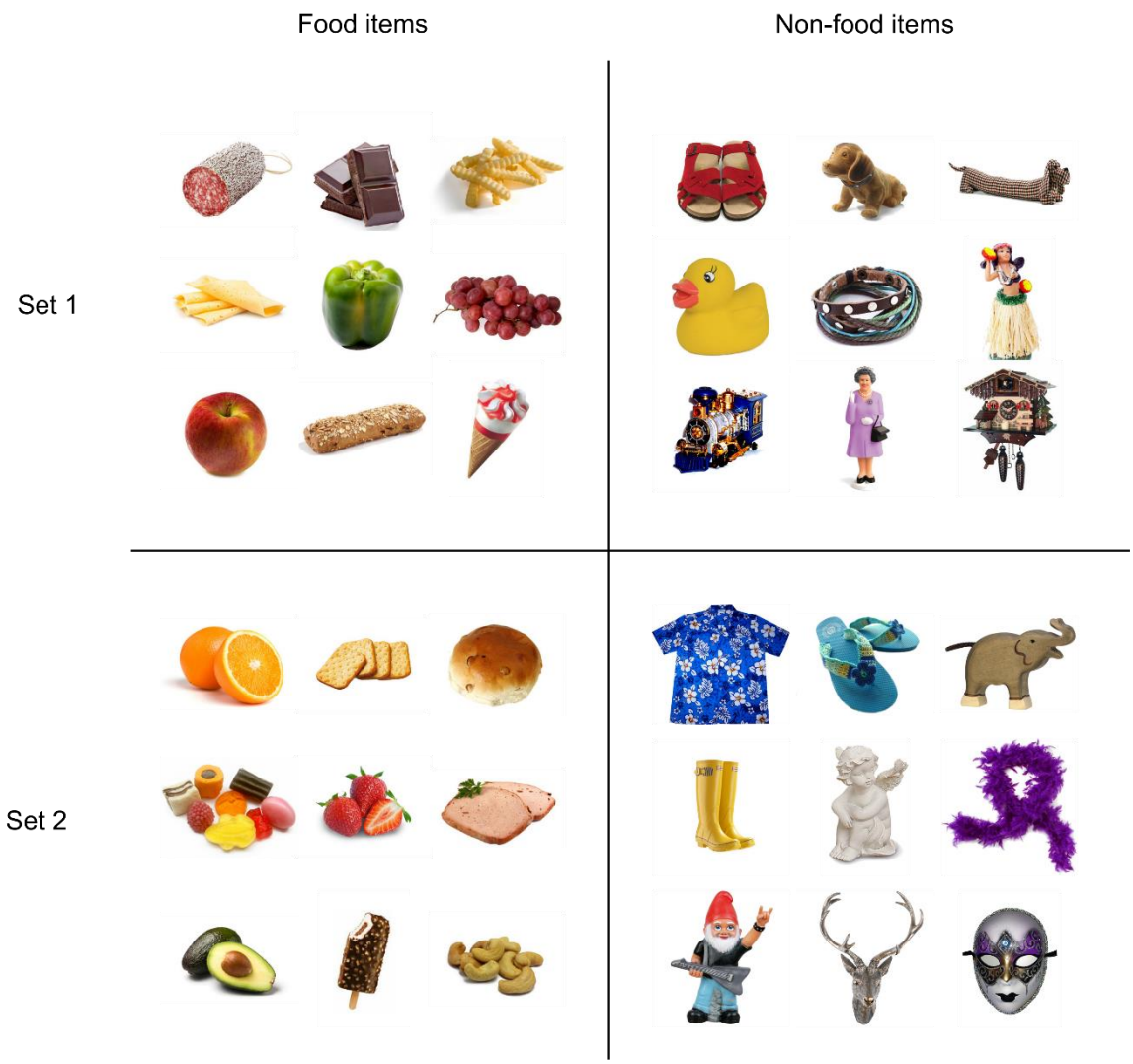

Figure 4 | Example stimuli

Examples of food and non-food items from set 1 and set 2.

2.1.4. Group classification

In this first study, participants were separated into two groups based on their peripheral insulin sensitivity. There are different means to assess this parameter, many of which are based on exogenous infusion of glucose or insulin, such as the glucose tolerance test or the insulin suppression test. Another measure is provided by the homeostatic model of insulin resistance (HOMA-IR), a mathematical model using fasting plasma glucose and insulin concentrations, see (I).

$$HOMA - IR = \frac{Glucose\ (mmol * l^{-1})\ x\ Insulin\ (\mu U * ml^{-1})}{22.5} \qquad \text{(I)}$$

In the present study, group definition was performed using this HOMA-IR, derived from the blood samples collected before administration of the nasal spray. Participants with a HOMA-IR score below 2 (Gayoso-Diz et al., 2013) on both scanning days were assigned to the 'normal insulin resistance' group (NIR), participants with a score equal to or larger than 2 were assigned to the 'increased insulin resistance' group (IR).

2.1.5. Statistical analyses

Paired *t*-tests were used to investigate potential differences between PL and IN sessions regarding blood parameters, hunger state and fasting duration. Independent samples *t*-tests and chi-square tests were used to compare the two groups. Interactions and differences between groups, sessions and time-points were analyzed using repeated measures analyses of variance (rmANOVAs). Relations between peripheral insulin sensitivity, behavioural performances and DCM parameters estimates were analyzed using Pearson correlations. We tested two-sided.

2.1.6. MRI and fMRI data acquisition and pre-processing

All imaging data were acquired on a Siemens Trio 3T scanner (Erlangen, Germany) at the Institute for Systems Neuroscience in Hamburg. For all images, a 32-channel head coil was used. Functional data were obtained using a multiband (number of bands = 2) echo-planar imaging (EPI) sequence. Slices of each volume were positioned at an oblique orientation, approximately 30° steeper than the anterior commissure–posterior commissure axis. An additional structural image (magnetization prepared rapid acquisition gradient echo (MPRAGE)) was acquired for functional pre-processing. See Tbl. 2 for a list of relevant fMRI imaging parameters.

Table 2 | Scan parameters

Relevant parameters of the EPI and the MPRAGE sequence.

	EPI	MPRAGE
Repetition time (TR) (ms)	2260	2300
Echo time (TE) (ms)	30	2.96
Flip angle	80°	12°
Number of slices	60	240
Voxel size (mm)	1.5 x 1.5 x 1.5	1 x 1 x 1

EPI, echo-planar imaging; MPRAGE, magnetization prepared rapid acquisition gradient echo; TE, echo time; TR, repetition time

Structural and functional data were analysed using SPM12 (Welcome Department of Cognitive Neurology, London, UK) and custom scripts in MATLAB (Version 2017a, Mathworks, Natick, MA, USA).

To eliminate T1 saturation effects, the first five EPIs were discarded. Subsequently, all functional volumes were realigned using rigid body motion correction ('realign and unwarp'). Each participant's individual structural T1 image was coregistered to the respective mean functional image generated during realignment. Functional images were then spatially normalized using unified segmentation and normalization, via the NewSegment routine in SPM, into a standard stereotactic space (Montreal Neurological Institute (MNI) template). Finally, the images were smoothed with a 4-mm Full Width at Half Maximum (FWHM) isotropic Gaussian kernel.

2.1.7. FMRI data analyses and ROI definition

A two-level random effects approach utilizing the general linear model (GLM) as implemented in SPM12 was used for statistical fMRI analyses. For each participant, onsets of food and non-food stimuli presentation were modelled as separate regressors convolving delta functions with a canonical hemodynamic response function. Analyses of the fMRI data were time-locked to the onset of stimulus presentation. Additionally, subjective combined preference values, ranging from 1 to 8, were included as parametric modulators in the model, separately for food and non-food regressors. Data from the placebo and the insulin sessions were defined as separate sessions; both sessions were entered into a single model.

As a next step, the GLM denoise toolbox for Matlab (Kay, Rokem, Winawer, Dougherty, & Wandell, 2013) was used to improve the signal-to-noise ratio. By conducting principal component and cross-validation analyses on voxel time-series, noise regressors were identified that were unrelated to the experimental paradigm. These individual noise regressors were then entered as regressors of no interest into the first-level model.

For each participant, contrast images for each regressor of interest were entered into second-level random-effect ANOVA models including the factors stimulus type (food/non-food), session (PL/IN) and group (NIR/IR). We report results corrected for family-wise error (FWE) due to multiple comparisons. This correction was conducted at the peak level within small volume regions of interest (ROIs) for which we had priori hypotheses or at the whole-brain level for exploratory reasons. Based on aforementioned central insulin findings in animals and our specific interest in its role in reward processing, we focused our analyses on the NAc and the VTA. To this end, we created ROIs as spheres with a 4mm radius, centred on the bilateralized peak voxels in the NAc (±12, 10, -8) and the VTA (±4, -14, -12) derived from 670 imaging studies on reward, as determined by a meta-analysis conducted on the neurosynth.org platform (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011) (status September 2016, Fig. 5).

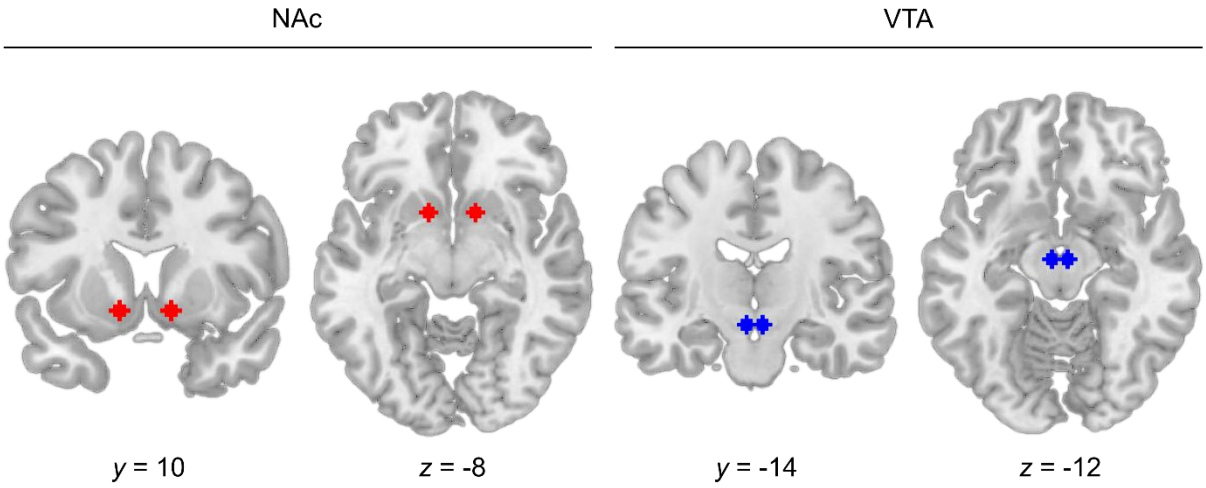

Figure 5 | Study 1: Regions of interest

Based on findings in animals of the effects of central insulin and its role in reward processing, in this study, the NAc and the VTA were selected as regions of interest.
NAc, nucleus accumbens; VTA, ventral tegmental area

2.1.8. Dynamic causal modelling

For effective connectivity analyses, we used the DCM software as implemented in SPM12 and a Bayesian model reduction approach (Friston et al., 2016). Principal Eigenvariate time-series were extracted from unilateral ROIs, i.e. the NAc and the VTA, adjusted for all effects of interest. To modulate the effects of insulin in a single model, time series were concatenated over the two experimental days. For every participant, a full DCM model was set up. This full model comprised three factors: (i) fixed connections between the VTA and the NAc, that is, extrinsic forward and backward connections between two regions, as well as their respective intrinsic self-connections (A matrix), (ii) contextual modulation of these connections by insulin (B matrix) and (iii) exogenous inputs, specifically visual stimuli, as driving inputs into the nodes (C matrix). This full model was defined and estimated for each participant. In the next step, post-hoc model selection was used to create and test all 2,048 possible models (two driving inputs and four possible modulatory effects on four endogenous connections) in an unbiased way. To identify the winning model at the group level, the evidence of each (reduced) model was pooled over all subjects. With Bayesian parameter averaging, magnitudes and probabilities of all selected coupling parameters, as well as the magnitudes and effects with which these connections are modulated, were calculated. Finally, we performed one-sample *t*-tests with Bonferroni correction on the Bayesian parameter averages to determine which parameters differed significantly from zero.

We repeated the procedure separately for NIR and IR groups. In addition to the Bayesian parameter averages across all participants, this method provides the single participant's individual parameters for the optimal model, which were extracted and entered into correlation analyses including behavioural measures.

2.2. Results

2.2.1. Task overview

All participants underwent a two-day fMRI scanning procedure, separated by at least one week (8.85 ± 3.94 days). After an overnight fast of at least 10 hours, on each scanning day participants arrived in the morning between 7:30 a.m. and 10:30 a.m. As insulin follows a diurnal rhythm (Carroll & Nestel, 1973; Jacobs et al., 1997; Saad et al., 2012), measurements were only taken in the morning. Fasting glucose levels confirmed fasting state in all participants on both scanning days. IN and PL sessions did not differ in regard to participants' blood markers, average hunger ratings or fasting duration (all $T(47) < 1.353$, all $p > 0.182$, Tbl. 3).

Table 3 | Study 1: PL versus IN

Placebo and insulin sessions were compared regarding participants' blood parameters, fasting duration and hunger ratings. Values indicate mean and SD.

	PL	IN	*T*	*p*
Fasting duration (hours)	12.76 ± 1.41	12.89 ± 1.39	0.647	0.521
Hunger rating (pre_scan)	3.80 ± 2.41	3.93 ± 2.19	0.427	0.672
Blood				
Insulin (pre_scan) ($pmol*l^{-1}$)	55.20 ± 30.00	56.15 ± 26.10	0.263	0.794
Glucose (pre_scan) ($mmol*l^{-1}$)	4.77 ± 0.44	4.75 ± 0.40	0.397	0.693
C-peptide (pre_scan) ($nmol*l^{-1}$)	0.61 ± 0.19	0.59 ± 0.19	1.353	0.182
Leptin (pre_scan) ($\mu g*l^{-1}$)	9.31 ± 10.78	8.68 ± 10.83	1.303	0.199

IN, insulin; PL, placebo; pre_scan, blood sample taken before the scan

2.2.2. Insulin groups

Forty-eight normal to overweight non-diabetic volunteers participated in the study and were separated into two groups based on their insulin sensitivity as measured via the homeostatic model assessment for insulin resistance using a cut-off of < 2 (Gayoso-Diz et al., 2013). This assessment indicated normal insulin sensitivity in n = 28 participants (NIR), and increased insulin resistance in n = 20 participants (IR). Importantly, in all IR participants, normal HbA1c values confirmed the exclusion of diabetes. These individuals are at an increased risk for T2D (Bonora et al., 2002), yet elevated insulin release might still compensate for their reduced insulin sensitivity (Tbl. 4). Across all participants, we observed high correlations between HOMA-IR and BMI ($r = 0.686$, $p < 0.001$), body fat ($r = 0.553$, $p < 0.001$) and waist circumference ($r = 0.604$, $p < 0.001$).

We compared NIR and IR participants regarding their age, gender, and days between sessions (Tbl. 4) and did not find differences between the two groups (all $T(46) < 0.381$, all $p > 0.705$). None of the questionnaires scores describing the participants' mood, personality, eating behaviour or physical activity differed significantly between the two groups (all $T(46) < 1.703$, all $p > 0.095$). This underlines that in spite of their increased insulin resistance and body composition, participants in the IR group did not exhibit unusual eating behaviour, did not suffer from mood disorders, had comparable levels of sensitivity to punishment and reward, and were not significantly less physically active.

Compared to NIR participants, IR participants had significantly higher scores in all body measurements, specifically BMI, waist, and body fat (all $T(46) > 3.310$, all $p < 0.002$). They also showed significantly elevated levels in all adiposity-related blood parameters at baseline (i.e.

during the PL session before the scan), specifically in glucose, insulin, leptin and C-peptide levels (all $T(46) > 2.682$, all $p < 0.010$).

Table 4 | Study 1: Sample characteristics

Comparison of groups with respect to demographics, anthropometrics, questionnaire scores and blood parameters. Values indicate group means with standard deviations.

	NIR (n = 28)	IR (n = 20)	*T*	*p*
Age (years)	25.67 ± 3.45	26.05 ± 3.14	0.381	0.705
Gender (female/male)	14/14	11/9		0.732
Days between sessions	8.71 ± 3.54	9.05 ± 4.52	0.289	0.774
Body measures				
BMI ($kg*m^{-2}$)	23.59 ± 3.94	29.40 ± 4.98	4.505	< 0.001
Waist (cm)	78.29 ± 10.23	88.33 ± 10.54	3.310	0.002
Body fat (%)	22.59 ± 7.75	32.01 ± 9.14	3.853	< 0.001
Questionnaires				
BDI	6.04 ± 4.48	6.80 ± 6.12	0.500	0.619
DEBQ: external eating	3.05 ± 0.46	3.03 ± 0.54	0.139	0.890
DEBQ: restraint eating	2.27 ± 0.76	2.56 ± 0.65	1.367	0.178
DEBQ: emotional eating	2.10 ± 0.75	1.99 ± 0.77	0.502	0.618
YFAS: loss of control	0.07 ± 0.38	0.30 ± 0.66	1.525	0.134
YFAS: unsuccessful quitting	1.29 ± 0.85	1.15 ± 0.99	0.508	0.614
YFAS: time demand	0.04 ± 0.19	0.20 ± 0.52	1.533	0.132
YFAS: constraint	0.14 ± 0.45	015 ± 0.49	0.052	0.958
YFAS: negative consequences	0.11 ± 0.31	0.30 ± 0.47	1.703	0.095
YFAS: tolerance	0.18 ± 0.48	0.10 ± 0.45	0.578	0.566
YFAS: withdrawal	0.14 ± 0.59	0.40 ± 0.82	1.263	0.213
YFAS: clinical significance	0.00 ± 0.00	0.15 ± 0.49	1.629	0.110
EPQ: psychoticism	3.79 ± 2.39	3.05 ± 1.93	1.135	0.262
EPQ: neuroticism	4.00 ± 2.82	3.95 ± 2.95	0.060	0.953
EPQ: extraversion	8.96 ± 3.07	9.00 ± 2.99	0.040	0.968
SPSR: susceptibility to punishment	9.21 ± 5.04	8.00 ± 5.12	0.805	0.425
SPSR: susceptibility to reward	11.50 ± 3.60	12.68 ± 4.37	1.015	0.315
IPAQ: total scored sum	5654.83 ± 5897.11	6968.18 ± 4501.60	0.836	0.407
Blood				
HOMA-IR (pre_PL)	1.20 ± 0.51	2.45 (1.08)	5.391	< 0.001
Glucose ($mmol*l^{-1}$) (pre_PL)	4.64 ± 0.40	4.96 (0.43)	2.682	0.010
Insulin ($pmol*l^{-1}$) (pre_PL)	40.13 ± 16.56	76.31 (32.14)	5.099	< 0.001
Leptin ($\mu g*l^{-1}$) (pre_PL)	5.05 ± 4.99	15.27 (13.70)	3.635	0.001

C-peptide (nmol*l^{-1}) (pre_PL)	0.54 ± 0.16	0.72 (0.17)	3.740	0.001
HbA1C (%) (Screening day)	4.79 ± 0.89	4.27 (1.64)	1.425	0.161

BDI, Beck Depression Inventory; BMI, body mass index; DEBQ, Dutch Eating Behaviour Questionnaire; EPQ, Eysenck Personality Questionnaire; HbA1c, haemoglobin A1c; HOMA-IR, homeostatic model of insulin resistance; IPAQ, International Physical Activity Questionnaire; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; pre_PL, measurement before application of nasal spray, on the day of the PL sessions; SPSR, Sensitivity to Punishment and Sensitivity to Reward Questionnaire; YFAS, Yale Food Addiction Scale

2.2.3. Comparability of sessions and effects of INI on blood parameters

Analyses on pre-scan C-peptides and leptin levels, as well as overnight fasting times demonstrated no differences between sessions within the groups, nor did they reveal significant group by session interactions (Tbl. 5; all $F < 1.082$, all $p > 0.304$).

Table 5 | Study 1: Comparison of PL and IN within and between groups

Differences between the sessions within groups, interactions of group and sessions. For easier readability, *T* values and exact *p* values were omitted here.

	NIR			IR			
	PL	IN	*p*	PL	IN	*p*	int
C-peptide (nmol*l^{-1})	0.54 ± 0.16	0.49 ± 016	ns	0.72 ± 0.17	0.72 ± 0.14	ns	ns
Leptin (µg*l^{-1})	5.05 ± 4.99	4.80 ± 4.29	ns	15.27 ± 13.70	14.11 ± 14.52	ns	ns
Time fasted (h)	12.79 ± 1.62	12.76 ± 1.46	ns	12.73 ± 1.09	13.08 ± 1.31	ns	ns
Pulse (bpm)	91.91 ± 11.85	94.91 ± 16.69	ns	95-02 ± 14.65	99.33 ± 15.15	ns	ns
Respiration (breaths per minute)	13.93 ± 1.71	13.37 ± 2.20	ns	13.81 ± 1.92	13.82 ± 2.24	ns	ns

IN, insulin; int ~ interaction; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; PL, placebo; ns ~ not significant

Plasma insulin levels decreased over time during the placebo sessions in the NIR group (Tbl. 6; $T(27) = 2.602$, $p = 0.015$), and during the insulin session in the IR group ($T(19) = 3.332$, $p = 0.004$). This resulted in a significant time point by session interaction in the IR group (Tbl. 6; $F(1,27) = 15.451$, $p = 0.001$), and a significant time point by session by group interaction ($F(1,46) = 4.441$, $p = 0.041$). At the same time, glucose levels increased slightly in the NIR group during the placebo

session (Tbl. 6; $T(27) = 2.517$, $p = 0.018$). After the scans, participants reported to feel hungrier, with a significant or trending to significant increase in hunger ratings (Tbl. 6; NIR, PL: $T(27) = 4.228$, $p < 0.001$, IN: $T(27) = 1.677$, $p = 0.105$; IR, PL: $T(19) = 3.234$, $p = 0.005$, IN: $T(19) = 5.000$, $p < 0.001$).

Table 6 | Study 1: Pre- and post-scan measures during PL and IN

Differences between time points within sessions and groups, time point by session interactions, and time point by session by group interactions. For simplicity, *T* values and exact *p* values were omitted here but can be found in the accompanying text.

	NIR							IR						
	PL			IN			*p*	PL			IN			*p*	int
	pre	post	*p*	pre	post	*p*	int	pre	post	*p*	pre	post	*p*	int	int
Insulin (pmol*l-1)	40.13 ± 16.56	35.39 ± 14.16	*	41.24 ± 14.03	45.58 ± 16.77	ns	**	76.31 ± 32.14	68.54 ± 31.34	ns	77.01 ± 24.88	66.45 ± 25.58	**	ns	*
Glucose (mmol*l-1)	4.64 ± 0.40	4.78 ± 0.31	*	4.68 ± 0.41	4.69 ± 0.30	ns	ns	4.96 ± 0.43	4.99 ± 0.45	ns	4.86 ± 0.38	4.86 ± 0.35	ns	ns	ns
Hunger rating	3.85 ± 2.40	5.07 ± 2.64	***	4.25 ± 2.03	5.00 ± 2.37	ns	ns	3.72 ± 2.49	5.06 ± 2.41	**	3.53 ± 2.29	5.16 ± 2.17	***	ns	ns

IN, insulin; int ~ interaction; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; PL, placebo; post, after completion of scans; pre, before application of nasal spray; * ~ $p < 0.05$, ** ~ $p < 0.01$, *** ~ $p < 0.001$, ns ~ not significant

In the MR scanner, measures of cardiac signals, recorded with a finger clip placed on the index finger of the left hand, and respiratory signals, recorded with a chest belt placed around the umbilical region, were assessed to exclude general insulin effects on peripheral body functions. Due to wrong positioning and technical issues, these vital function data were only analysable in 42 participants. RmANOVAs yielded no significant effects of group or condition on heart rate or respiration (all $F(1,40) < 2.792$, all $p > 0.103$) (Fig. 6).

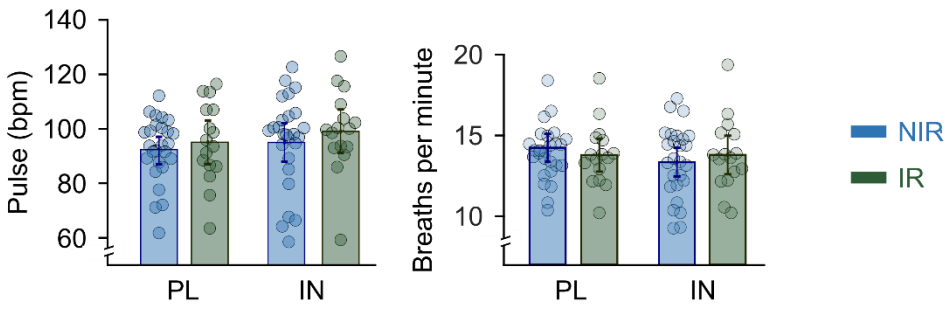

Figure 6 | Study 1: Autonomic data

Individual data, group means and 95% confidence interval (CI) of heart rate and respiration recorded during PL and IN, in the NIR and the IR group.

bpm, beats per minute; IN, insulin; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; PL, placebo

2.2.4. Behavioural results

2.2.4.1. Food item preference is decreased in IR during PL

To characterize the baseline conditions, we first analysed data from the placebo session. NIR and IR participants liked food items significantly more than non-food items, both when considering their binary as well as parametric responses (binary: $F(1,46) = 137.624$, $p < 0.001$; parametric: $F(1,46) = 110.598$, $p < 0.001$). During the binary choice, NIR participants more frequently responded 'yes' for food items (relative to non-food items) compared to IR individuals ($F(1,46) = 5.491$; $p = 0.023$) (Fig. 7a), an effect that also showed a trend in significance when considering the parametric responses ($F(1,46) = 3.337$; $p = 0.074$).

In all participants, fasting insulin levels were below the critical cut-off of 174 pmol*l^{-1} ('Williams Textbook of Endocrinology—12th Edition', 2011). We investigated whether plasma insulin levels obtained before and after scanning were directly related to food preference values, i.e. whether peripheral insulin had an influence on food liking. This analysis revealed insulin levels to be correlated with food preference scores only in the NIR group. In this group, those individuals with higher levels of plasma insulin reported lower preference for food items (pre_scan insulin x mean parametric rating food: r = -0.428; $p = 0.023$, post_scan insulin x mean parametric rating food: r = -0.486, $p = 0.009$), with correlations differing significantly between the groups (IR group: pre_scan insulin x mean parametric rating food: r = -0.088, $p = 0.712$; between groups: Fisher's $Z = 1.736$; $p = 0.041$; post_scan insulin x mean parametric rating food: r = -0.172, $p = 0.469$; between groups: Fisher's $Z = 2.241$; $p = 0.013$; Fig. 7b). In both groups, there was no such effect for non-food items (all $r < 0.302$, all $p > 0.118$).

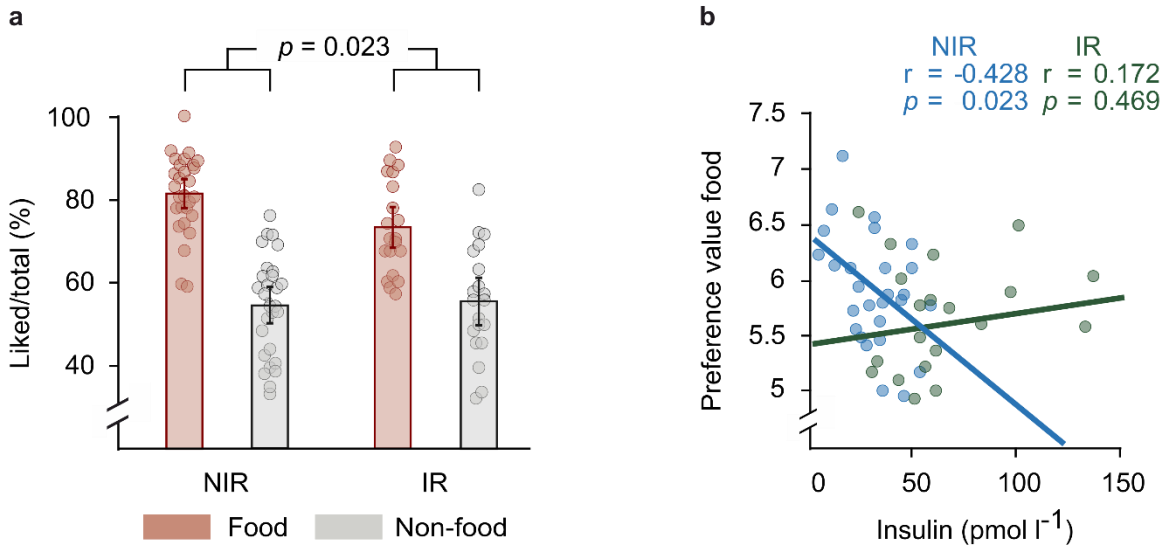

Figure 7 | Study 1: Behavioural results in the placebo condition

(a) Groups means of percentage of liked food and non-food items during PL demonstrate reduced food value scores in the IR group. Individual data are overlaid onto mean values and 95% confidence intervals (CI). (b) Correlation between participants' post-scan plasma insulin levels and preference values for food items during PL. Only in the NIR group, plasma insulin predicted preference values.

IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance

2.2.4.2. Application of INI reduces food values only in NIR

Next, we tested for the effects of INI on behavioural preference ratings. A rmANOVA including the factors item (food/non-food), session (PL/IN) and group (NIR/IR) revealed a significant item by session by group interaction ($F(1,46) = 5.133$; $p = 0.028$). Closer examination of this finding indicated food preference ratings to be significantly reduced after INI in the NIR group, which was also mirrored in a significant item by session interaction within this group ($F(1,27) = 7.376$; $p = 0.011$). No such effect could be observed on non-food items in this group, nor were there any significant effects in the IR group (Fig. 8a).

In line with this, HOMA-IR scores were negatively correlated with insulin-mediated changes in food preference scores: Individuals with lower HOMA-IR scores showed stronger reduction of food values following INI application than individuals with higher HOMA-IR scores ($r = -0.297$; $p = 0.040$; Fig. 8b).

To rule out the possibility that these changes were primarily driven by differences in body composition, we repeated the analyses including BMI, body fat or waist, respectively, as a

covariate. Of note, irrespective of these body measures, group interactions remained significant (BMI: $F(1,45) = 6.494$; $p = 0.014$; body fat: $F(1,45) = 6.997$; $p = 0.011$; waist: $F(1,45) = 5.764$; $p = 0.021$).

In contrast to the placebo session, neither pre-, nor post-scan insulin levels explained any significant variability in food liking scores in NIR individuals or IR individuals (all r > -0.292, all $p < 0.131$).

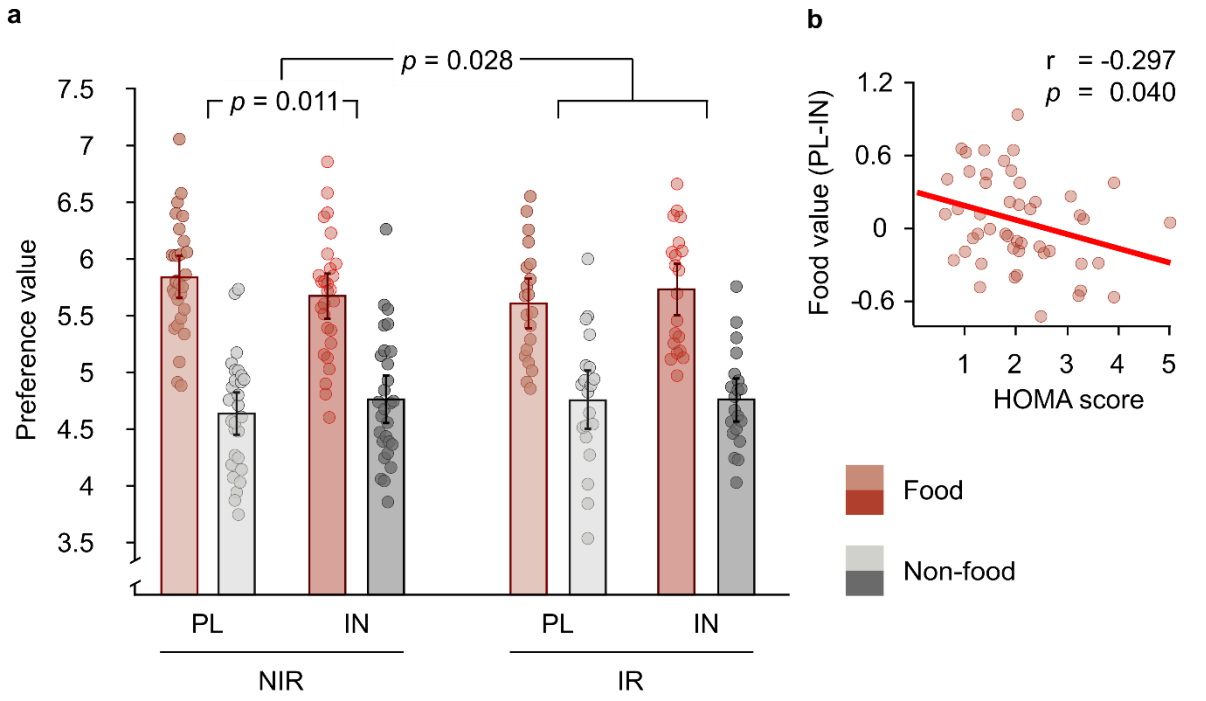

Figure 8 | Study 1: Behavioural insulin effects

(a) Individual data, group means and 95% CI of preference values for food and non-food stimuli during the placebo (PL) and the insulin session (IN), in the group with normal insulin resistance (NIR) and in the group with increased insulin resistance (IR). A rmANOVA revealed significantly reduced preference values specifically for food items under INI only in the NIR group, while food values tend to increase in the IR group. (b) INI-mediated changes in food preference scores ($food_{PL - IN}$) were directly correlated to individual peripheral insulin sensitivity as defined by the HOMA-IR index across all participants.

IN, insulin; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; PL, placebo

2.2.5. Neural results

2.2.5.1. Food valuation activates hedonic and metabolic neurocircuits

To investigate how INI influenced neural activation, specifically in the mesolimbic reward circuitry, we analysed blood oxygenation level-dependent (BOLD) activity measured during the preference task. Comparisons of BOLD responses to food compared to non-food items in the placebo session yielded highly significant activations across all participants in a large network of reward-related brain regions including the VTA, the amygdala, the insula and the OFC (Tbl. 7, Fig. 9a). These general activation patterns did not differ between groups.

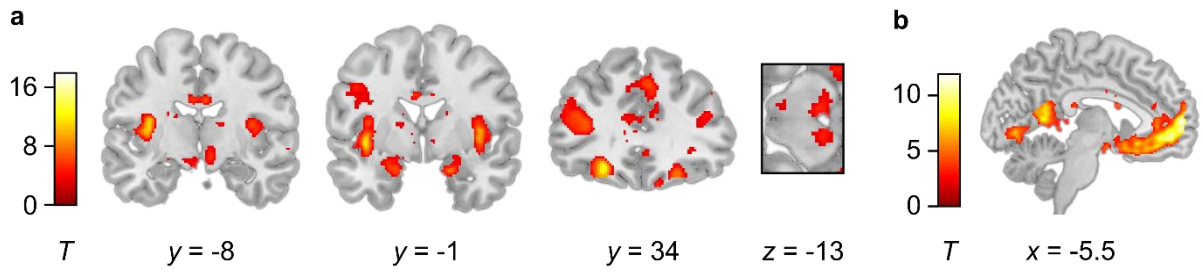

Figure 9 | Study 1: Paradigm-induced activation patterns

(a) Stronger activation in the insula, amygdala, OFC and the VTA was observed following food compared to the non-food stimulus presentation across both groups. For details see Tbl. 7 'Paradigm-induced activation patterns during PL – Food > non-food'. (b) Activation on the vmPFC, the posterior cingulate cortex and the bilateral NAc correlated significantly with food and non-food preference values across all participants. For details see Tbl. 7 'Paradigm-induced activation patterns during PL – Parametric modulation by food and non-food preference values'.
The coloured voxel-based statistical parametric mapping (SPM) results are overlaid on a normalized canonical image (ch2better-template) available in the MRIcron software (display threshold $p < 0.005$ uncorrected).

Table 7 | Study 1: Peak coordinates and statistics of fMRI analyses

MNI coordinates, *Z* and *T* values are reported for peak voxels and local maxima within each cluster. All $p < 0.05$ FWE corrected.

		MNI (peak)					
Brain region	Side	*x*	*y*	*z*	Cluster size	*Z*	*T*
Paradigm-induced activation patterns during PL							
Food > non-food							
Middle frontal gyrus	L	-24	34	-13	636	Inf	12.75
	R	21	29	-19	693	Inf	9.87
Inferior frontal gyrus	L	-36	34	14	1493	6.18	6.65
Superior frontal gyrus	R	4	34	40	846	5.85	6.24
Medial frontal gyrus	R	12	11	-18	Same cluster	5.13	5.40
Anterior insula	L	-38	5	-12	2523	Inf	17.84
	R	39	8	-12	2849	Inf	15.52
Amygdala	R	20	-1	-22	Same cluster	7.07	7.78
VTA	L	-4	-13	-12	47	4.72	4.93
	R	6	-12	-13	15	3.93	4.06
Postcentral gyrus	L	-60	-19	32	1151	7.28	8.06
Middle cingulate gyrus	R	4	-19	30	1362	6.58	7.15
Posterior cingulate gyrus		0	-30	32	Same cluster	6.68	7.28
Inferior temporal gyrus	L	-56	-52	-19	544	7.21	7.97
Lingual gyrus	L	-12	-94	-4	757	5.45	5.77
Parametric modulation by food and non-food preference values							
Superior frontal gyrus	L	-4	59	6	9527	Inf	11.87
Anterior cingulate gyrus	L	-10	47	-6	Same cluster	Inf	11.51
NAc	L	-10	8	-6	6	3.38	3.46
	R	9	8	-6	19	3.93	4.05
Middle temporal gyrus	L	-64	-43	-8	2294	Inf	6.23
Posterior cingulate cortex	L	-9	-54	14	1531	7.83	8.82
Cerebellum	R	45	-60	-40	576	5.97	6.38
Lingual gyrus	L	-12	-74	-12	813	5.15	5.41
Parametric modulation, $NIR_{food>non\text{-}food} > IR_{food>non\text{-}food}$							
NAc	L	-12	8	-8	26	3.85	3.96
Neural insulin effects (PL > IN)							
Parametric modulation, $NIR_{food>non\text{-}food} > IR_{food>non\text{-}food}$							
NAc	L	-12	8	-8	17	3.50	3.58
	R	10	8	-7	10	3.10	3.16
VTA	L	-4	-12	-14	7	3.13	3.19

IR, participants with increased insulin resistance L: left, NAc, nucleus accumbens; NIR, participants with normal insulin resistance; PL, placebo; R: right; VTA, ventral tegmental area

2.2.5.2. NAc food value signals are reduced in IR

In a second step, regions were tested for that show a positive correlation between the strength of the BOLD response and subjective preference values in both food and non-food conditions, i.e. which encode subjective liking values. This analysis revealed a strong activation of the valuation network (Kable & Glimcher, 2007; Jan Peters & Büchel, 2010), including the vmPFC, the posterior cingulate cortex and the bilateral NAc (Fig. 9b). Food-specific valuation signals, identified through a comparison of the correlation of BOLD activation and food preference values versus BOLD activation and non-food preference values, were identified in the bilateral NAc in NIR individuals. However, no significant activation differences emerged for participants in the IR group, resulting in a significant group interaction in the left NAc (Fig. 10).

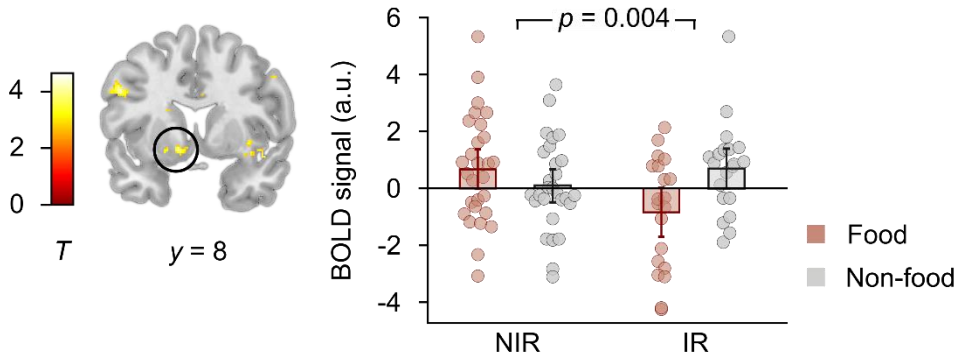

Figure 10 | Study 1: Neural group differences at baseline

Food-specific value signals in the left NAc were stronger in the NIR group than in the IR group. For more details see Tbl. 7 'Paradigm-induced activation patterns during PL - Parametric modulation, $NIR_{food>non\text{-}food} > IR_{food>non\text{-}food}$'. Plotted contrast: $NIR_{food > non\text{-}food} > IR_{food > non\text{-}food}$. The bar graph shows individual data, group means and 95% CI of mean parameter estimates extracted from the peak of the left NAc. The coloured voxel-based statistical parametric mapping (SPM) results are overlaid on a normalized canonical image (ch2better-template) available in the MRIcron software (display threshold $p < 0.005$ uncorrected).

IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance

2.2.5.3. INI reduces mesolimbic food value signals only in NIR

Next, we examined the effects of INI on neural value signals in the reward circuit. In a first step, we focused on general changes in value signals. To this end, we compared parametric activation

patterns during PL, pooled over the food and non-food conditions, with those patterns evoked during IN. We did not observe significant differences across and between groups.

Next, we investigated INI effects on food-specific valuation responses (food > non-food). Importantly, here we found a significant group interaction in the NAc (Tbl. 7; Fig. 11a) and the left VTA (Tbl. 7; Fig. 11b), showing that while central insulin reduced food-specific valuation signals in the NIR group in these structures, in IR individuals here neural signals increased.

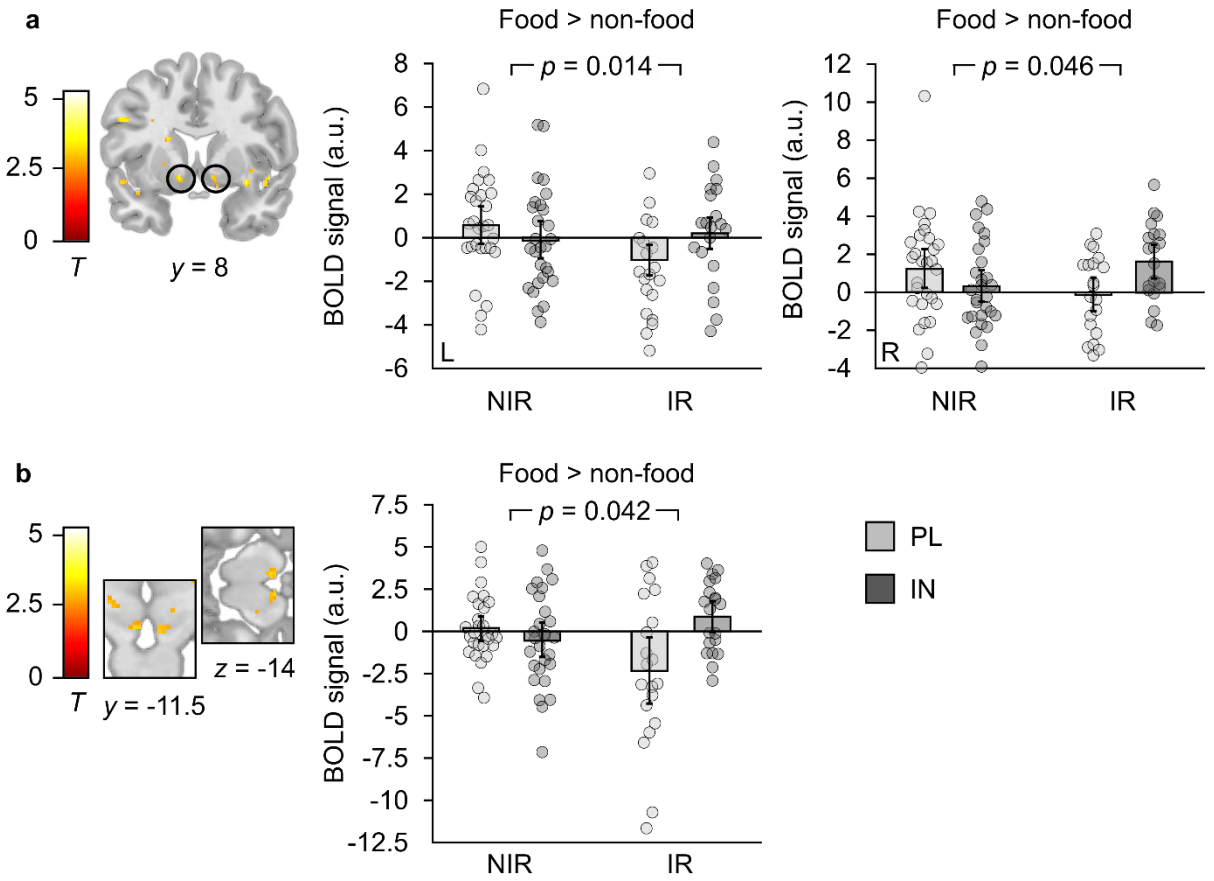

Figure 11 | Study 1: Neural insulin effects

Analyses of food value signals from PL and IN yielded significant group by session interactions in the bilateral NAc (a) and the left VTA (b). For more details see Tbl. 7 'Neural insulin effects (PL > IN) - Parametric modulation, $NIR_{food>non\text{-}food}$ > $IR_{food>non\text{-}food}$'. In both regions, these food-specific value signals were decreased only in the NIR group following INI, while signals increased under INI in the IR group. The bar graphs show individual data, group means and 95% CI of mean parameter estimates extracted from the peak of the left and right NAc, and the left VTA from the comparison $NIR_{PL > IN}$ > $IR_{PL > IN}$. The coloured voxel-based statistical parametric mapping (SPM) results are overlaid on a normalized canonical image (ch2better-template) available in the MRIcron software (display threshold $p < 0.005$ uncorrected).

IN, insulin; IR, participants with increased insulin resistance; NIR, participants with normal insulin resistance; PL, placebo

2.2.5.4. Dynamic causal modelling

Finally, we investigated whether the observed neural changes were related to changes in connectivity between the regions. Based on findings in animals (Stuber & Wise, 2016), a specific focus here was placed on whether central insulin modulates forward, backward or bidirectional projections between the VTA and the NAc. To this end, we used DCM analyses on adjusted BOLD time-series from the VTA and the NAc.

As the insulin-related GLM results were specifically pronounced in the left hemisphere, we first focused on the left VTA and NAc, but repeated all analyses also in the right hemisphere, were results were very similar (Tbl. 8). For DCM analyses, a full model was defined and inverted for each participant that included all connections between the regions, intrinsic connections, food and non-food stimuli as driving inputs, and potential modulatory insulin inputs (Fig. 12a).

Table 8 | Study 1: DCM parameter estimates

Bayesian parameter estimates (with SD) of fixed-connections and modulation through insulin in the winning model across all participants for the left (_L) and the right (_R) hemisphere. *P* values significant after Bonferroni-correction for 7 parameters of interest (in the left hemisphere) are printed in bold.

Parameter		Estimate	*T*	*p*
Fixed coupling – between regions				
VTA_L	→ NAc_L	0.05 ± 0.14	2.474	**0.007**
NAc_L	→ VTA_L	0.33 ± 0.07	32.662	**< 0.001**
VTA_R	→ NAc_R	-0.03 ± 0.07	2.969	**0.005**
NAc_R	→ VTA_R	0.02 ± 0.14	0.990	0.327
Fixed coupling – within regions				
VTA_L		-0.74 ± 0.14	36.621	**< 0.001**
NAc_L		-0.23 ± 0.14	11.382	**< 0.001**
VTA_R		-0.08 ± 0.14	3.959	**< 0.001**
NAc_R		-0.10 ± 0.14	4.949	**< 0.001**
Modulation by insulin – between regions				
VTA_L	→ NAc_L	-0.49 ± 0.62	5.476	**< 0.001**

VTA_R	→ NAc_R	-0.23 ± 0.35	4.553	**< 0.001**
NAc_R	→ VTA_R	0.00 ± 0.42	0	1
Modulation by insulin – within regions				
VTA_L		-1.73 ± 0.83	14.441	**< 0.001**
NAc_L		-1.97 ± 0.69	19.781	**< 0.001**
VTA_R		-1.65 ± 0.76	15.041	**< 0.001**
NAc_R		-1.68 ± 0.49	23.754	**< 0.001**

NAc, nucleus accumbens; VTA, ventral tegmental area

Bayesian model selection and reduction (Friston et al., 2016) was then used to identify the model with the best evidence out of all possible combinations of the above described parameters (Fig. 12c). The winning model comprised reciprocal positive connections between the VTA and the NAc, and negative intrinsic connections in both regions (Fig. 12b, c).

Most importantly, the winning model also provided evidence for a negative modulation by insulin on the forward projections from the VTA to the NAc and on the self-connection in both regions, but did not indicate modulatory effects on the backward projection. Using one-sample *t*-tests, we confirmed that all of the four parameters quantifying (self-)connections were significantly different from zero, which is consistent with the assumption that these two mesolimbic regions are strongly interconnected (Haber & Knutson, 2009; Russo & Nestler, 2013). The parameters describing insulinergic modulation were also significantly different from zero (all $p < 0.007$ Bonferroni-corrected for multiple comparisons).

To investigate whether the results from the Bayesian model selection differ between the NIR and IR groups, we repeated the post-hoc model selection, separately for the two groups. In the NIR group, the identified winning model was identical to the selected model across all participants. In contrast, in the IR group, modulation of extrinsic connections by insulin was no longer selected to explain neural activity in mesolimbic ROIs, whereas intrinsic self-connections of both regions were still negatively modulated by insulin (Fig. 12e).

To test whether behavioural effects of insulin on food preference were directly associated with insulin effects on mesolimbic connectivity, we correlated individual modulatory parameters of the winning model (insulinergic modulation the projection from the VTA to the NAc) with food value reduction under insulin per subject. This analysis yielded a significant negative association ($r = -0.440$; $p = 0.002$), indicating that the stronger the inhibition of the forward connection from the VTA to the NAc was, the stronger was food value reduction under INI (Fig. 12d). Insulinergic

effects on intrinsic connections did not correlate with behavioural findings (all r < -0.176, all p > 0.232).

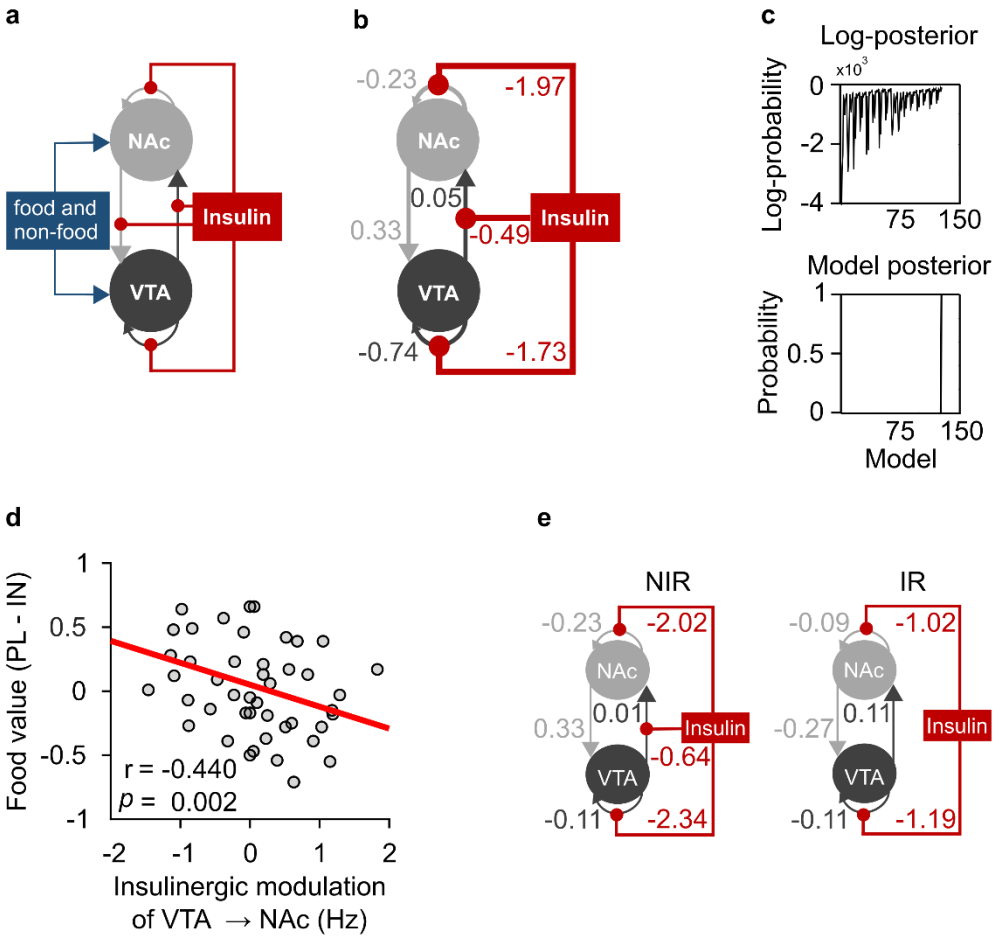

Figure 12 | Study 1: DCM results

(a) For every participant, a full model was defined and inverted that included all (intrinsic) self-connections and (extrinsic) forward and backward connections, food and non-food stimuli as driving inputs, and insulin modulation of all connections. (b) The reduced model with the highest evidence across all participants as identified through post-hoc optimization included insulinergic modulation of the forward connection from the VTA to the NAc and of both self-connections. Numbers indicate Bayesian parameter averages. (c) (Log-)Posterior probabilities of all possible models examined in the left hemisphere. The winning model had a posterior probability of (almost) 1, suggesting that this reduced model had more evidence than any other variant. The next most probable model's probability was very low at almost 0. (d) Individual parameter estimates of INI modulation on the forward connection from the VTA to the NAc were directly related to insulin-mediated changes of food preference scores ($food_{PL-IN}$). Specifically, inhibitory modulation of this connection predicted stronger decrease of food values relative to non-food

values under INI across all participants. (e) The winning model selected within each group: In the IR group, there was no significant modulation by INI on the VTA to NAc connection.
IR, participants with increased insulin resistance; NAc, nucleus accumbens; NIR, participants with normal insulin resistance; VTA, ventral tegmental area

2.3. Discussion

The findings of the first study reveal distinct effects of central insulin on behaviour and brain activity in individuals with normal and with increased peripheral insulin resistance. In healthy participants with normal HOMA-IR scores, INI specifically decreased food palatability ratings, while it had no effect on non-food ratings. This reduced preference scores for food were directly associated with decreased food value signals in the VTA and the NAc. Insulin-mediated depression of dopaminergic activity in the VTA, paralleled by decreased salience of food stimuli, had previously been described in animals (Könner et al., 2011; Labouèbe et al., 2013; Mebel et al., 2012) – the present findings validate and extend these works also in humans.

In one such animal study, insulin injection induced long-term depression (LTD) of excitatory synapses onto VTA dopaminergic neurons, potentially attenuating dopamine release in target regions of the mesolimbic system and selectively reducing the preference for contextual cues associated with food reinforcement, as can be measured via conditioned place preference (Labouèbe et al., 2013). This insulin-mediated reduction in food anticipatory behaviour relies on alterations in the subjective valuation of stimuli, which is encoded in striatal sub-regions (Haber & Knutson, 2009). In the present study, we can demonstrate that INI reduces food value signals in the NAc also in humans, and with the help of effective connectivity analyses, we provide evidence that this is mediated by insulinergic modulation of the connection from the VTA to the NAc.

Concurrent with this insulinergic inhibition of the connection from the VTA to the NAc, NAc food value signals show a decrease (in the NIR group) following insulin application, with the strength of the inhibition being directly linked to the extent of the behavioural food value reduction. This is possibly a consequence of reduced dopaminergic activity, as animal studies suggest. These studies showed that following a disinhibition of dopaminergic VTA neurons by the LHA, dopamine was released into the NAc (Stuber & Wise, 2016). Insulin was found to induce LTD of excitatory synapses onto dopaminergic VTA neurons (Labouèbe et al., 2013), thus inhibiting dopaminergic output. Taking into account, the findings of the present study might point towards similar processes in the human brain, with insulin signalling in mesolimbic circuits via inhibition of the VTA, thereby reducing the salience to food cues in the NAc.

Under baseline conditions (i.e. in the PL session) in the NIR group, plasma insulin levels predicted food preference scores. Insulin is released from beta cells in the pancreas following food consumption and as a possible subsequent action in the CNS, it might reduce the incentive salience of food in fed compared to fasted states (Cameron, Goldfield, Finlayson, Blundell, & Doucet, 2014). Peripheral insulin levels return to baseline levels approximately six to eight hours after consumption of food (Suckale & Solimena, 2008). However, the present finding suggests that in the participants in the NIR group, who all had fasted for at least ten hours, the amount of insulin in the periphery might directly be related to central insulin levels and its effects here, and that central insulin effects possibly outlast peripheral effects of insulin.

Importantly, the present study also included a group of individuals with increased peripheral insulin resistance as assessed via the homeostatic model for insulin resistance (Matthews et al., 1985; Wallace, Levy, & Matthews, 2004). In addition to impaired transport of insulin across the BBB in these individuals (Heni et al., 2014), which was not a focus of the present study and was therefore excluded through the use of intranasal application, central dysfunction of insulin has been discussed to occur in obesity (Ketterer et al., 2011; Oh, Boghossian, York, & Park-York, 2013).

On the one hand, results of the present study show that during baseline measurements, food preference ratings were reduced in the IR group as compared to the NIR group – a behavioural finding that was directly mirrored by decreased NAc food value signals in this group. This observation is in line with the frequently discussed reward deficiency theory of obesity. According to this theory, individuals with hypo-dopaminergic function in the brain reward circuity overeat to compensate for decreased activation in these regions. In animals, when dopaminergic receptors are blocked, an increase in food consumption can be observed (Volkow et al., 2011; G.-J. Wang et al., 2001) and when pharmacological dopamine application was ended, increased weight gain was observed in rodents (Reinholz et al., 2008). Conversely, mice that frequently overate showed reduced dopamine signalling in the striatum (Tellez et al., 2013).

Additionally, studies in humans found that obese individuals show less dopamine D2 (Kessler, Zald, Ansari, Li, & Cowan, 2014) and μ-opioid (Karlsson et al., 2015) receptor availability in the striatum, as well as decreased responses to food stimuli in this region (Babbs et al., 2013; Stice, Spoor, Bohon, Veldhuizen, & Small, 2008; Stice & Yokum, 2016). The Taq1A minor (A1) allele of the gene codifying dopamine receptors 2 and 3, which associated with lower DRD2/3 density, has been found to exist in higher frequencies in obese subjects (Dang et al., 2016), which is in line with earlier findings describing decreased D2/3 receptor binding in the striatum in participants with morbid obesity (G.-J. Wang et al., 2001). However, more recently, in a large sample of adult human subjects (n = 130, age between 18 and 81 years), no such direct association between DRD2/3 and BMI was found after controlling for age distribution (Dang et al., 2016). When comparing

participants with milder forms of obesity (i.e. a BMI between 30 and 40 $kg*m^{-2}$) to normal-weight participants, equivalent D2/3 receptor density were observed (Eisenstein et al., 2015). This calls into question whether indeed obesity per se is related to dopamine D2/3 reductions (Vainik, García-García, & Dagher, 2019), or whether other factors such as obesogenic diet are necessary to reveal the full effects of the dopamine D2/3 reduction on obesity (Formolo et al., 2019).

Nevertheless, the findings of the present study confirm that participants with heightened insulin resistance and body status show reduced responses to food cues, both on a behavioural and a neural level. Here, prolonged overconsumption might have resulted in perpetually elevated levels of insulin, in turn leading to chronical downregulation of mesolimbic regions.

The absence of a correlation of peripheral insulin levels and food preference at baseline and the fact that INI did not result in a decrease of food preference values in this group further suggest central insulin resistance in this group. These findings cannot be explained by body status (BMI, fat mass or waist circumference), demonstrating that insulin resistance leads to changes in food valuation independently of obesity. In the present study, there was a high correlation between the different measures of obesity and HOMA-IR, yet some variance remained unexplained. This might be due to the shortcomings of the different measures of obesity (Huxley, Mendis, Zheleznyakov, Reddy, & Chan, 2010; Prentice & Jebb, 2001; Smeets et al., 2019), but it might also be related to the fact that factors such as hypertension (Pollare, Lithell, & Berne, 1990) or vitamin D insufficiency (Moschonis et al., 2018) contribute to insulin resistance independently of obesity. Thus, obese individuals can be insulin sensitive as well as insulin resistant (T. McLaughlin et al., 2002), and, conversely, insulin-resistant individuals can be obese or non-obese.

In line with findings regarding the behaviour and neural responses, effective connectivity analyses revealed further differences between the groups in the present study. When only including participants from the IR group, the connectivity model with the highest evidence no longer included an insulin-mediated inhibition of the connection from the VTA to the NAc. This is in agreement with findings in animals showing that in a state of permanently increased peripheral levels of insulin, the capacity of central insulin to cause a synaptic depression of VTA dopamine neurons is reduced (S. Liu et al., 2013).

To sum up, the first study of the present thesis provides evidence that in individuals with normal peripheral insulin sensitivity, insulin alters the hedonic valuation of food stimuli through its effects in the CNS, specifically through modulation of mesolimbic regions and pathways. In participants with increased peripheral insulin resistance, we found neurobehavioural evidence for specific reward deficiency and could show the absence of insulin-mediated effects, shedding light on pathophysiological reward regulation promoting metabolic disorders.

3. Study 2: Valence signatures and food choice modulation in the human amygdala

3.1. Materials and methods

3.1.1. Participants

Data of thirty volunteers (age: mean ± SD = 25.07 ± 2.50 years; range = 21 – 30 years; 14 female, Tbl. 9) were analysed in the second study of this thesis. This set of volunteers is a subsample of the participants described in chapter 2.1.1. As this second study focused solely on valence processing and food choice in healthy individuals, from the larger sample described above, participants were selected who were below the critical cut-off for overweight or obesity, following guidelines provided by the World Health Organization (waist circumference ≤ 94 cm for men and ≤ 80 cm for women (*Waist Circumference and Waist-hip Ratio*, 2011)). Waist circumference has been discussed to better describe the distribution of body fat compared to BMI (Huxley et al., 2010; Prentice & Jebb, 2001) and to be a better predictors of disease risk factors (Klein et al., 2007; Nazare et al., 2015; Savva et al., 2000).

For the selection processes and exclusion criteria, see chapter 2.1.1.

Table 9 | Study 2: Sample characteristics

Age, gender, HbA1c values, and body measures of the participants included in the second study.

	Age (years)	Gender (f/m)	HbA1c (%)	BMI (kg/m²)	Waist (cm)
n = 30	25.07 ± 2.50	14/16	4.13 ± 1.90	23.17 ± 3.35	f: 69.54 ± 5.66 m: 82.48 ± 7.34

BMI, body mass index; HbA1c, haemoglobin A1c

3.1.2. Experimental design

For the second study of this thesis, data from the PL session (see study 1) and an additional study day ($sess_{want}$) were analysed. In the following, the PL session will be termed $sess_{like}$ (Fig. 13a).

During $sess_{want}$, participants arrived in the morning between 7:30 a.m. and 10:45 a.m. after an overnight fast of at least ten hours. Anthropometric measurements were taken, and participants rated their current feelings of hunger. To confirm fasted state in all participants also on this study day, we took blood samples to analyse blood glucose levels.

In the scanner, during $sess_{want}$, participants indicated whether they wanted to eat one mouthful of the presented food item at that very moment ("Yes, I want to eat this now" vs. "No, I do not want to eat this now"), and then specified these answers on a scale from 1 to 4, resulting in decision values ranging from 1, the strongest rejection, to 8, the strongest wanting to consume the food (Fig. 13b). Here, participants saw 140 food items – the 70 food items shown during $sess_{like}$ plus the 70 food items shown during IN. Only stimuli identical with $sess_{like}$ were analysed to address the research questions of this study. Ecological validity of single-trial consumption decisions was ensured by informing participants beforehand that after the scanning session they would have to eat a mouthful of a randomly selected food item for which they indicated that they want to eat it. Both liking and the wanting task are adapted versions of previously established and validated behavioural paradigms (Finlayson, King, & Blundell, 2007; Hare et al., 2011; Tibboel et al., 2011).

Paradigm set-up and timing during $sess_{want}$ was identical to $sess_{like}$ (see chapter 2.1.3 and Fig. 3).

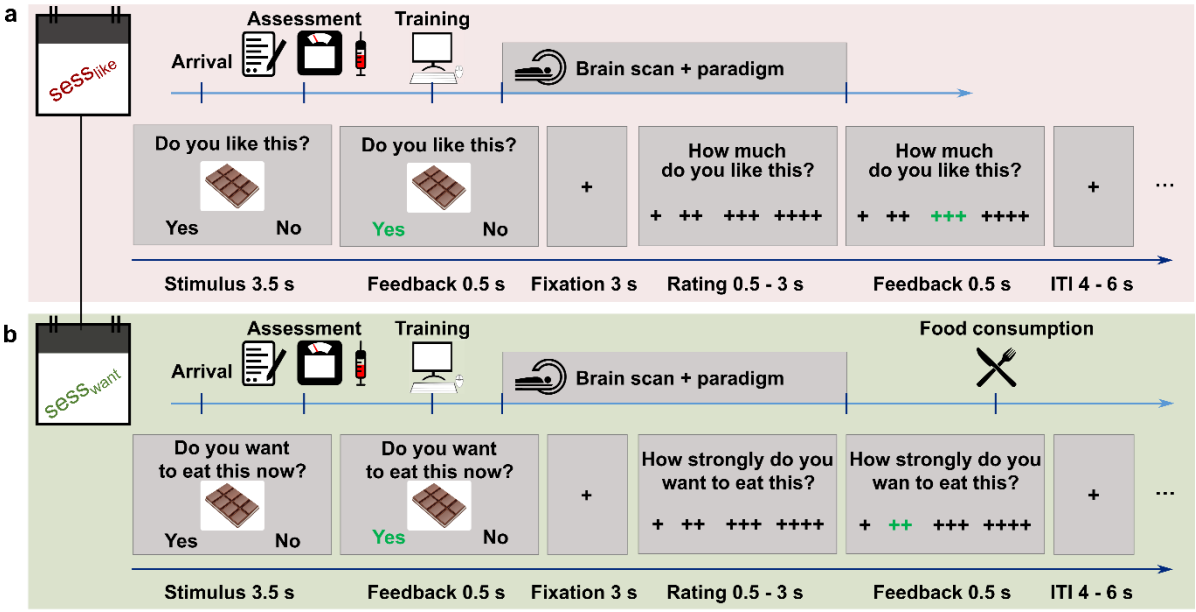

Figure 13 | Study 2: Design and experimental tasks

On both experimental days, anthropometrics and feelings of hunger were assessed before scanning, and blood samples were taken. (a) During $sess_{like}$, participants rated their liking of food items. (b) During $sess_{want}$, participants had to indicate whether they want to consume the depicted food at that moment. $Sess_{want}$ was followed by a consumption phase where participants had to eat one randomly selected food they had indicated as "wanted".

ITI, inter-trial interval

3.1.3. Statistical analyses

Paired t-tests were used to investigate potential differences between $sess_{like}$ and $sess_{want}$ regarding fasted glucose levels, hunger state and fasting duration. Interactions between behavioural liking and wanting-ratings and macronutrient content were analysed using Pearson correlations. One-sample *t*-tests were used to test correlation coefficients for significance. We tested two-sided and Bonferroni correction was used for multiple-comparison correction when addressing our research hypotheses. Specifically, correlations between macronutrients and valence-/willingness to eat-ratings were corrected for five comparisons each (corrected p-value: 0.01), and multivariate analyses were corrected for two regions of interest (corrected p-value: 0.025).

3.1.4. MRI and fMRI data acquisition and pre-processing

For a description of data acquisition and pre-processing, see chapter 2.1.6. and Tbl. 2.

3.1.5. ROI definition

ROIs for the amygdala and the occipital pole/primary visual cortex (V1) were defined in MNI space based on the Harvard–Oxford Structural Probability Atlas distributed with the Functional MRI of the Brain Software Library (FSL) neuroimaging analysis software package (Harvard Center for Morphometric Analysis; http://www.cma.mgh.harvard.edu/fsl_atlas.html; Fig. 14). Masks were thresholded at 50% probability. Individual bilateral ROIs were then constructed for each participant from the overlap of these anatomically defined regions and participant's individual activation pattern during $sess_{like}$. To this end, a first-level GLM that included all 70 food items was calculated for every participant. Contrast images representing paradigm-induced activation were thresholded at 0.9, and surviving voxels entered the respective ROIs.

To test our neural hypotheses regarding brain activation during $sess_{want}$, we further defined ROIs of the NAc and the vmPFC (Fig. 14). The NAc was defined in MNI-space based on the FSL Harvard Oxford structures atlas (threshold 50% probability), for the vmPFC we used a 10mm sphere centred on the peak voxel (0, 46, -6) derived from 143 imaging studies reporting "vmPFC", as determined by a meta-analysis conducted on the neurosynth.org platform (status May 2018). For $sess_{want}$, a separate GLM was set up that included all 70 food items identical with $sess_{like}$, and again the paradigm-induced activation was thresholded at 0.9 and served for ROI definition of the NAc and the vmPFC. In line with the definition of individual amygdala and V1 ROIs, both NAc and vmPFC ROIs were individualized by combining the respective anatomically defined region with the individual activation patterns during $sess_{want}$.

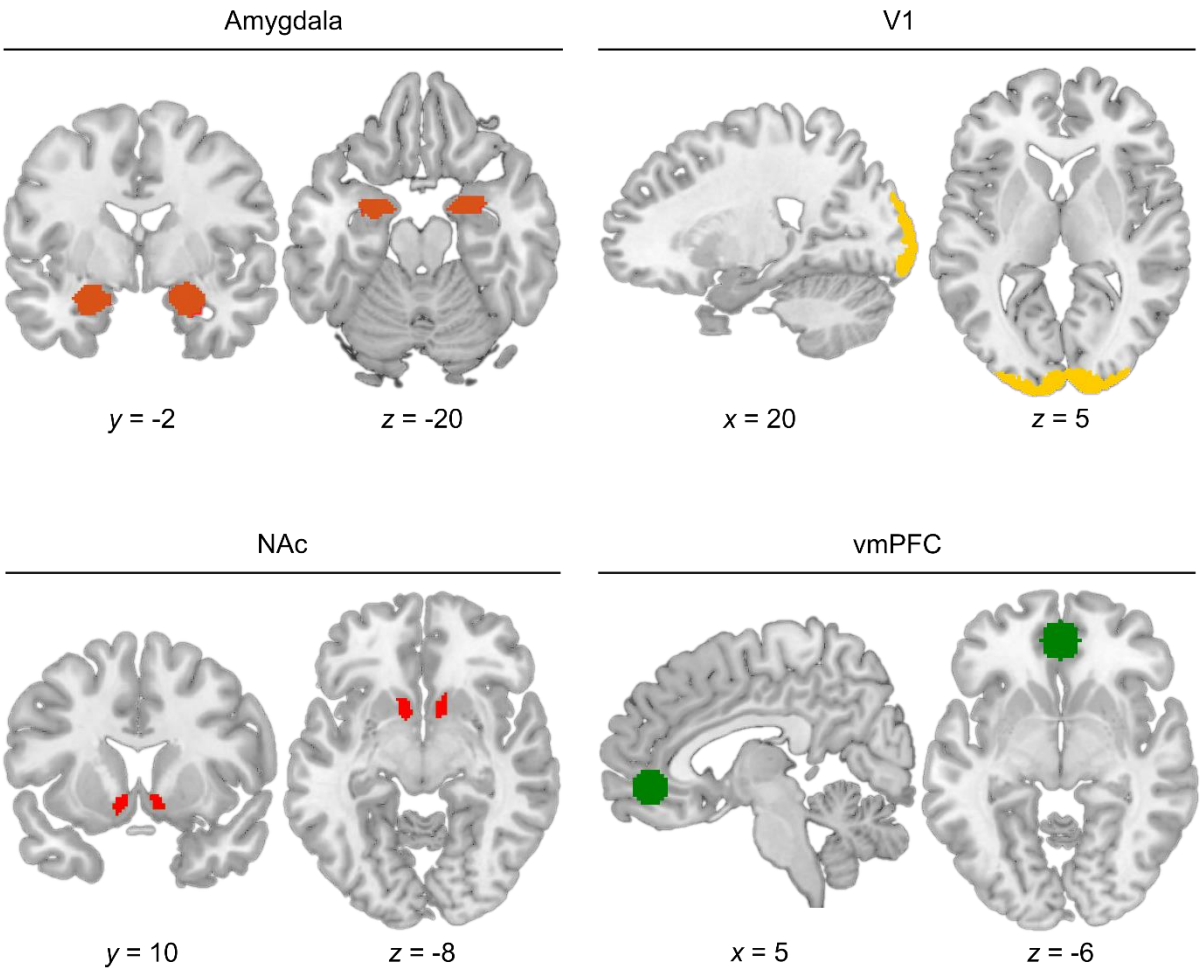

Figure 14 | Study 2: Regions of interest

Based on findings in animals on the role of the amygdala in valence processing, this region constituted the key region of interest. The occipital pole served as a control region. The NAc and the vmPFC were included as further regions of interest to investigate the neural processes accompanying consumption decisions.

NAc, nucleus accumbens; vmPFC, ventromedial prefrontal cortex; V1, primary visual cortex

3.1.6. FMRI data analyses

3.1.6.1. Univariate analyses

A two-level random effects approach utilizing the GLM as implemented in SPM12 was employed for statistical univariate imaging analyses. First-level models for every participant were set up independently for each scanning day. Each of the two models per subject included all 70 food items and a parametric modulator coded for the subjective combined liking or wanting value assigned to the food.

Second-level random-effects group contrast maps were then tested for significance in one-sample *t*-tests and within-subject ANOVAs on the single-subject contrasts of interest. We report results corrected for FWE due to multiple comparisons. We conducted this correction at the peak level within small volume ROIs for which we had a priori hypotheses or at the whole-brain level for exploratory reasons.

3.1.6.2. Representational similarity analysis

Data pre-processing for RSA was identical to pre-processing for univariate analyses until the final smoothing step, which was omitted in order to preserve high levels of spatial information.

For every participant, a first-level GLM was calculated on the unsmoothed functional data, which modelled every liking rating separately as one regressor. Since we were interested in neural representations of palatability, we averaged the signal across all items that received the same liking rating. From resulting contrast images per regressor, *t* values were extracted from each voxel within the individualized bilateral amygdala and V1 ROIs resulting in one vector summarizing all voxels per rating, which represents the activity patterns associated with that value in a confined brain region.

These vectors were then used to create subject-wise representational dissimilarity matrices (RDMs) per brain region through pairwise comparisons of neural activity patterns for each condition (measured as 1 minus Pearson's *r*). We calculated the (Spearman) correlations of these RDMs with a model RDM that quantified distances between patterns to increase linearly and continuously. If a certain rating had not been given to any of the food items, this rating was omitted in both the brain and the model RDM.

To statistically examine how well the model RDM described neural encoding of valence, Spearman's rank correlation coefficients were computed between all values above the diagonal of the brain and model RDMs (Kriegeskorte et al., 2008). A one-sample *t*-test of Fisher *z*-transformed correlation coefficients was used to test significance across subjects.

3.1.6.3. Pattern reinstatement analysis

To test for neural liking valuation in the amygdala and in the V1 during food wanting decisions, activation patterns from $sess_{want}$ of each single food item were compared to liking-specific activation profiles from $sess_{like}$. Single-item multivoxel patterns from $sess_{want}$ were therefore correlated with both averaged multivoxel patterns from $sess_{like}$ of all items with the same liking rating (r_{same_liking}) (excluding the item being tested) and averaged multivoxel patterns from $sess_{like}$

of all other items (r_{other_liking}). The difference between both correlations ($dist_{brain} = r_{same\_liking} - r_{other\_liking}$) was considered as an indicator for specific palatability processing during food wanting decisions.

The behavioural impact of liking on wanting was quantified as the distance between liking and wanting rating per item [$dist_{behav} = abs(rating_{liking} - rating_{wanting})$]. This behavioural distance was related to the neural indicator for specific palatability processing. The resulting correlations between $dist_{brain}$ and $dist_{behav}$ per subject were Fisher *z*-transformed and tested for significance with one-sample *t*-test.

3.1.6.4. Identification of positive and negative valence-encoding components

In order to identify positive and negative value coding amygdala sub-clusters, we transformed each participants' *t* values from the parametric liking analysis into a correlation coefficient per voxel, using the Computational Anatomy Toolbox (CAT12, http://www.neuro.uni-jena.de/cat/, see (II)). Amygdala voxels were then segregated based on negative (i.e. stronger activation for unpleasantness) and positive (i.e. stronger activation for pleasantness) coefficients.

$$r = \frac{\text{sign}(t)}{\sqrt{\frac{df}{t^2} + 1}} \qquad \text{(II)}$$

(df = effective residual degrees of freedom)

3.1.6.5. Dynamic causal modelling

We used the DCM software implemented in SPM12 and a Bayesian model reduction approach for effective connectivity analysis (Friston et al., 2016). The principal Eigenvariate time-series were extracted from ROIs of the amygdala sub-clusters, the NAc and the vmPFC, adjusted for effects of interest.

In a first step, a model of fixed connections was set up that comprised reciprocal connections between the appetitive sub-cluster of the amygdala (amy_app) and the NAc, amy_app and vmPFC, the aversive sub-cluster of the amygdala (amy_avers) and the NAc, amy_avers and vmPFC, and NAc and vmPFC, in addition to intrinsic connections within each region. Due to their spatial proximity, connections between amygdala sub-clusters were omitted. This model was defined and estimated for each participant. Next, a post-hoc model selection was used to create and test all possible reduced models in an unbiased way (Friston & Penny, 2011; Rosa, Friston, & Penny,

2012). With Bayesian parameter averaging, magnitudes and probabilities of each parameter were calculated (Kasess et al., 2010).

The fully connected model, which was identified as the most probable model in this step, was then further specified by testing the effect of liking values on network connectivities. To this end, another model was set up that allowed modulation of liking values on each connection, while keeping endogenous connections constant. This model was again defined and estimated for every participant, and all potential models of contextual modulation were tested through post-hoc optimization.

One-sample *t*-tests on the Bayesian parameter averages were performed and Bonferroni corrected to determine which parameters differed significantly from zero.

3.2. Results

3.2.1. Behavioural data

The food liking session ($sess_{like}$) was followed by the eating decision session ($sess_{want}$) after an average of 14.13 days (SD = 6.55 days). On both days, participants came to the laboratory after an overnight fast of at least 10 hours (fasting duration $sess_{like}$: 12.85 ± 1.48 h, $sess_{want}$: 12.85 ± 1.29 h). Fasting glucose levels confirmed fasting states in all participants on both scanning days (glucose $sess_{like}$: 4.74 ± 0.46 mmol*l^{-1}, $sess_{want}$: 4.75 ± 0.41 mmol*l^{-1}). Fasting duration and glucose levels did not differ between study days (all $T(29) < 0.206$, all $p > 0.839$). Accordingly, hunger ratings were similar on both days (hunger $sess_{like}$: 4.00 ± 2.45, $sess_{want}$: 4.17 ± 2.18, $sess_{like}$ vs. $sess_{want}$: $T(29) = 0.344$, $p = 0.733$).

Of the 70 food stimuli presented, participants liked on average 76.79% (SD = 11.20%), while they wanted to consume 42.01% (SD = 18.83%) of the food items (see Fig. 15a for the distribution of answers per rating). Specifically, of all the liked food items, participants wanted to consume on average 52.16% (SD = 19.85%, see Fig. 15b for the distribution of wanting ratings per liking category). Liking ratings were not significantly correlated with caloric ($T(29) = 0.180$, $p = 0.858$), sugar ($T(29) = 0.837$, $p = 0.409$), complex carbohydrates ($T(29) = 0.312$, $p = 0.757$), fat ($T(29) = 0.086$, $p = 0.933$) or protein content ($T(29) = 2.62$, $p = 0.014$, n.s. ($p = 0.070$) after Bonferroni-correction). Also wanting ratings were not correlated with any macronutrient or caloric content (all $T(29) < 1.617$, all $p > 0.117$), but were significantly correlated with liking ratings across all participants ($T(29) = 40.34$, $p < 0.001$).

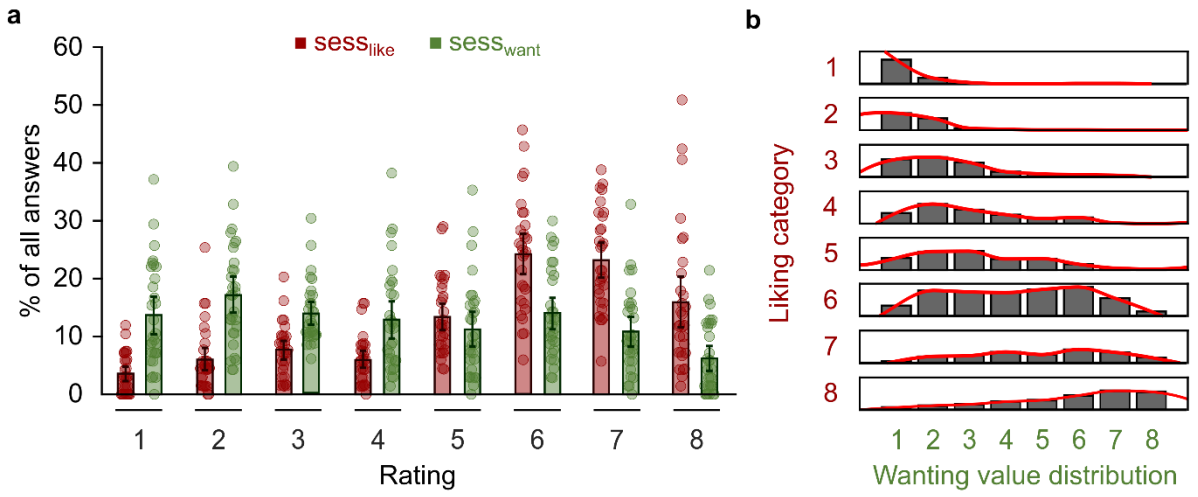

Figure 15 | Study 2: Behavioural data

(a) Individual data, group means and 95% CI of parametric hedonic and decision values ranging from 1 to 8. (b) Average distribution of wanting values (%) for each liking category.

While hunger was not correlated with food liking (r = 0.278, p = 0.137), there was a significantly positive correlation of hunger ratings during $sess_{want}$ with food wanting (r = 0.626, p < 0.001). Accordingly, correlations of hunger ratings with average wanting and liking ratings differed significantly (z = 1.651, p = 0.049; Fig. 16), indicating a differential impact of metabolic state on food palatability and the desire to consume food.

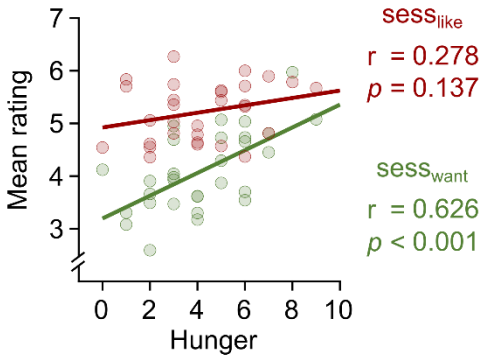

Figure 16 | Study 2: Impact of hunger on food liking and wanting

While hunger was not related to parametric liking ratings, it significantly correlated with parametric wanting ratings.

3.2.2. Liking patterns in the amygdala

To assess palatability-specific activity patterns in the amygdala in each subject, we created individual, bilateral ROIs from a univariate analysis providing all voxels within an anatomical amygdala mask that were generally engaged during explicit food liking evaluation. The size of individual ROIs ranged from 443 to 1178 voxels (mean ± SD: 1054.00 ± 150.72 voxels). In the next step, food items with identical liking ratings (ranging from 1 = "no - not at all" to 8 = "yes - very much") were sampled into separate regressors before estimating another GLM. Extracted *t* values of each condition per voxel within individual amygdala ROIs were then used to create subject-wise RDMs (Walther et al., 2016) through pairwise comparisons of neural activity patterns for each liking rating (Fig. 17a). We then calculated the correlations of these RDMs with a model that quantified the similarity of liking ratings from strong disliking to strong liking in a linear fashion (model RDM; Fig. 17a). A one-sample *t*-test of Fisher *z*-transformed correlation coefficients revealed strong evidence for the differential coding of palatability in the amygdala ($T(29) = 2.848$, $p = 0.008$; Fig. 17c). As a control, we also examined activity patterns in the primary visual cortex (V1, Fig. 17b). Results revealed no reliable evidence for palatability coding in this region ($T(29) = 0.166$, $p = 0.869$; Fig. 17c).

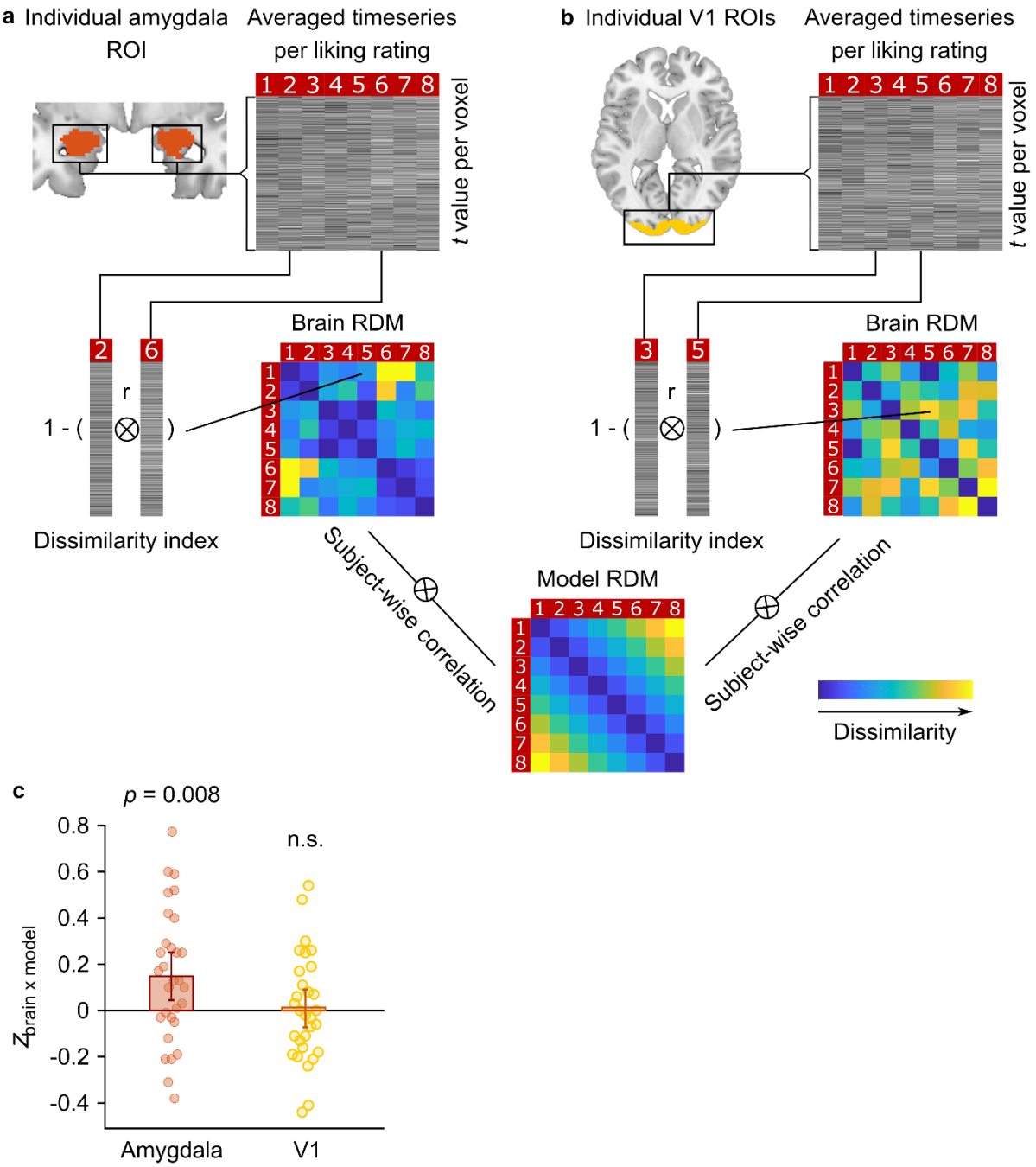

Figure 17 | Study 2: Multivariate liking coding

Example analysis pipeline for a single subject in the amygdala (a) and – as a control – the V1 (b): Within individual ROIs, *t* values per voxel were extracted for each liking rating. RDMs per subject were created through pairwise comparisons of neural activity patterns for each liking rating. Neural RDMs were then correlated with a model RDM that quantified the similarity of food liking patterns assuming a continuous, linear valence coding. (b) Group result: Individual data, mean and 95 % CI of Fisher *z*-transformed correlation coefficients between brain and model RDMs in the amygdala and the primary visual cortex.

RDM, representational similarity matrix; ROI, region of interest; V1, primary visual cortex

3.2.3. Modulation of food choices by amygdala valence assignment

On average 37.20% variability of food wanting decisions at $sess_{want}$ could be explained by liking ratings from $sess_{like}$, indicating a strong but not full mediation of eating decisions by food liking. To identify neural liking valuation in the amygdala during explicit food choices we compared pattern activation from $sess_{want}$ of each food item to liking-specific activation profiles from $sess_{like}$. Single-item multivoxel patterns from $sess_{want}$ were therefore correlated with both averaged multivoxel patterns from $sess_{like}$ of all other items with the same liking rating (r_{same_liking}) and averaged multivoxel patterns from $sess_{like}$ of all other items (r_{other_liking}). The difference between both correlations ($dist_{brain} = r_{same\_liking} - r_{other\_liking}$) was considered as an indicator for pattern specificity of palatability processing in the amygdala during $sess_{want}$ (Fig. 18a).

We further assumed that the reinstatement of liking patterns in the amygdala during consumption decisions predicted the behavioural impact of liking on food wanting which was quantified by the distance between liking and wanting ratings per item [$dist_{behav} = abs(rating_{liking} – rating_{wanting}$], Fig. 18b). To test this assumption we correlated the indicator for liking valuation in the amygdala $dist_{brain}$ with the behavioural distance score $dist_{behav}$ for each item (Fig. 18b), and found a significant negative correlation on the group level that was different from zero ($T(29) = 2.784$, $p = 0.009$, Fig. 18c). This indicates that implicit valuation processing as indexed by multivoxel activity in the amygdala modulates food choices. To further rule out that such an impact was only driven by unspecific neural processes during the liking and the wanting session (e.g. salience), we re-ran this analysis in the primary visual cortex. This control analysis revealed no significant brain-behaviour association ($T(29) = 0.694$, $p = 0.493$; Fig. 18c).

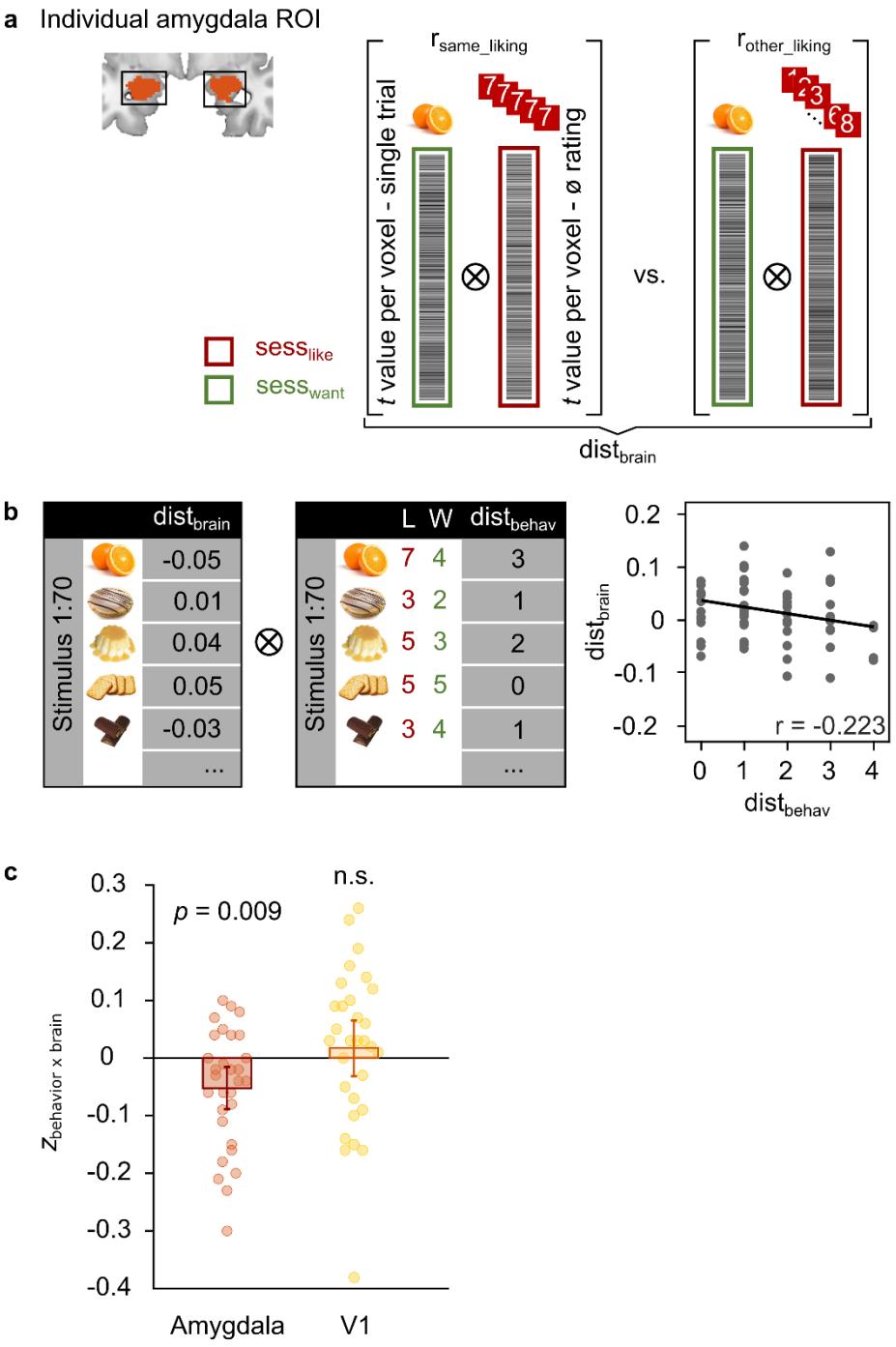

Figure 18 | Study 2: Neural identification of liking valuation

Example analysis pipeline of a single subject: (a) *T* values of single items in $sess_{want}$ were extracted from individual amygdala ROIs. Each of these single item patterns was correlated to average multivoxel patterns from $sess_{like}$ of all other items that had received the same liking rating (r_{same_liking}) and to average multivoxel patterns from $sess_{like}$ of all other items (r_{other_liking}). The difference between these two was calculated and represents an indicator for specific palatability processing ($dist_{brain}$). (b) $Dist_{brain}$ was related to a distance score between the two behavioural ratings [$dist_{behav}$ = abs($rating_{liking}$ – $rating_{wanting}$)]. (c) Group result: Individual data, mean and 95%

CI of Fisher *z*-transformed correlation coefficients between brain and behaviour for the amygdala and the primary visual cortex.
L, liking; ROI, region of interest; V1, primary visual cortex; W, wanting

3.2.4. Segregation of appetitive and aversive signals

As a control and in order to potentially identify positive and negative coding in different amygdala subareas (Beyeler et al., 2016; J. Kim et al., 2016; O'Neill et al., 2018; Paton et al., 2006) we also applied a univariate approach to our data from $sess_{like}$ including subjective liking values from 1 to 8 as parametric regressor of food items in the model. Group statistics on the second level revealed no significant parametric modulation of mean amygdala responses (Tbl. 10). Thus, whole spectrum palatability information can be detected in multivariate pattern analyses, but not as a strictly linear component of the average univariate response in the amygdala. Next, we transformed each individuals' *t* values from the aforementioned parametric analysis into correlation coefficients per voxel. This allowed us to segregate amygdala voxels for each subject based on negative (i.e. stronger activation for unpleasantness) and positive (i.e. stronger activation for pleasantness) coefficients. Segregation resulted in comparable numbers of voxels encoding positive (mean ± SD: 537.07 ± 206.81 voxels) and negative (mean ± SD: 516.93 ± 244.35 voxels) valence with no difference between valences ($T(29) = 0.346$, $p = 0.732$). Clusters spatially followed an anterior to posterior topography (Fig. 19a), with predominantly posterior voxels activated by palatable foods while predominantly anterior voxels rather responded to unpalatable food cues (Fig. 19b).

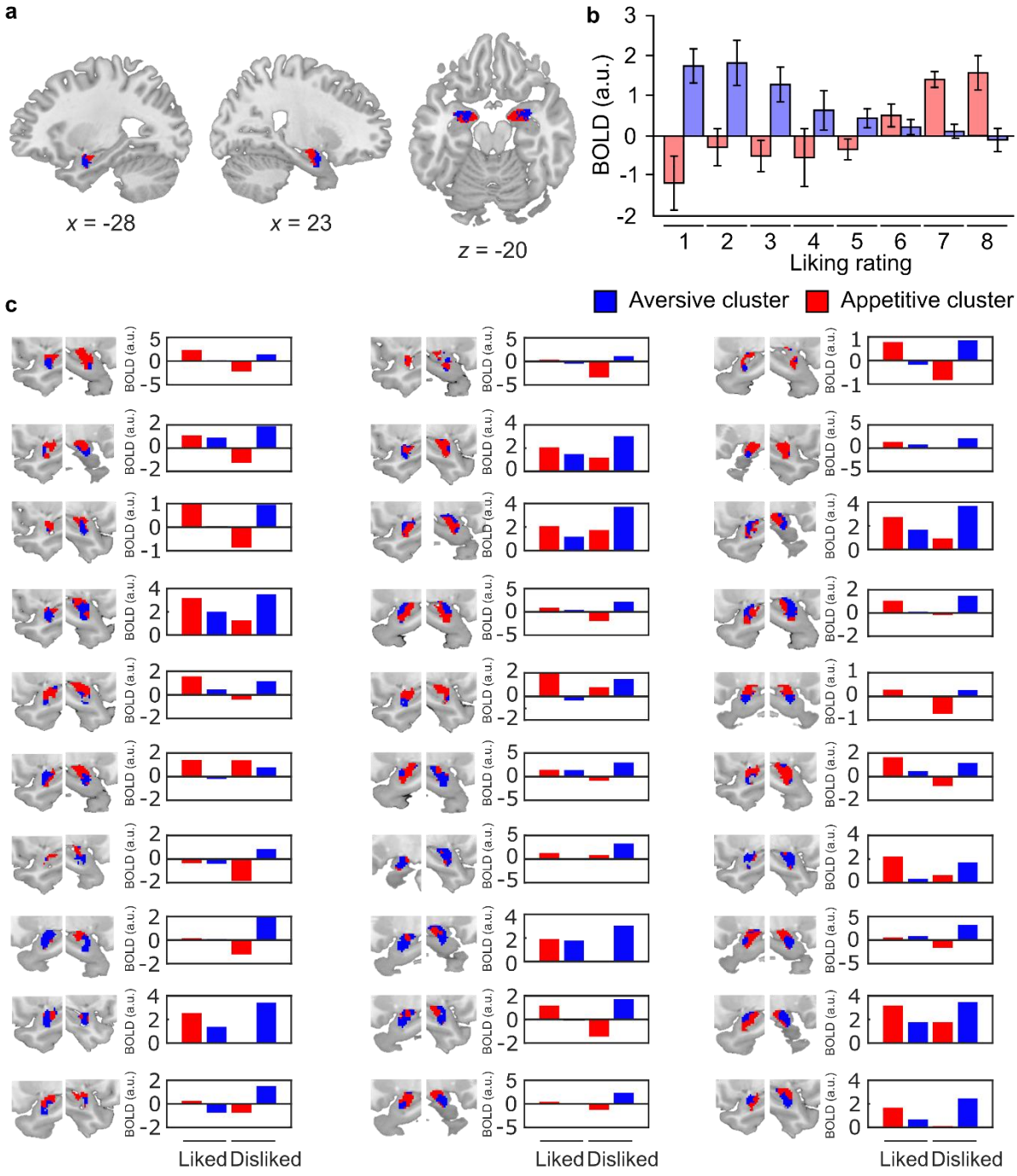

Figure 19 | Study 2: Spatial distribution of valence coding in the amygdala

(a) Across all participants, activity in more posterior regions within the amygdala increased with pleasantness (appetitive cluster, red), while activity in more anterior regions increased with unpleasantness (aversive cluster, blue). Voxels are shown in red if the majority of the participants exhibited positive correlation with liking values in that voxel, and in blue if the majority of the participants showed negative correlation with liking values in that voxel. (b) The bar graph shows group means and SEM of mean parameter estimates of each liking rating, extracted from individuals' appetitive and aversive amygdala sub-clusters. (c) Individual sub-clusters with mean brain signals in response to liked and disliked food items.

Table 10 | Study 2: Peak coordinates and statistics of fMRI analyses

Statistically significant peaks are listed whose activity correlated significantly with liking and decision values in $sess_{like}$ and in $sess_{want}$, respectively (parametric analysis). MNI coordinates, *Z* and *T* values represent peak voxels from a whole-brain random-effects analysis ($p < 0.05$ FWE corrected).

		MNI (peak)					
Brain region	Side	*x*	*y*	*z*	Cluster size	*Z*	*T*
$sess_{like}$ (parametric modulation by liking rating)							
Precuneus	L	-19	-54	12	1218	5.48	7.35
vmPFC		0	44	-7	711	4.67	5.76
$sess_{want}$ (parametric modulation by wanting rating)							
Middle frontal gyrus	L	-40	40	12	3911	5.44	7.27
vmPFC		0	38	-7	705	4.99	6.14
NAc	L	-8	5	-6	28	4.03	4.72
	R	10	14	-4	10	3.71	4.24
$sess_{want}$ (parametric modulation by liking rating)							
vmPFC		0	4-	-2	540	4.77	5.95

L: left; NAc, nucleus accumbens; R: right; vmPFC, ventromedial prefrontal cortex

3.2.5. Amygdala modulation of the vmPFC-NAc network

Ultimately, we investigated whether and how liking values of food are integrated into neural value computation during food choices. To this end, we first aimed to confirm the proposed role of the vmPFC-NAc network in food choice modulation. We therefore analysed brain signals measured during $sess_{want}$ using a two-level random effects model with wanting values ranging from 1 to 8 as parametric modulator on the individual level. Group statistics at the second level yielded a strong parametric modulation in the vmPFC and the NAc ($p < 0.05$ FWE corrected, Fig. 20, Tbl. 10), i.e. BOLD signals in these regions correlated positively with food wanting. No significant results were observed in the amygdala. Next, we tested whether food liking values directly modulated brain activity during $sess_{want}$. For this purpose, we repeated the parametric analysis but used the liking ratings as a parametric modulator. Results revealed a significant modulation in the vmPFC ($p < 0.05$ FWE corrected) but neither in the NAc nor in the amygdala (Tbl. 10).

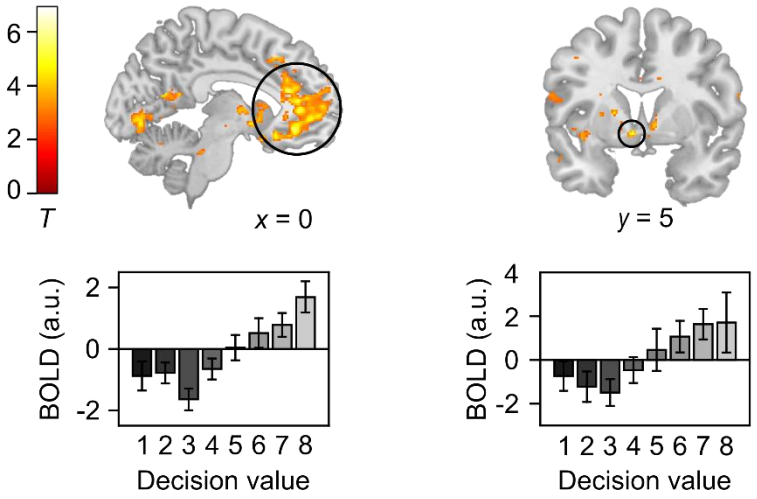

Figure 20 | Study 2: Neural representation of decision values

Regions in which the correlation with the decision value was significant across all participants included the vmPFC and the bilateral NAc. For more details see Tbl. 10 '$sess_{want}$ (parametric modulation by wanting rating)'. Activations are overlaid on a standard anatomical template image (ch2better.nii, MRIcron; display threshold $p < 0.005$ uncorrected).

Based on above findings on valence-specific amygdala sub-clusters and recent animal data (Beyeler et al., 2018; J. Kim et al., 2016; Seo et al., 2016) we then used dynamic causal modelling to investigate whether food liking modulates the connectivity between amygdala sub-clusters and the vmPFC-NAc network during food choices. In an initial model, we allowed for all connections between amygdala sub-clusters, the NAc and the vmPFC. Bayesian model reduction identified the model with the best evidence by comparing the evidence for all possible reduced models of connectivities (Friston et al., 2016). The winning model included all reciprocal connections with a posterior probability of almost 1 (Fig. 21a). In a second step, this model was refined by allowing parametric liking values to modulate coupling of the connections between regions while keeping the endogenous connectivity constant. Note that these modulation parameters capture the degree to which variations in item valence modulate network dynamics. Again, Bayesian model reduction was used to identify the best model of this modulation. This model selection indicated liking values to positively modulate the connections from the appetitive sub-cluster of the amygdala to the NAc, and to the vmPFC. At the same time, there was a negative modulatory effect of liking on the connection from the aversive sub-cluster to the vmPFC. No other modulatory effects were included in this winning model that had a posterior probability of 0.99 (Fig. 21b).

Using one-sample *t*-tests, we confirmed that each of the parameters quantifying connections and modulatory effects was significantly different from zero. Because we tested 17 parameters of

interest (14 endogenous plus 3 modulatory) we applied Bonferroni correction (α = 0.05/17 = 0.0029) to one-sample *t*-tests. Descriptive statistics of all intrinsic and modulatory parameters can be found in Tbl. 11.

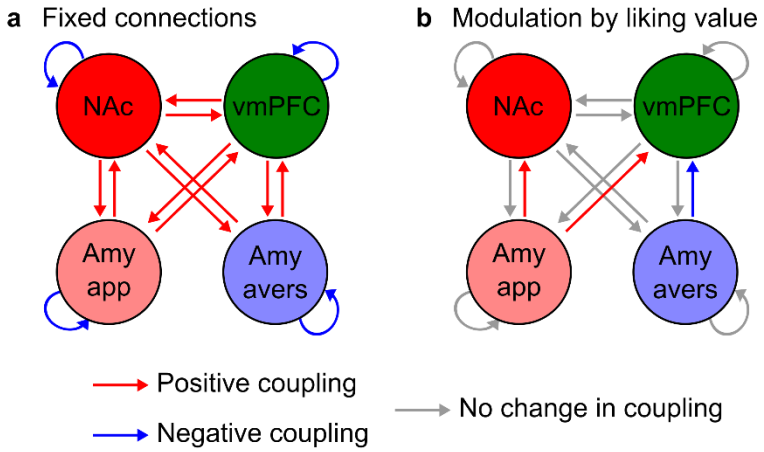

Figure 21 | Study 2: DCM results

(a) The most probable model of fixed connections as identified through post-hoc optimization included reciprocal connections between all nodes (except for within-amygdala connections, which were not included in the initial model due to spatial proximity). (b) Liking values positively modulated the connections from the appetitive sub-cluster of the amygdala to the NAc, and to the vmPFC, and negatively modulated the connection from the aversive sub-cluster to the vmPFC. Amy app, appetitive amygdala sub-cluster; Amy avers, aversive amygdala sub-cluster; NAc, nucleus accumbens; vmPFC, ventromedial prefrontal cortex

Table 11 | Study 2: DCM parameter estimates

Statistically significant connectivity estimates and their standard deviations are given for the averaged model parameters obtained from Bayesian model averaging. *T* values refer to one-sample t-tests. *P* values significant after Bonferroni correction for multiple comparisons (17 parameters of interest) are printed in bold.

Parameter		Estimate	*T*	*p*
Fixed coupling – between regions				
amy_app	→ NAc	0.06 ± 0.03	10.954	**< 0.001**
	→ vmPFC	0.14 ± 0.03	25.560	**< 0.001**
amy_avers	→ NAc	0.06 ± 0.05	6.573	**< 0.001**
	→ vmPFC	0.27 ± 0.05	29.577	**< 0.001**

NAc	→ amy_app	0.22 ± 0.03	40.166	**< 0.001**
	→ amy_avers	0.39 ± 0.02	106.806	**< 0.001**
	→ vmPFC	0.12 ± 0.03	21.909	**< 0.001**
vmPFC	→ amy_app	0.28 ± 0.04	38.341	**< 0.001**
	→ amy_avers	0.08 ± 0.09	4.869	**< 0.001**
	→ NAc	0.31 ± 0.08	21.224	**< 0.001**
Fixed coupling – within regions				
amy_app		-1.03 ± 0.01	564.154	**< 0.001**
amy_avers		-1.04 ± 0.02	284.816	**< 0.001**
NAc		-0.94 ± 0.01	514.859	**< 0.001**
vmPFC		-0.94 ± 0.02	257.430	**< 0.001**
Modulation by liking value – between regions				
amy_app	→ NAc	0.22 ± 0.21	5.738	**< 0.001**
	→ vmPFC	0.50 ± 0.11	24.896	**< 0.001**
amy_avers	→ vmPFC	-0.23 ± 0.22	5.726	**< 0.001**

Amy app, appetitive amygdala sub-cluster; Amy avers, aversive amygdala sub-cluster; NAc, nucleus accumbens; vmPFC, ventromedial prefrontal cortex

3.3. Discussion

In the second study of this thesis, distributed palatability representations in the human amygdala were identified using a multivariate analysis approach. Beyond that, we used identified patterns to track implicit liking valuation during food choices. During the explicit rating of palatability, liking values were differentially encoded across the whole spectrum through spatial patterns supporting a unidimensional bipolar model of valence encoding in the amygdala (Fontanini et al., 2009; Jin et al., 2015; Sadacca et al., 2012). The reinstatement of these valence-specific multivoxel amygdala patterns directly predicted the impact of food attractiveness on single item choices. Moreover, our data indicate that projections from appetitive and aversive amygdala sub-clusters differentially incorporated hedonic values into prefrontal-accumbal circuits during decisions on food. These findings underline the role of amygdala subsets in engaging valence-specific behavioural responses during food choices even in an implicit liking-valuation context.

Our results demonstrate that unique, spatially segregated patterns in the amygdala encode the entire subjective valence dimension from palatability to unpalatability. Specifically, the amygdala showed stronger pattern correlations among food cues of similar palatability and weaker correlations among food cues of dissimilar palatability. This regionally distributed valence coding

is in agreement with the lack of a significant result using a conventional univariate fMRI approach on averaged amygdala signals and is in line with previous findings in humans where a representational similarity approach revealed multivoxel valence coding of different odors (Jin et al., 2015). Importantly, our data indicate that this dimensional coding is also relevant for liking values in response to visual food cues. Indeed, the amygdala receives sensory input from all modalities and thus has been related to the affective valuation of many different stimuli (Anders, Eippert, Weiskopf, & Veit, 2008; Baxter & Murray, 2002; Zerubavel, Bearman, Weber, & Ochsner, 2015). In the context of taste and palatability valuation, relevant networks including the NTS (Price & Amaral, 1981), the VPM (Nakashima et al., 2000; Turner & Herkenham, 1991) and the primary gustatory cortex (Aggleton, Wright, Rosene, & Saunders, 2015; Mufson et al., 1981) are strongly interconnected with the amygdala. Recent findings indicate that these gustatory circuits already respond to food-predicting cues and that such cue-related activity mediates the expression of food approach behaviour (Kusumoto-Yoshida, Liu, Chen, Fontanini, & Bonci, 2015). Recent findings demonstrate visual food stimuli to evoke amygdala responses even in the absence of conscious awareness of food (Sato, Kochiyama, Minemoto, Sawada, & Fushiki, 2019). Moreover, automatic value encoding in the amygdala in response to visual food stimuli has been identified previously also in valence-independent tasks (Mormann, Bausch, Knieling, & Fried, 2017). Thus, one could speculate that amygdala responses upon the sight of a visual food stimulus represent value information irrespective of the task.

Intriguingly, the more amygdala activity patterns during eating decisions resembled palatability-specific patterns, identified during explicit liking, the more liking values overlapped with wanting values, i.e. the stronger food liking modulated individual decisions to consume this food item. Animal and human studies have distinguished brain networks more involved with motivating the desire for the food, i.e. food "wanting", versus those processing the hedonic properties of food, i.e. food "liking". How these two mechanisms act in concert to modulate eating behaviour, however, is less understood (Berridge & Kringelbach, 2015; Volkow et al., 2011). The amygdala seems to represent an optimal target for studying such interaction given its role in the valuation of palatability (Fontanini et al., 2009; Jin et al., 2015; Piette et al., 2012; L. Wang et al., 2018), the learning of food preferences (Agustín-Pavón et al., 2011; Risco & Mediavilla, 2014), and the triggering of valence-specific behavioural responses (O'Neill et al., 2018). Especially the opioid receptor-rich BLA is thought to convey the hedonic value of rewards into goal-directed actions probably based on incentive learning (Balleine, Killcross, & Dickinson, 2003; S.-H. Wang, Ostlund, Nader, & Balleine, 2005) which is further supported by pharmacological studies using the opioid antagonist naltrexone (E. A. Murray et al., 2014; Wassum et al., 2009).

The present study emphasizes that food palatability not always triggered consumption decisions as also indicated by a substantial within-subject variability of pattern reinstatement. It is possible

that other mechanisms, such as metabolic processes including endocrine signals (see study 1), (learned) habits on food intake (Furlong, Jayaweera, Balleine, & Corbit, 2014; Lingawi & Balleine, 2012; Naughton, McCarthy, & McCarthy, 2015; Volkow & Wise, 2005) or restrained eating strategies (Hare et al., 2009; Hollmann et al., 2012) cause this variability. Our approach allowed for the neural tracking of value-driven food choice behaviour on a trial-by-trial basis and thus was sensitive to variability of food valuation processes during eating decisions.

Based on recent evidence in animals (O'Neill et al., 2018; L. Wang et al., 2018) we were further interested whether observed pattern activation in the amygdala at least in part results from topographically separated sub-clusters representing the coding of positive versus negative valence. Such positive value-coding neurons have been shown to increase their firing rate to conditioned stimuli predicting reward but decrease their firing in response to aversive cues while negative value-coding neurons demonstrate the opposite pattern (Belova, Paton, & Salzman, 2008). To follow up on this, we segregated amygdala voxels based on their single-voxel response to palatable versus unpalatable food cues. This procedure resulted in topographically distinct amygdala clusters that spatially overlapped with recent data in rodents where rewarding stimuli primarily activated the posterior BLA while aversive stimuli primarily activated the anterior BLA (J. Kim et al., 2016). In a confirmatory manner, extracted BOLD signals from these sub-clusters demonstrated increased signals in the appetitive cluster in response to palatable food cues and decreased signals in response to unpalatable food cues while the opposite signal pattern emerged in the aversive cluster, fitting aforementioned data in monkeys (Belova et al., 2008) and suggesting different codes for pleasantness and unpleasantness within the same set of voxels.

Using dynamic causal modelling, a method that was already employed in the first study, we could show that these positive and negative amygdala subsets differentially mediate hedonic values to the vmPFC-NAc network. In line with the proposed role of this network in valuation processing (Bartra et al., 2013; Clithero & Rangel, 2014; Kable & Glimcher, 2007), the BOLD response in both the NAc and the vmPFC was significantly correlated with food wanting values. The predominantly dopaminergic cells-containing NAc is considered a key-structure in the regulation of reward-appetitive behaviour (Haber & Knutson, 2009; Sescousse, Caldú, Segura, & Dreher, 2013) and is directly connected to homeostatic networks and metabolic signals ((Berridge, 2009; Berthoud, 2011; Volkow et al., 2011), also see study 1). Accordingly, direct stimulation and optogenetic manipulations in rodents have identified a metabolic-reward feeding regulation network comprising hypothalamic-mesolimbic pathways in which descending LHA efferents can activate dopaminergic midbrain structures such as the VTA (Wise, 1978). VTA activation can increase dopamine release into the NAc and thus probably the incentive salience of food (see study 1) which may then be incorporated into prefrontal value computation (Haber & Knutson, 2009; Salamone & Correa, 2012) triggering appetitive behaviour. However, the NAc also receives

glutamatergic innervations from the (basolateral) amygdala and activation of this pathway has been shown to promote cue-driven motivated behaviour, i.e. sucrose intake (Stuber et al., 2011). Thus, one could speculate that palatability assignment in the amygdala mediates food decisions across at least two routes: direct pathways to the vmPFC (Seo et al., 2016) as well as modulation of dopaminergic salience signals in the NAc (Stuber et al., 2011). Importantly, these findings were restricted to the appetitive amygdala sub-cluster which agrees with animal data (Beyeler et al., 2016; Namburi et al., 2015) showing that BLA neurons projecting to the NAc preferentially respond to a reward-predictive cue. In contrast, connections from the aversive cluster to the vmPFC were negatively modulated by palatability, i.e. during decisions about unpalatable food, this projection became more activated. Previous studies in animals found negative valence coding neurons of the BLA projecting predominantly to the CeA (Beyeler et al., 2018, 2016; Namburi et al., 2015). Moreover, there is evidence for GABAergic projections from the CeA to the vmPFC (Seo et al., 2016). Thus, even though limited spatial resolution in the presently used fMRI sequences did not allow for investigating within-amygdala connectivity, one might speculate that activation of the pathway originating in the aversive subset inhibits vmPFC value signals.

The vmPFC receives inputs and thus stimuli information from many other brain regions that then may be integrated into food decisions. For instance, recent findings demonstrated individuals' belief on different nutritional components of foods to be represented in different subregions of the OFC and integrated within medial OFC value computation (Suzuki, Cross, & O'Doherty, 2017). Moreover, even though we studied non-overweight non-dieters, health aspects of different foods may have engaged dorsolateral PFC mediated top-down control to regulate food intake (Hare et al., 2009, 2011; Ochner et al., 2012). Our data complement these findings by providing another mechanism triggering food choices in the human brain helping to understand how expectations of pleasantness can mediate appetitive eating behaviour. Specifically, the multivariate approach helps to identify the impact of hedonic valuation on food decisions and thus to explain a relevant amount of variability within a complex neurobehavioral framework of eating behaviour. The second study of this thesis provides strong evidence that the amygdala plays an important role in the integration of palatability into consumption decisions.

4.1. Integration of the results

Both parts of the present thesis unveil important contributors to hedonic food processing and the formation of consumption decisions. In our affluent society, many food items are engineered to provide maximum taste experience, and most consumption decisions are driven by the palatability we associate with food. The present thesis provides evidence that under normal circumstances the neural processing of such hedonic aspects is influenced by the hormone insulin, that aberrant central insulin functioning plays a role in dysfunctional eating behaviour, and provides one mechanism how hedonic aspects are further integrated into food choices.

In the first study, across all participants, viewing food pictures as compared to non-food pictures activated a large network including the VTA, the amygdala, the insula and the OFC – regions which are related to general reward processing and to processing of taste and food-related cues. Using univariate analysis protocols, hedonic and motivational processing of visual food stimuli was found to rely on the valuation network, e.g. the precuneus and the vmPFC. A large number of studies, using different techniques, species, and stimulus types, consistently link activity within these regions with the computation of stimulus values (Padoa-Schioppa, 2011; Rangel & Hare, 2010; Wallis & Kennerley, 2010).

In contrast to these very strong value-related activations in the vmPFC, neural findings concerning the NAc were more specific in the studies at hand. Its involvement in hedonic food valuation could be found in dissociation to non-food cues in participants with normal peripheral insulin sensitivity, and in motivational processing of food cues. The NAc is considered an important interface for reward, motivation and action, and contains sub-regional opioidergic hotspots encoding hedonic aspects, as well as dopaminergic hotspots encoding motivation (Castro & Berridge, 2014; Mitchell et al., 2018; Smith et al., 2011). The present thesis supports these findings by showing its involvement not only in stimulus-specific hedonic processing, but also in the encoding of decision values, through integration of information from the VTA and from the appetitive sub-cluster of the amygdala.

The main aim of the first study was to identify how central insulin modulates these reward networks. Here, specific effects were found on reward-related brain regions, the VTA and the NAc, which were down-regulated following INI application, and on connections within these regions. Although insulin receptors have been described in the amygdala as well (Areias & Prada, 2015; Oh et al., 2013), the first study did not identify effects of INI on general or value-related amygdala activation. Again, one possible explanation might be the unsuitability of univariate analyses, as were used in the first study, to appropriately capture valuation processes in the amygdala.

However, through multivariate analyses, not only hedonic valuation processes in the amygdala were unveiled in the second study, but also direct effects of value patterns on the behavioural impact of hedonic aspects on consumption decisions were identified. This suggests that, as was found in animals, both positive and negative valence is encoded in this region, and that this information contributes to decisions on food consumption.

All in all, the present studies show that hedonic values of food cues are processed in the vmPFC, the NAc and the amygdala, either in the form of overall activation or through multivoxel patterns, and that insulin, i.e. consumption-related signals from the periphery, affects value signalling through modulation of the mesolimbic pathway. Feeding-relevant consumption decisions are formed in partly overlapping circuits, i.e. the NAc and the vmPFC, which additionally receive information on hedonic aspects from different sub-parts of the amygdala. Thus, the present thesis adds important aspects to the understanding of feeding behaviour.

4.2. Limitations of the present studies and outlook to new studies

The two paradigms at hand, hedonic valuation and consumption decisions, capture important aspects of feeding behaviour, and the stimuli used in the present study covered a wide range of foods. Nevertheless, food-related behaviour involves a more complex set of processes. Multivariate patterns accompanying the smelling of odours or actual food consumption might add interesting insights to palatability patterns in response to visual food cues identified in the second study of the present thesis. Additionally, assessing the propensity to exert effort to obtain food instead of subjective reports of the participants' food wanting might present a more detailed, realistic measure of food motivation, and tracking participants' eye movements to determine their attentional focus could allow for conclusions about unconscious processes.

Another important factor in feeding behaviour is self-control, and since the PFC seems to be sensitive to central insulin (Kullmann et al., 2015), and top-down projections exist between the PFC and the amygdala (Delli Pizzi et al., 2017; Motzkin, Philippi, Wolf, Baskaya, & Koenigs, 2015; Viviani, 2014), an experimentally modified influence of self-control on hedonic processing, and its interactions with insulin, might be of particular interest. This could be achieved by directing the participants' attention to health aspects, at the time when they are evaluating food stimuli, or prior to completion of paradigms in the form of informational or training sessions. It additionally might help to explain occasions where palatability did not trigger consumption decisions. Further, such approaches could validate the governmental introduction of e.g. a traffic light rating system providing information on the amount of saturated fats, sugar and salt are in commercially

available foods, and could provide a justification for self-control trainings in the battle against obesity.

Particularly the state of hunger influences food-related behaviours. Importantly, hunger levels were stable across all study days and participants met the recommended duration of 12 hours of fasting (Smeets et al., 2019). Interestingly, insulin did not affect hunger ratings in any of the two groups of the first study, which is in line with the finding that although insulin affects the salience of food cues, it does not change the motivation to approach and consume food in animals (Labouèbe et al., 2013). Future studies might aim at confirming this in humans by investigating the effect of insulin on food wanting and motivation. In the same vein, food processing in the amygdala also strongly depends on the physiological state (LaBar et al., 2001; Siep et al., 2009), so that future studies might aim at comparing hedonic valuation processes in this region between hungry and sated states.

The first study shows that in individuals with impaired peripheral insulin functioning, also central insulin processes are altered. Future studies might complement these findings with a longitudinal approach, to test if central insulin functioning is a predictor of future weight gain or loss, thus adding to early studies finding that peripheral insulin resistance at baseline was unrelated to weight loss (T. McLaughlin, Abbasi, Carantoni, Schaaf, & Reaven, 1999; T. McLaughlin et al., 2001), and if weight loss can restore insulin functioning in the CNS.

Viewing of food cues, or the perspective of upcoming food consumption can elicit insulin release, which is also called the pre-absorptive cephalic phase of insulin secretion (Eliasson, Rawshani, Axelsen, Hammarstedt, & Smith, 2017; Powley & Berthoud, 1985). This in itself might have led to complex interactions between our food paradigm and endogenous insulin and glucose metabolism, also in the insulin session, and might be a plausible explanation of complex group interactions in plasma insulin concentrations observed in the present study, in addition to possible slight dose-dependent permeation of INI into the circulation (Ott et al., 2015). To better account for this, future studies might assess C-peptide levels also after nasal spray application, providing information about endogenous insulin production.

It should be noted, too, that central insulin effects are very complex. In a regionally-dependent fashion, insulin can increase dopamine signalling via cholinergic interneurons (Schoffelmeer et al., 2011; Stouffer et al., 2015). However, through non-invasive fMRI analyses conclusions on this can only be drawn in an indirect manner. First studies have now used INI in combination with positron emission tomography (PET), which provides quantitative information on the receptor occupancy, binding, and regional brain distribution of the targeted pharmaceuticals, to investigate the effects of central insulin on dopaminergic activity in humans (Blum et al., 2019; Kullmann et

al., 2019). Unfortunately, in these studies samples sizes here were very small, and results were not linked to behaviourally relevant outcomes.

In summary, future studies might add to the present results by making use of other stimulus modalities, e.g. food consumption, of other response assessments, e.g. eye movements or grip force, or of other methods, e.g. PET. Experimentally altering the influence of self-control or physiological state would also contribute to the understanding of food control and to the development of efficient treatment protocols in the prevention or reversal of obesity.

5. Abstract

In western societies, with a large variety of palatable food continuously on offer, there is rarely any occasion to eat something not tasty when being hungry. The amygdala is a key structure in palatability processing, but how it modulates the effect of implicit valence assignment on food choices is less studied. Following food consumption, the human body is equipped with a variety of processes downregulating the salience of food cues, e.g. the hormone insulin, which is thought to act at the neural interface between metabolic and hedonic drives to eat. Yet how exactly this hormone affects CNS food cue processing is not fully understood.

Here, we investigate three separate fMRI data sets, acquired while overnight fasted participants performed explicit food liking tasks on the first two days and an explicit decision task on the third day. We make use of a pharmacological approach to test for the effects of INI, and we apply pattern-based multivariate approaches to investigate amygdala contribution.

We can show that through modulation of mesolimbic pathways, INI changes the value of food cues during liking valuation. Specifically, INI reduces ratings of food palatability in individuals with normal insulin sensitivity, accompanied by a change in value signals in mesolimbic regions. In insulin-resistant participants, reduced food preference values and aberrant central insulin action are observed.

In a slightly different set of participants, representational similarity analysis showed a differential coding of hedonic values from low to high palatability in the amygdala during explicit liking ratings. More importantly, the reinstatement of these individual liking patterns within trials during the explicit food choice task predicted the correlation between food pleasantness and eating decisions. Further exploration of amygdala liking patterns revealed topographically segregated subareas representing appetitive versus aversive liking values.

We applied effective connectivity analyses to all datasets, to not only describe changes in behaviour and neural activity, but to also shed a light on the underlying network structures. These analyses reveal insulinergic inhibition of the projection from the VTA to the NAc. Importantly, the strength of this modulation directly predicts the decrease of palatability ratings, linking neural findings to behaviour. Furthermore, during eating decisions, liking values positively modulated projections from the appetitive amygdala subarea to both the NAc and the vmPFC while projections from the aversive amygdala subarea to the vmPFC were negatively modulated by individual liking, showing how hedonic information is fed into the network computing overall decision values during dietary choice.

These results demonstrate how central insulin modulates the cross-talk between homeostatic and hedonic feeding systems and provide a mechanism of how hedonic food values can mediate appetitive eating decisions in humans.

6. Zusammenfassung

In unserer westlichen Welt, in der ständig eine große Auswahl an leckeren Lebensmitteln zur Verfügung steht, gibt es selten den Anlass, etwas zu essen, das nicht schmackhaft ist. Die Amygdala ist eine wichtige Region in der Verarbeitung von Palatabilität, doch wie sie den Einfluss von impliziter Valenzzuweisung auf die Nahrungsauswahl moduliert, ist weniger erforscht. In Folge auf Nahrungsaufnahme ist der menschliche Körper mit einer Vielzahl von Prozessen ausgestattet, die die Salienz von Nahrungsmitteln herunterregulieren, so zum Beispiel das Hormon Insulin, das an der neuronalen Schnittstelle zwischen metabolischem und hedonischem Essenstrieb wirkt. Wie genau sich dieses Hormon auf die hedonische Verarbeitung von Lebensmitteln im zentralen Nervensystem auswirkt, ist jedoch noch nicht vollständig geklärt.

In dieser Dissertation werden drei separate fort-Datensätze untersucht. Diese wurden erhoben, während Teilnehmer, die zuvor über Nacht gefastet hatten, verschiedene Lebensmittel hinsichtlich ihrer Palatabilität bewerteten (Tag 1 und 2), beziehungsweise eine explizite Entscheidungsaufgabe ausführten (Tag 3). Ein pharmakologischer Ansatz wird angewendet, um die Auswirkung von intranasalem Insulin zu untersuchen, und musterbasierte multivariate Ansätze werden genutzt, um Prozesse in der Amygdala zu entschlüsseln.

Die Studien ergeben, dass intranasales Insulin den hedonischen Wert von Essen durch die Modulation von mesolimbischen Signalwegen beeinflusst. Insbesondere verringert intranasales Insulin die hedonische Bewertung von Lebensmitteln bei Personen mit normaler peripherer Insulinsensitivität, begleitet von einer Abnahme der hedonischen Signale in mesolimbischen Regionen. Bei insulinresistenteren Versuchsteilnehmern werden insgesamt niedrigere Bewertungen von Essensreize und eine abnormale Wirkung von zentralem Insulin beobachtet.

In der zweiten Studie gibt die Analyse der repräsentativen Ähnlichkeiten zwischen neuronalen Mustern Hinweise auf eine unterschiedliche Kodierung hedonischer Werte in der Amygdala. Darüber hinaus zeigt sich, dass das Wiederauftreten dieser individuellen neuronalen ‚Geschmacksmuster' während expliziter Konsumentscheidungen den Zusammenhang zwischen Schmackhaftigkeit und Essentscheidungen prädiziert. Weitere Untersuchungen dieser neuronalen Amygdala-Muster ergeben topografisch getrennte Teilbereiche in dieser Struktur, die appetitliche gegenüber aversiven Essenseigenschaften repräsentieren.

Zusätzlich wendeten wir effektive Konnektivitätsanalysen auf alle Datensätze an, um nicht nur Effekte im Verhalten und in der neuronalen Aktivität zu beschreiben, sondern auch die zugrundeliegenden Netzwerkverbindungen zu beleuchten. Diese Analysen zeigen eine insulinerge Hemmung der Verbindung vom ventralen Tegmentum zum Nucleus Accumbens. Das Ausmaß dieser Modulation prädiziert dabei direkt die Abnahme der Geschmacksbewertung und

stellt so eine Verbindung zwischen neuronalen Befunden und Verhaltensergebnissen her. Bei Lebensmittel mit positiver Valenz verstärkte sich die Verbindung vom appetitlichen Amygdala-Untercluster zum Nucleus Accumbens und zum ventromedialen präfrontalen Kortex. Lebensmittel mit negativer Valenz hingegen modulierten die Projektion vom aversiven Amygdala-Untercluster zum ventromedialen präfrontalen Kortex auf negativ Weise.

Diese Ergebnisse zeigen, wie Insulin die Interaktion des homöostatischen und nicht-homöostatischen Systems moduliert und weisen einen Mechanismus auf, wie hedonische Aspekte Essentscheidungen beim Menschen beeinflussen.

7. Abbreviations

AgRP	-	agouti-related protein
amy_app	-	appetitive sub-cluster of the amygdala
amy_avers	-	aversive sub-cluster of the amygdala
BBB	-	blood-brain barrier
BDI	-	Beck Depression Inventory
BLA	-	basolateral amygdala
BMI	-	body mass index
BOLD	-	blood oxygenation level-dependent
bpm	-	beats per minute
CAT	-	Computational Anatomy Toolbox
Cb	-	cerebellum
CeA	-	central amygdala
CI	-	confidence interval
CNS	-	central nervous system
CP	-	caudate putamen
CSF	-	cerebrospinal fluid
DAMGO	-	Tyr-D-Ala-Gly-(me) Phe-Gly-ol
DAT	-	dopamine reuptake transporter
DEBQ	-	Dutch Eating Behaviour Questionnaire
dlPFC	-	dorsolateral prefrontal cortex
EPI	-	echo-planar imaging
EPQ	-	Eysenck Personality Questionnaire
fMRI	-	functional magnetic resonance imaging
FSL	-	Functional MRI of the Brain Software Library
FWE	-	family-wise error
FWHM	-	full width at half maximum
GABA	-	gamma-Aminobutyric acid
GLM	-	general linear model
GLP-1	-	glucagon-like peptide 1
HbA1c	-	haemoglobin A1c
HOMA-IR	-	homeostatic model of insulin resistance
Hpc	-	hippocampus
Hy	-	hypothalamus
IN	-	insulin session
INI	-	intranasal insulin

IPAQ	-	International Physical Activity Questionnaire
IR	-	increased insulin resistance-group
ITI	-	inter-trial interval
LHA	-	lateral hypothalamic area
LTD	-	long-term depression
MAO	-	monoamine oxidase
MCH	-	melanin-concentrating hormone
MNI	-	Montreal Neurological Institute
MVPA	-	multivariate/multivoxel pattern analysis
NAc	-	nucleus accumbens
NIR	-	normal insulin resistance-group
NPY	-	neuropeptide y
NTS	-	nucleus tractus solitarii
OFC	-	orbitofrontal cortex
PBN	-	parabrachial nucleus
PET	-	positron emission tomography
PFC	-	prefrontal cortex
PL	-	placebo session
POMC	-	propiomelanocortin
PVN	-	paraventricular nucleus
PYY3–36	-	peptide YY
RDM	-	representational dissimilarity matrix
rmANOVA	-	repeated measures analysis of variance
RN	-	raphe nucleus
RSA	-	representational similarity analysis
SD	-	standard deviation
SPSR	-	Sensitivity to Punishment and Sensitivity to Reward Questionnaire
T2D	-	type 2 diabetes
V1	-	primary visual cortex
VPM	-	ventral posteromedial nucleus of the thalamus
VTA	-	ventral tegmental area
YFAS	-	Yale Food Addiction Score

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10. References

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11. Acknowledgements

First, I would like to thank my supervisor PD Dr. Stefanie Brassen for her all support, her countless advice, her close supervision, and her guidance throughout the last years. This doctorate might have taken longer that initially anticipated, but I wholeheartedly hope that it was worth the while for everyone involved.

Moreover, I am very grateful for the support from my colleagues at the Department of Systems Neuroscience and beyond, in particular to Judith Hettel, Paul Francke and Katrin Gießen, who have supported the challenging research project over the course of the years and contributed to the success of data collection, and to Sebastian M. Schmid and Arjen Alink for their support in the process leading to my publications. Special thanks also go to all of the student assistants, who got up bright and early for assistance during the experiments, who rarely ever gave up hope during difficult blood collections and who showed great patience in handling more complicated participants. I also would like to thank Christian Büchel for his support in experiment conceptualization, data analysis, I would like to thank Jürgen Finsterbusch for his help with everything related to MRI, and our radiographers Katrin Bergholz, Kathrin Wendt and Waldemar Schwarz for their help in acquiring the imaging data.

Furthermore, I would like to thank Prof. Simone Kühn and Prof. Dr. Ulrike Krämer for their disposition to assess my thesis (yeah to an all-female-team!).

Finally, I would like to thank my family and friends for their endless support, constant encouragement, strong shoulders and open ears.

12. Curriculum Vitae

Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

13. Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

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