Decoding the control of food intake

Insights from the habenula and hypothalamus

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GENERAL INTRODUCTION

The burden of obesity

Obesity has become a global health problem with epidemic proportions. In 2016, more than 1.9 billion adults were overweight, of which over 650 million were obese (WHO, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight on 15-2-2023). Overweight is defined as having a body mass index (BMI) between 25 and 30, whereas obesity is defined as having a BMI greater than 30. A high BMI often results from an excessive amount of body fat, which can have detrimental effects on a person's health. Obesity not only affects people in wealthy nations but also affects developing countries: the majority of the world's population lives in countries where more people decease from overweight and obesity than from being underweight [1, 2]. Obesity is a major risk factor for the development of type 2 diabetes mellitus (T2D), as it can cause insulin resistance by impairing the body's ability to produce and use insulin effectively [3]. In turn, this can result in high blood sugar levels, which, over time, damages blood vessels and nerves and increases the risk for complications like heart disease, stroke, and kidney damage [4].

The pathophysiology of overweight and obesity is complex and includes genetic, environmental, nutritional, metabolic, cultural, and psychosocial factors [5]. Essentially, obesity is caused by a disbalance between caloric consumption and energy expenditure. The obesity epidemic is exacerbated by the widespread availability of highly palatable, calorie-dense, processed, convenient and affordable food options. Additional contributors are a general increase in sedentary behaviors and perceived stress levels [6-9]. Obesity can be both prevented and treated by adopting a healthy eating regimen and engaging in regular physical exercise. However, adapting lifestyle changes that promote and maintain weight loss after becoming overweight or obese is very challenging, and has low success rates [10]. Thus, understanding the processes that mediate the development of obesity and diabetes is crucial to optimize prevention strategies. This knowledge will help individuals, healthcare providers, and policymakers, to deal with these metabolic disorders on individual and societal levels.

The brain controls energy homeostasis

The brain plays a crucial role in regulating caloric intake by integrating central and peripheral information related to energy levels. It can trigger or inhibit food seeking and consumption behavior rapidly and effectively. Notably, even the mere smell or thought of food can initiate feelings of hunger. During and following food consumption, the brain receives information via nutrient signaling, which includes glucose, triglycerides, fatty acids and amino acids that reach the brain via the blood. Additionally, peripheral sensors, located in the mouth, stomach, and intestines, provide direct neural input to the brain, relaying information including taste or expansion of the stomach. In addition, hormones like insulin (secreted by the pancreas), ghrelin (secreted by the stomach), and leptin (secreted from adipose tissue) circulate in the periphery and influence brain processes. Caloric intake and the processing of nutrients are also regulated by the biological clock, which blunts these processes during the inactive period while promoting them during the active period [11-13]. The circadian timing of the caloric intake is therefore an important aspect of metabolic (dys)regulation, as research has shown that eating at the wrong time of the day (when one is supposed to sleep or rest) promotes weight gain and metabolic disease, in both rodents and humans [14-17]. Indeed, shift workers have increased rates of obesity [18].

A main integration site of metabolic information in the brain is the hypothalamus, which regulates key functions like body temperature, thirst, hunger, and energy expenditure to maintain energy homeostasis [19]. Obesity and other metabolic disorders can arise when the energy homeostasis becomes dysregulated in the brain. In subjects with obesity, brain sensitivity to hormones like leptin and insulin decreases, reducing the response to satiety signals. Such resistance to leptin or insulin can disrupt the feedback that normally maintains energy homeostasis, leading to increased appetite and reduced energy expenditure [20, 21]. In addition, reward-related or hedonic eating behaviors can also be disturbed in subjects with obesity. Brain reward-related signaling can alter the reinforcing value of food and increase motivation for reward-seeking behavior beyond homeostatic needs [22-24]. Neurons in reward-related brain regions can be activated by high-caloric foods containing fat and/or sugar [25, 26], subsequently promoting excessive intake. In the next paragraphs, the brain circuits underlying homeostatic- and reward-related consumption behavior will be discussed in more detail.

Homeostatic-related brain circuitry controlling caloric intake

Homeostatic-related brain circuits control food intake by integrating signals concerning energy homeostasis, whereas reward-related regions control food intake driven by hedonic or reinforcing aspects. The brainstem and hypothalamus, especially the arcuate nucleus, are key homeostatic-related brain regions that control consummatory behavior. The arcuate nucleus is uniquely located, at the ventral side of the hypothalamus and in close proximity of the third ventricle and median eminence, where there is a blood-brain-barrier-free connection [27]. This complex organization allows first-order neurons in the arcuate nucleus to directly receive information about peripheral nutrients and metabolic signals [28]. These first-order neurons subsequently project to other hypothalamic nuclei, such as the paraventricular nucleus (PVN) and lateral hypothalamus (LH) [29], as well as to the brainstem [30]. The LH is an important regulator of homeostatic feeding, by integrating input from several other hypothalamic areas. Additionally, the LH also expresses many receptors, including leptin and insulin receptors, and harbors glucose-sensitive neurons, enabling it to directly respond to the consumption of specific nutrients [31-36]. Nutrient-related signals from the gastrointestinal tract are also transferred to the hypothalamus via multiple pathways, including the vagal nerve and the brainstem [30]. These homeostatic circuits enable the brain to stimulate food seeking behavior or consumption when the energy balance is low, and vice versa. These homeostatic-related brain circuits interact with

hedonic or reward-related regions, which can drive food seeking (appetitive) or consummatory behaviors while the energy balance does not require so. In addition, many of the peripheral signals related to energy balance, such as ghrelin, leptin and insulin can also affect reward-related circuitry directly as these hormone receptors are localized in these areas as well [37-40].

Reward-related brain circuitry controlling caloric intake

Reward-related or hedonic brain circuits can drive caloric intake beyond homeostatic needs. This can occur by altered reinforcing value of a specific food or increased motivation to seek or work for palatable foods [22-24]. Dopamine is a crucial neurotransmitter in reward-related consumption behavior, and is involved in motivation ('wanting'), learning, and movement [22, 41-45]. Dopamine is released from dopaminergic neurons within the ventral segmental area (VTA) and substantia nigra (SN), which project throughout the brain, including limbic areas, the striatum, (lateral) hypothalamus and cortical areas [46-51]. An important regulator of dopaminergic activity within the VTA is the lateral habenula (LHb) [52-54].

On a neurobiological and psychological level, reward processes can be divided in three components: 'liking', 'wanting', and 'learning', all of which involve dopamine signaling [22, 41-44]. Liking encompasses the pleasurable or hedonic experience associated with a food reward, displayed as affective facial expressions immediately after consumption [55-57]. 'Liking' is encoded by hedonic hotspots in limbic structures such as the ventral pallidum (VP) and nucleus accumbens (NAc; part of ventral striatum) [58-60], which are responsive to opioids and endocannabinoids [44, 61-63] and can be influenced by satiety state or learned taste preferences or aversions [64]. 'Wanting' refers to incentive salience, and represents the motivational component and desirability that drives an organism to approach a reward. Manipulations of dopaminergic neurotransmission can alter incentive salience bidirectionally, without affecting 'liking' [41, 65-67]. Finally, 'learning' involves the formation of associations between rewards and cues, enabling us to predict when to expect a reward and facilitate an approach to obtain the predicted reward. This associative learning is thought to be driven by the reward prediction error (RPE), encoded by dopaminergic neurons [68, 69]. The RPE entails that dopaminergic activity increases at unexpected stimuli, whereas firing stops whenever stimuli are expected; crucial for learning associations. In relation to food intake, learning processes are important as they teach what foods to consume and which to avoid [70-72]. Although it is generally accepted that dopamine is involved in both learning and motivation, how one signal can encode both remains a topic of investigation and discussion [45]. All of the above discussed processes are valid for reward in general, as well as especially for food reward.

Thus, reward-related brain areas, especially dopaminergic circuits, can drive overconsumption by altering incentive salience ('wanting'), hedonics ('liking') or learning processes regarding food, leading to eating beyond homeostatic needs [44]. Interestingly, neuroendocrine signals such as ghrelin, insulin and leptin have been reported to affect food reward, assisting the reward circuit to appropriately respond to food-related signals [73]. Also, stress-related signals can influence the reward system by increasing the incentive salience, possibly underlying emotionally driven overconsumption, or comfort eating [8, 74, 75]. In that regard, serotonin (5-HT) signaling is reported to be involved as well, although it has received considerably less attention than dopamine [76-79]. Historically, homeostatic and hedonic or reward-related brain regions were often discussed separately. However, it has been postulated that these circuits are overlapping, with ventricular neurons (ARC, PVN) directly sensing peripheral signals and specialized in feeding behavior [50]. Monoaminergic nuclei such as VTA and SN (dopamine) and dorsal raphe (DR; serotonin) have a role in feeding, but also more general functions like reward arousal, movement, and motivation [50]. Finally, intermediate nuclei such as LH, central amygdala (CeA) and LHb are located between monoaminergic and ventricular areas, involved in both feeding specific and general behaviors such as reward [50]. The next part will focus on the LH, LHb and CeA, which play a significant role in consumption behavior and reward-related behavior in general, thus making them of interest in the context of obesity development and prevention.

The lateral hypothalamus (LH)

The LH as integrator of homeostatic and reward-related information

The LH is located anterior to the VTA and posterior to the preoptic area and is a heterogeneous structure with a wide range of genetically distinct cell populations [80]. Historically, the LH is a well-described brain region in the control of caloric intake. Electric stimulation of this area increases caloric consumption, whereas lesions reduce caloric consumption [81-83]. The LH plays an important role in the regulation of homeostatic feeding, but activation of LH is also rewarding [84, 85], revealing a role for LH in reward processing [86, 87]. Electrical stimulation of the LH can become aversive if continued for too long or with a too high intensity [88, 89], which possibly could be explained by a GABAergic pathway to the VTA that is differentially requited with different stimulation frequencies [90]. Activation of this LH^{GABA}->VTA connection produces conditioned place preference [90], whereas activation of the glutamatergic LH->VTA connection produces conditioned place aversion [91, 92]. Regarding feeding behavior, stimulation of the LH->VTA connection increases compulsive sucrose consumption, but only stimulation of the GABAergic projection affects caloric consumption [93]. This indicates that the LH->VTA connection is important in reward-seeking behavior, possibly by signaling the drive component of motivation [92]. Currently, the LH is considered an integrator of homeostatic and reward-related information [94, 95], ideally positioned with input from (other) hypothalamic regions [96-98] and intermediate nuclei such as NAc and amygdala [99-101]. The homeostatic input includes nutrient information, such as fat and sugar, that have been shown to affect neurotransmission within the LH [102-104].

Subpopulations within the LH and their role in caloric consumption and reward

Overarching the more than 30 different subpopulations in the LH, which likely differ in connectivity and function, are populations of neurons defined by releasing the neurotransmitters GABA or glutamate [80]. These GABAergic and glutamatergic populations seem to have opposing roles in regulating caloric consumption: inhibition of LH^{GABA} neurons decreases caloric consumption and reduces motivation to work for a food reward [105], while ablation of LH^{Glut} neurons increases caloric consumption and weight gain [106]. In contrast, stimulation of LH^{GABA} neuronal activity increased caloric consumption [107], but also consummatory behavior of non-caloric or non-relevant stimuli [108]. Focusing on the effects of the specific subpopulations can be counterintuitive and more complex. For example, stimulating glutamatergic orexin or melanin-concentrating hormone (MCH) subpopulations drives feeding [109-115], while stimulation of the overall LH^{Glut} neuron population does the opposite [106]. Similarly, stimulating LH^{GABA} neurons, including GABAergic galanin-expressing neurons [116], drives feeding [105, 108], while stimulation of GABAergic neurotensin neurons reduces feeding [95, 117]. Unraveling how molecularly defined subpopulations in the LH respond to dietary factors will advance the field in understanding this complex and heterogeneous brain region.

The lateral habenula (LHb)

Anatomy, input and output

The habenula is an evolutionary well-conserved bilateral small epithalamic nucleus [118-120]. It can be divided in a lateral and medial part with differences in anatomy and gene expression [121-126]. The lateral habenula (LHb) contains mostly glutamatergic neurons, and some sparse GABAergic neurons [122, 127], which exhibit spontaneous activity in a circadian pattern with increased activity during the active phase [128-130]. The LHb acts as a hub between the limbic forebrain and midbrain structures, integrating cognitive, emotional and sensory processes and regulating monoaminergic nuclei in the midbrain, including dopamine and serotonin [131, 132].

The LHb regulates dopaminergic signaling primarily via direct, glutamatergic projections to both glutamatergic and dopaminergic neurons in the VTA [133]. Additionally, there is a pathway for indirect inhibition, from LHb to GABAergic neurons in the rostromedial tegmental nucleus (RMTg) [134], *e.g.* the tail of the VTA, which in turn inhibits VTA dopaminergic neurons[135]. This connectivity enables the LHb to bidirectionally modulate VTA dopaminergic activity, which is suggested to play a role in avoidance and aversive behaviors [134-139]. In addition to modulation of the dopaminergic system, the LHb projects to the DR and median raphe (MnR) to modulate the serotonergic system either directly or indirectly via the RMTg [135, 139-145]. In turn, the DR and MnR send reciprocal projections back to the LHb [146-148]. In the rat, individual LHb neurons project to either serotonergic or dopaminergic nuclei, and only a small percentage projects to both [149]. Next to dopamine and serotonin, the habenula also modulates other (non)monoaminergic nuclei, such as norepinephrine and acetylcholine signaling by the lateral and medial habenula respectively [131, 150].

The input to the LHb originates from a wide range of brain regions, including LH [106, 151, 152], basal forebrain [153], endopeduncular nucleus (EPN) (rodent analogue of globus pallidus) [148, 154-156], ventral pallidum [157, 158], (medial) prefrontal cortex (mPFC) [159], amygdala [160, 161] and reciprocal connections from VTA and DR/MnR [146, 162, 163]. Interestingly, various inputs to the LHb have been suggested to co-release GABA and glutamate, and this synaptic GABA/glutamate balance is thought to be important in regulating mood and behavior [151, 164-166]. Together, this widespread input to the LHb provides cognitive, emotional and sensory information to modulate the monoaminergic systems and direct behavior.

Habenula function and its circuitry

Historically, research using primates focused on the LHb encoding a negative reward prediction error. While dopamine neurons encode a reward prediction error, lateral habenula neurons encode a negative reward prediction error, meaning that LHb neurons decrease firing upon reward, and increase activity upon punishment or omission of an expected reward [52]. This negative reward prediction error is most likely encoded by the EPN input to the lateral habenula, a connection that has been shown to be co-releasing GABA and glutamate [154, 164]. The habenular firing pattern reflects a negative regulation of reward processing, which could also include caloric rewards. Interestingly, rodent studies found that the LHb firing pattern is altered when stress is involved. Stress induced a decrease in AMPA receptor transmission leading to synaptic depression, transforming rewarding activity into punishment-like activity [167, 168]. This could explain why the LHb has been associated with a range of psychiatric disorders, especially major depressive disorder [169-171]. Rodent and fish models for depression revealed increased habenular activity [172-174], which could be normalized with the NMDA receptor antagonist ketamine [174, 175]. Furthermore, antidepressant treatment also alters GABA/glutamate co-release in synaptic input onto the LHb [164]. In humans, this hyperactivity of the habenula has also been observed in depressed individuals [176-179]. In therapy-refractory patients, the first successful pilots with deep brain stimulation of the LHb are being performed [180, 181], indicating a causal relation between disturbed habenula activity and affective disorders.

In addition to its role in encoding stress, aversion, negative reward and mood, the LHb is increasingly recognized for its involvement in a wide range of processes, including sleep regulation, pain processing, motor suppression and memory processes (for review, see Hu, Cui [182]). The availability of opto- and chemogenetics, which allow for the precise manipulation of specific connections, has helped unravel the role of LHb input and output in motivated behavior. For example, glutamatergic input from the LH to the LHb is important in aversion, as stimulation or inhibition of this LH->LHb projection induces real-time place preference or aversion, respectively [106, 151]. Furthermore, studies indicate that the LH->LHb connection is involved in pain and voluntary ethanol consumption, and behavior to escape aversive stimuli [152, 183, 184]. The glutamatergic LH neurons are mostly targeting LHb neurons that project to the VTA/RMGg [185], which in turn are reported to synapse predominantly on mPFC projecting VTA neurons [134]. This suggests that a LH -> LHb -> VTA/RMTg -> mPFC circuit might be involved in encoding aversion [134, 185]. The LHb to VTA projection is the main output from the LHb, and has been shown to be stress-responsive in both humans and mice [137, 186]. Optogenetic activation of this specific pathway leads to strongly conditioned aversive behavior, both in an active and passive manner [137]. Another stress-sensitive LHb pathway is the input from the basolateral amygdala (BLA), which is thought to influence depressive behaviors through orexin and MCH receptors [187]. Additionally, projections from the central amygdala (CeA) to the LHb have been shown to affect pain perception or binge-like ethanol consumption [160, 161]. These amygdalar inputs to the LHb are considered potential candidates for transmitting aversive and emotional state information, attracting significant attention in recent research.

Potential role of LHb in negative (palatable) feeding regulation

As discussed previously, the LH is involved in both homeostatic and hedonic feeding behavior, as well as taste information processing. Interestingly, when stimulating LH glutamatergic neurons projecting to the LHb, mice consumed less Ensure®, a highly palatable and highly caloric solution. Thus, the glutamatergic LH->LHb pathway is negatively regulating palatable food intake [106]. Inhibition of this pathway does not change the preference for sweet taste, but does increase tolerance for aversive tastes, signaling a role in aversive taste processing [188, 189]. Finally, this LH->LHb connection has been observed to be modulated by leptin and ghrelin signaling in opposite directions, and is more active during fasted than sated states, together with the RMTg->VTA projection [189, 190]. These reports suggest a role for the LHb in feeding regulation, especially the input originating from the LH. Regarding the LHb in general, stimulation of GABAergic receptors in the LHb did not affect consumption of a standard laboratory diet (chow) or consumption of a sweetened-fat diet, whereas it did promote locomotor activity. On the other hand, μ-opioid stimulation in the LHb increased chow intake, but decreased sweetened-fat intake [191]. For the medial habenula, a role for opioid signaling was observed as well, with increased μ-opioid binding after exposure to palatable food in the rats that gained weight but not in those that did not [192]. Others reported a glutamatergic projection from the basal forebrain to the LHb, which was involved in aversion, but also limited palatable food intake when activated in a fasted state [193]. Recently, neuropeptide Y (NPY) signaling within the LHb and specifically input from the CeA has been reported to regulate stress-induced palatable feeding [194]. In human studies, associations between weight loss and habenula connectivity have been found [195-197]. These recent findings suggest that LHb might play a role in limiting food intake, which makes it a potential therapeutic target for obesity.

Notably, the habenula might also play a role in glucose metabolism, in addition to its role in regulating food intake. Food intake and glucose metabolism are related, as brain circuits not only control food intake to match energy status but also regulate glycaemic levels. Through direct and indirect projections to peripheral organs, the brain can alter glucose production (gluconeogenesis), glucose uptake or the release of glucoregulatory hormones in the circulation [198, 199]. For example, a bilateral lesion of the rat habenula affects cerebral glucose utilization[200]. More recently, it was reported that lesioning the LHb improved glucose metabolism in rats with streptozocin-induced type 2 diabetes by increasing insulin sensitivity and inhibiting gluconeogenesis [201]. The habenula might also be underlying the increased prevalence of diabetes in people who smoke nicotine [202, 203]. The diabetes-associated transcription factor *Tcf7l2* [204-207] mediates increased blood glucose levels upon nicotine exposure [208, 209], and is expressed in the medial habenula [210]. This habenular *Tcf7l2* is thought to be involved in the stimulatory effects of nicotine through a habenula-pancreas axis, emphasizing a potential role of (medial) habenula in a nicotine-induced increase in blood glucose [210]. In a human imaging study, habenula volume was reported to be associated with HbA1c, a long-term glucose marker, in individuals who smoke [211], adding translational weight to the involvement of habenula in glucose regulation. In conclusion, recent work suggests a role for the LHb in the negative regulation of (palatable) feeding behavior, possibly in combination with effects on glucose regulation, although more research is needed to confirm this and study the circuits that could be involved.

The central amygdala in control of caloric intake

One of the possible LHb circuits in the context of (palatable) feeding behavior, is input from the CeA, the last intermediate nucleus that will be discussed in this thesis. The CeA is part of the amygdaloid complex, together with the basolateral nucleus (BLA), which both play a role in conditioned fear and anxiety. The CeA is considered the main amygdalar output nucleus, while BLA is the input nucleus [212]. The CeA is a heterogeneous, striatum-like brain structure[213, 214], consisting mostly of GABAergic neurons and historically well-known for defensive responding, fear conditioning and anxiety [212, 215]. The CeA receives input from feeding-related areas, like the brainstem and PVN [216-218], and gustatory areas like the parabracial nucleus and insular cortex [219, 220]. Additionally, it is part of the reward circuitry receiving dopaminergic input from VTA and SN [221, 222]. This connectivity with gustatory, feeding and reward centers allows the CeA to process salience, valence, sensory input and emotional states and use this information to select actions and drive motivation and decisions [223, 224].

Early lesion studies showed that the CeA is a crucial regulator of food intake [225, 226]. Recent technical developments allow for specific targeting of CeA cell populations expressing various neuropeptides and receptors to study their role in feeding behavior, such as somatostatin (*Sst*), NPY, protein kinase C – delta (*Pkc-δ*), tachykinin 2 (*Tac2*) or neurotensin (*Nts*). These molecularly defined subpopulations of CeA neurons have different effects on feeding behavior.

For example, inhibiting dopamine receptor 1 (Drd1) expressing neurons in the CeA reduces food intake in a food-restricted state [227]. These *Drd1+* neurons consist of 3 different subtypes co-expressing *Sst*, *Nts* or *Tac2*, which do not affect feeding behavior when inhibited individually [227]. However, caloric consumption did increase Fos expression in these CeA *Sst*, *Nts* and *Tac2* neurons [227]. Another population that positively modulates feeding is serotonin 2A receptor (*Htr2a*) neurons, that get activated during feeding and drive caloric intake when activated. These neurons are negative for *Pkc-δ*, show partial overlap with *Sst* neurons and act as positive reinforcers [228]. Moreover, another neuron population involved in promoting food consumption is the CeA neurons expressing prepronociceptin (*Pnoc*), the precursor to orexigenic opioid-like nociceptin. These *Pnoc* neurons partially overlap with *Sst* neurons, but not with other CeA populations like *Nts*, *Tac2* and *Htr2a*[229]. During feeding, *Pnoc* neurons are active, scaled to the caloric content, and they only partially respond to satiety. Inhibition of this *Pnoc* neuronal population reduces selectively high-fat food consumption, which leads to weight loss[229]. Fat intake can also be driven by opioid signaling in the CeA, but only when energy status is low, and not when driven by palatability [230]. In contrast to these CeA neuron populations driving caloric intake, there are also CeA neuron populations involved in caloric intake inhibition. For example, CeA *Pkc-δ*$^{+}$ neurons show increased activity when satiated [227] and can bi-directionally modulate food intake [231]. These neurons reduce feeding when stimulated, and receive feeding-suppressing input from the parabrachial nucleus [220, 231, 232]. Another limiting effect on caloric intake is by pituitary adenylate cyclase activating polypeptide (PACAP) in the CeA. Infusion of PACAP in the CeA reduces food intake, possibly through reducing appetitive motivational drive [233]. In addition, also the insular cortex to CeA pathway acts like a brake on feeding, by reducing food intake when stimulated [219]. Thus, several subpopulations in the CeA can modulate caloric intake, with especially *Pnoc* neurons selectively driving palatable feeding.

In conclusion, specific subpopulations within the CeA can modulate feeding behavior, by changing motivational drive or consummatory behaviors. These often depend on satiety state, as satiety is a large contributor to motivational drive [227, 228, 234, 235]. In addition to direct effects on consummatory behavior, the CeA can increase motivation for reward [236-238] or modulate existing reward values, possibly through an efferent to the ventromedial prefrontal cortex (vmPFC) [238, 239]. Overall, the CeA forms associations between environmental stimuli and outcomes [240, 241], a reward function depending on dopaminergic input from VTA and SN[221, 242]. Similarly to the LHb, the CeA also displays a reward prediction error, with increased Fos expression in CeA neurons after omission of expected rewards [243]. When considering the LHb as a negative regulator of food intake, and that it processes emotional and aversive states, it would be very likely that it receives input from the CeA. Indeed, there have been reports of a CeA to LHb connection, with some mentioning that it is a glutamatergic *Sst+* pathway [160], and others that it is a GABAergic NPY 1 receptor (NPY1R) expressing projection [161]. Chemogenetic inhibition of the GABAergic population to the LHb from the CeA significantly blunted binge-like consumption of 20% EtOH and did not blunt the binge-like consumption of 3% sucrose [161]. Interestingly, a recent study reported that there is a NPY1R expressing CeA->LHb pathway, crucial in stress-induced palatable feeding [194]. In this thesis we focus on *Drd1*+ neurons within the CeA (and BLA), their projections to other brain regions, and their effects on feeding behaviors.

Thesis Outline

The overall aim of this thesis is to study the role of the lateral hypothalamus, (lateral) habenula and an input from the central amygdala to the lateral habenula in caloric consumption and how this affects obesity development (**Figure 1**).

To study mechanisms of caloric intake in relation to obesity, an obesogenic animal model was used that mimics feeding patterns observed in humans with obesity. In **chapter 2** we describe the free-choice high-fat high-sugar (fcHFHS) diet, which is a palatable diet paradigm commonly used in our studies. The fcHFHS diet consists of four diet components: pellets of standard laboratory diet (chow), a bottle of tap water, a bottle of 30% sucrose solution, and a dish with beef tallow. In this review, we stress the importance of the choice component and the advantages of using this diet paradigm to model human obesity and its development. We

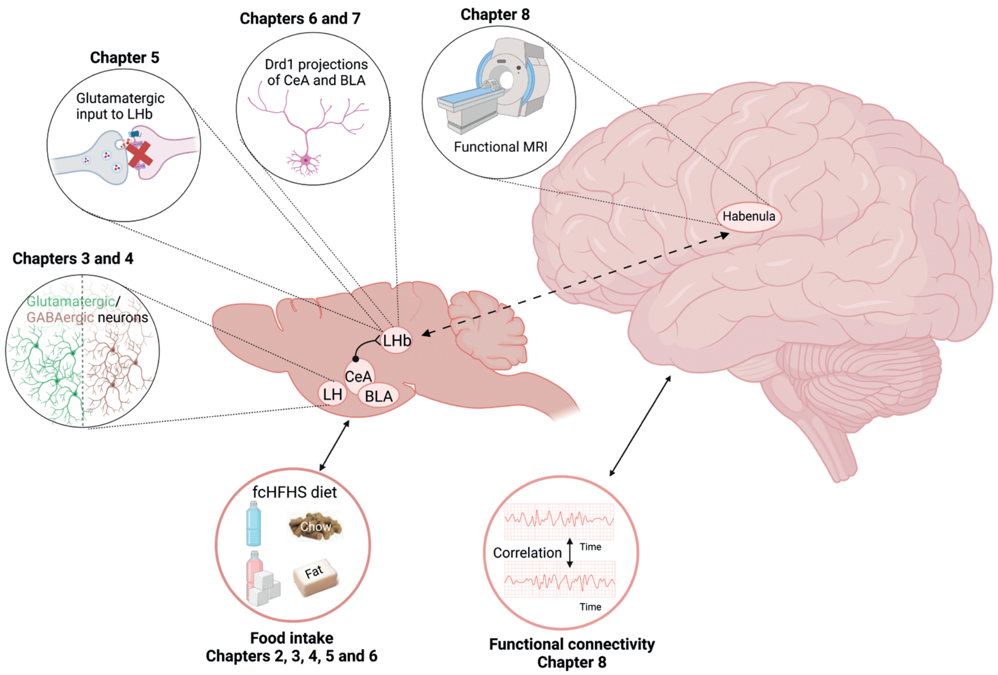

Figure 1. Overview of the content of this thesis. Abbreviations: LHb: lateral habenula, LH: lateral hypothalamus, CeA: central amygdala, BLA: basolateral amygdala, MRI: magnetic resonance imaging, fcHFHS: free-choice high-fat high-sugar, Drd1: dopamine receptor 1.

discuss the implementation of this model, the literature regarding effects on body composition, consummatory and motivational behavior, and (molecular) adaptations in the brain in response to the diet.

In chapter 2 we discuss how fat and sugar intake have interacting effects, which could originate in the LH, as this brain area is involved in the homeostatic regulation of caloric intake and in reward processing including food-motivated behavior. Therefore, in part I, we focus on the effects of fat and sugar consumption on two overarching neuronal populations in the heterogeneous LH: glutamatergic (**chapter 3**) and GABAergic (**chapter 4**) neurons. Using head-fixed two-photon endomicroscopy, we investigate activity of GABAergic and glutamatergic LH neurons upon water and sucrose drinking, in mice on a chow or free-choice high-fat diet (fcHFD). In **chapter 3**, we included a low-calorie sweetened solution, while in **chapter 4** we focused only on fat and sugar interactions.

In part II, we investigate the LHb, a small nucleus well known to be involved in aversion. Recently, it has been suggested that the LHb might also play a role in feeding behavior. In **chapter 5** we describe effects on (palatable) food intake after pharmacological inhibition of glutamatergic input to the LHb. For this, we used an AMPA receptor antagonist (CNQX), a NMDA receptor antagonist (AP5), and both combined.

Two-photon calcium imaging is a state-of-the-art technique used in neuroscience to visualize neural activity patterns. When neurons are active, there is an increase in the intracellular calcium concentration. Genetically encoded calcium indicators (GECIs), like GCaMP, utilize this physiological phenomenon by undergoing conformational changes upon binding with calcium ions (Ca^{2+}). Following viral injection, a GECI is expressed into a selective neuronal population. Then, fluorescence is recorded as a proxy of neural activity, using a head-fixed imaging system. Alternatively, a miniature microscope (miniscope) can be used to allow imaging in freely moving animals. GECIs do not emit light continuously; instead, they emit light at specific wavelengths in response to two-photon excitation, determined by their excitation and emission spectra. A light source, which can be a focused laser or LED, scans the tissue sequentially to construct the image by two-photon excitation. Finally, an imaging device is employed to collect and analyze the resulting fluorescence signal. The complexity of two-photon calcium imaging extends to the subsequent data analysis. The challenge lies in converting the observed visual changes in fluorescence into meaningful neuronal activity metrics. Identifying and extracting source information for each individual neuron – complicated by background noise and movement – is the first step. Other key steps in this process include tracking neurons over time, baseline correction to establish a reference level for fluorescence changes (often expressed as $\Delta F/F$), and, with a sufficiently high sampling rate, estimating the number of action potentials generated by neurons underlying the fluorescent changes. Overall, two-photon calcium imaging offers a powerful means to delve into the dynamics of neural activity, but it requires a comprehensive understanding of both the experimental and analytical aspects to extract meaningful insights from the collected data.

Next, we used a more projection-specific approach, by focusing on a *Drd1+* input to the LHb, originating from the CeA. It is known that *Drd1+* neurons within the CeA can negatively regulate food intake when food-restricted [227]. Indeed, in **chapter 6**, we first identify a *Drd1+* projection from the CeA to the LHb. This amygdalar nucleus is of interest, as the CeA is a known modulator of (palatable) food intake and sensitive to environmental context, and could be regulating food intake in a state-dependent manner through this CeA^{Drd1+} -> LHb pathway. In the same chapter, we use slice electrophysiology to determine whether this newly identified CeA^{Drd1+}->LHb projection is GABAergic or glutamatergic. In addition, we explore behavioral effects of manipulating this CeA^{Drd1+}->LHb projection using chemogenetics, focusing on satiety state specific effects on (palatable) food intake. The LHb is not the only projection area from CeA^{Drd1+} neurons, which we describe in more detail in **chapter 7**, together with projections from *Drd1+* neurons in the basolateral amygdala (BLA).

Finally, since a better mechanistic understanding of obesity is needed to provide better treatment and therapeutic targets, we included a translational study using functional magnetic resonance imaging (fMRI) data from the Human Connectome Project [244]. As specific inputs to the habenula affect caloric consumption, we hypothesized that resting state functional connectivity (rs-FC) of the habenula might be altered in obesity. In **chapter 8,** we investigate the relation between habenular rs-FC and BMI for regions of interest (ROI) related to reward and homeostatic brain circuits. As the habenula has also been suggested in glucose regulation

Magnetic resonance imaging (MRI) is a powerful and non-invasive technique used in humans both in research and clinical settings, but also in rodent and primate research. **Functional MRI** (fMRI) is a valuable tool to investigate patterns of brain activity and is based on the concept of increased blood flow towards active brain regions. Using the differential magnetic susceptibility of oxygenated and deoxygenated hemoglobin in the blood, their relative levels can be assessed, resulting in a **blood-oxygen level dependent (BOLD) signal.** This BOLD response is an indirect proxy for neural activity, and can be measured during rest, during a task, or following a stimulus. Using fMRI, **functional connectivity (FC)** can be determined, which is a measure of correlation between time series of BOLD signals of different brain areas, providing insights into the communication between these areas. When determined in rest, it is called resting state FC (rs-FC). The rsFC can be determined within one subject in different conditions, or across several individuals to find patterns or associations between the group rsFC and certain phenotypes or (cognitive) processes in a cross-sectional analysis. Importantly, FC cannot be used to express causal relationships, or directionality. Moreover, the fact that two regions are functionally connected does not imply that they are also structurally connected via white matter bundles. Using preclinical and more invasive methods, typically performed in rodents, allows us to determine directionality of structural and functional connections and manipulate specific projections to record subsequent effects on behavior. Therefore, for causality and specificity in terms of gene expression, circuits, or subregions, animal research is required, while fMRI is a highly valuable non-invasive tool for translational and clinical research.

in rodents, an exploratory analysis including a possible interaction with glucose regulation was also performed. Finally, due to its role in encoding a negative RPE, we determined the habenula blood-oxygen level dependent (BOLD) response during monetary punishment in a gambling task. We hypothesized that the BOLD response of the habenula upon punishment was altered in subjects with obesity.

References

1. Afshin, A., et al., *Health effects of overweight and obesity in 195 countries over 25 years.* New England journal of medicine, 2017. **377**(1): p. 13-27.
2. Bhurosy, T. and R. Jeewon, *Overweight and obesity epidemic in developing countries: a problem with diet, physical activity, or socioeconomic status?* The Scientific World Journal, 2014. **2014**.
3. Petersen, M.C. and G.I. Shulman, *Mechanisms of insulin action and insulin resistance.* Physiological reviews, 2018. **98**(4): p. 2133-2223.
4. Collaboration, E.R.F., *Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies.* The lancet, 2010. **375**(9733): p. 2215-2222.
5. Marti, A., et al., *Genes, lifestyles and obesity.* International Journal of Obesity, 2004. **28**(3): p. S29-S36.
6. Hu, F.B., *Sedentary lifestyle and risk of obesity and type 2 diabetes.* Lipids, 2003. **38**(2): p. 103-108.
7. Park, J.H., et al., *Sedentary lifestyle: overview of updated evidence of potential health risks.* Korean journal of family medicine, 2020. **41**(6): p. 365.
8. Dallman, M.F., *Stress-induced obesity and the emotional nervous system.* Trends in Endocrinology & Metabolism, 2010. **21**(3): p. 159-165.
9. Kyrou, I., G.P. Chrousos, and C. Tsigos, *Stress, visceral obesity, and metabolic complications.* Annals of the New York Academy of Sciences, 2006. **1083**(1): p. 77-110.
10. Ayyad, C. and T. Andersen, *Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999.* Obesity reviews, 2000. **1**(2): p. 113-119.
11. Nagai, K., et al., *Effect of bilateral lesions of the suprachiasmatic nuclei on the circadian rhythm of food-intake.* Brain research, 1978. **142**(2): p. 384-389.
12. Stoynev, A.G., O.C. Ikonomov, and K.G. Usunoff, *Feeding pattern and light-dark variations in water intake and renal excretion after suprachiasmatic nuclei lesions in rats.* Physiology & Behavior, 1982. **29**(1): p. 35-40.
13. Challet, E., *The circadian regulation of food intake.* Nature Reviews Endocrinology, 2019. **15**(7): p. 393-405.
14. Opperhuizen, A.L., et al., *Feeding during the resting phase causes profound changes in physiology and desynchronization between liver and muscle rhythms of rats.* European Journal of Neuroscience, 2016. **44**(10): p. 2795-2806.
15. De Goede, P., et al., *Time-restricted feeding improves glucose tolerance in rats, but only when in line with the circadian timing system.* Frontiers in endocrinology, 2019. **10**: p. 554.
16. de Goede, P., et al., *After-effects of time-restricted feeding on whole-body metabolism and gene expression in four different peripheral tissues.* Obesity, 2020. **28**: p. S68-S80.
17. Garaulet, M. and P. Gómez-Abellán, *Timing of food intake and obesity: a novel association.* Physiology & behavior, 2014. **134**: p. 44-50.
18. Antunes, L.d.C., et al., *Obesity and shift work: chronobiological aspects.* Nutrition research reviews, 2010. **23**(1): p. 155-168.
19. Williams, G., et al., *The hypothalamus and the control of energy homeostasis: different circuits, different purposes.* Physiology & behavior, 2001. **74**(4-5): p. 683-701.
20. Myers, M.G., M.A. Cowley, and H. Münzberg, *Mechanisms of leptin action and leptin resistance.* Annu. Rev. Physiol., 2008. **70**: p. 537-556.
21. Clegg, D.J., et al., *Consumption of a high-fat diet induces central insulin resistance independent of adiposity.* Physiology & behavior, 2011. **103**(1): p. 10-16.
22. Berridge, K.C., *'Liking'and 'wanting'food rewards: brain substrates and roles in eating disorders.* Physiology & behavior, 2009. **97**(5): p. 537-550.
23. Berthoud, H.-R., *Metabolic and hedonic drives in the neural control of appetite: who is the boss?* Current opinion in neurobiology, 2011. **21**(6): p. 888-896.
24. Berthoud, H.-R. and C. Morrison, *The brain, appetite, and obesity.* Annu. Rev. Psychol., 2008. **59**: p. 55-92.
25. DiFeliceantonio, A.G., et al., *Supra-additive effects of combining fat and carbohydrate on food reward.* Cell metabolism, 2018. **28**(1): p. 33-44. e3.
26. Van De Giessen, E., et al., *Free-choice and no-choice high-fat diets affect striatal dopamine D2/3 receptor availability, caloric intake, and adiposity.* Obesity, 2012. **20**(8): p. 1738-1740.
27. Rodríguez, E.M., J.L. Blázquez, and M. Guerra, *The design of barriers in the hypothalamus allows the median eminence and the arcuate nucleus to enjoy private milieus: the former opens to the portal blood and the latter to the cerebrospinal fluid.* Peptides, 2010. **31**(4): p. 757-776.
28. Valassi, E., M. Scacchi, and F. Cavagnini, *Neuroendocrine control of food intake.* Nutrition, metabolism and cardiovascular diseases, 2008. **18**(2): p. 158-168.
29. Schwartz, M.W., et al., *Central nervous system control of food intake.* Nature, 2000. **404**(6778): p. 661-671.
30. Blevins, J.E. and D.G. Baskin, *Hypothalamic-brainstem circuits controlling eating.* Frontiers in eating and weight regulation, 2010. **63**: p. 133-140.
31. Havrankova, J., M. Brownstein, and J. Roth, *Insulin and insulin receptors in rodent brain.* Diabetologia, 1981. **20**: p. 268-273.
32. Moriguchi, T., et al., *Neurons containing orexin in the lateral hypothalamic area of the adult rat brain are activated by insulin-induced acute hypoglycemia.* Neuroscience letters, 1999. **264**(1-3): p. 101-104.
33. Leinninger, G.M., et al., *Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding.* Cell metabolism, 2009. **10**(2): p. 89-98.
34. Elmquist, J.K., et al., *Distributions of leptin receptor mRNA isoforms in the rat brain.* Journal of Comparative Neurology, 1998. **395**(4): p. 535-547.
35. Yamanaka, A., et al., *Hypothalamic orexin neurons regulate arousal according to energy balance in mice.* Neuron, 2003. **38**(5): p. 701-713.
36. Oomura, Y., et al., *Glucose and osmosensitive neurones of the rat hypothalamus.* Nature, 1969. **222**(5190): p. 282-284.
37. Figlewicz, D., et al., *Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat.* Brain research, 2003. **964**(1): p. 107-115.
38. Figlewicz, D.P., *Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat: historical perspective.* Brain research, 2016. **1645**: p. 68-70.
39. Cone, J.J., J.E. McCutcheon, and M.F. Roitman, *Ghrelin acts as an interface between physiological state and phasic dopamine signaling.* Journal of Neuroscience, 2014. **34**(14): p. 4905-4913.
40. Skibicka, K.P., et al., *Ghrelin directly targets the ventral tegmental area to increase food motivation.* Neuroscience, 2011. **180**: p. 129-137.
41. Berridge, K.C., *The debate over dopamine's role in reward: the case for incentive salience.* Psychopharmacology, 2007. **191**: p. 391-431.
42. Berridge, K.C. and T.E. Robinson, *Parsing reward.* Trends in neurosciences, 2003. **26**(9): p. 507-513.
43. Berridge, K.C. and T.E. Robinson, *What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?* Brain research reviews, 1998. **28**(3): p. 309-369.
44. Berridge, K.C., T.E. Robinson, and J.W. Aldridge, *Dissecting components of reward:'liking','wanting', and learning.* Current opinion in pharmacology, 2009. **9**(1): p. 65-73.
45. Berke, J.D., *What does dopamine mean?* Nature neuroscience, 2018. **21**(6): p. 787-793.

46. Fields, H.L., et al., *Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement.* Annu. Rev. Neurosci., 2007. **30**: p. 289-316.
47. Arias-Carrión, O., et al., *Dopaminergic reward system: a short integrative review.* International archives of medicine, 2010. **3**(1): p. 1-6.
48. Baik, J.-H., *Dopamine signaling in reward-related behaviors.* Frontiers in neural circuits, 2013. **7**: p. 152.
49. Kreitzer, A.C. and R.C. Malenka, *Striatal plasticity and basal ganglia circuit function.* Neuron, 2008. **60**(4): p. 543-554.
50. Rossi, M.A. and G.D. Stuber, *Overlapping brain circuits for homeostatic and hedonic feeding.* Cell metabolism, 2018. **27**(1): p. 42-56.
51. Stuber, G.D. and R.A. Wise, *Lateral hypothalamic circuits for feeding and reward.* Nature neuroscience, 2016. **19**(2): p. 198-205.
52. Matsumoto, M. and O. Hikosaka, *Lateral habenula as a source of negative reward signals in dopamine neurons.* Nature, 2007. **447**(7148): p. 1111.
53. Ji, H. and P.D. Shepard, *Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABAA receptor-mediated mechanism.* Journal of Neuroscience, 2007. **27**(26): p. 6923-6930.
54. Omelchenko, N., R. Bell, and S.R. Sesack, *Lateral habenula projections to dopamine and GABA neurons in the rat ventral tegmental area.* European Journal of Neuroscience, 2009. **30**(7): p. 1239-1250.
55. Grill, H.J. and R. Norgren, *The taste reactivity test. II. Mimetic responses to gustatory stimuli in chronic thalamic and chronic decerebrate rats.* Brain research, 1978. **143**(2): p. 281-297.
56. Berridge, K.C. and M.L. Kringelbach, *Affective neuroscience of pleasure: reward in humans and animals.* Psychopharmacology, 2008. **199**: p. 457-480.
57. Steiner, J.E., et al., *Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates.* Neuroscience & Biobehavioral Reviews, 2001. **25**(1): p. 53-74.
58. Wheeler, R.A. and R.M. Carelli, *The neuroscience of pleasure. Focus on "Ventral pallidum firing codes hedonic reward: when a bad taste turns good".* Journal of Neurophysiology, 2006. **96**(5): p. 2175-2176.
59. Tindell, A.J., et al., *Ventral pallidum firing codes hedonic reward: when a bad taste turns good.* Journal of neurophysiology, 2006. **96**(5): p. 2399-2409.
60. Smith, K.S. and K.C. Berridge, *The ventral pallidum and hedonic reward: neurochemical maps of sucrose "liking" and food intake.* Journal of neuroscience, 2005. **25**(38): p. 8637-8649.
61. Peciña, S. and K.C. Berridge, *Hedonic hot spot in nucleus accumbens shell: where do μ-opioids cause increased hedonic impact of sweetness?* Journal of neuroscience, 2005. **25**(50): p. 11777-11786.
62. Mahler, S.V., K.S. Smith, and K.C. Berridge, *Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking'of a sweet reward.* Neuropsychopharmacology, 2007. **32**(11): p. 2267-2278.
63. Smith, K.S. and K.C. Berridge, *Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum.* Journal of neuroscience, 2007. **27**(7): p. 1594-1605.
64. Berridge, K.C., *Modulation of taste affect by hunger, caloric satiety, and sensory-specific satiety in the rat.* Appetite, 1991. **16**(2): p. 103-120.
65. Leyton, M., *The neurobiology of desire: Dopamine and the regulation of mood and motivational states in humans.* 2010.
66. Tindell, A.J., et al., *Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine.* European Journal of Neuroscience, 2005. **22**(10): p. 2617-2634.
67. Pecina, S., et al., *Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards.* Journal of Neuroscience, 2003. **23**(28): p. 9395-9402.

68. Schultz, W., P. Apicella, and T. Ljungberg, *Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task.* Journal of neuroscience, 1993. **13**(3): p. 900-913.
69. Montague, P.R., P. Dayan, and T.J. Sejnowski, *A framework for mesencephalic dopamine systems based on predictive Hebbian learning.* Journal of neuroscience, 1996. **16**(5): p. 1936-1947.
70. Grove, J.C., et al., *Dopamine subsystems that track internal states.* Nature, 2022. **608**(7922): p. 374-380.
71. Wise, R.A., *Role of brain dopamine in food reward and reinforcement.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2006. **361**(1471): p. 1149-1158.
72. Sclafani, A., *Learned controls of ingestive behaviour.* Appetite, 1997. **29**(2): p. 153-158.
73. Figlewicz, D.P. and A.J. Sipols, *Energy regulatory signals and food reward.* Pharmacology Biochemistry and Behavior, 2010. **97**(1): p. 15-24.
74. Tomiyama, A.J., *Stress and obesity.* Annual review of psychology, 2019. **70**: p. 703-718.
75. Yau, Y.H. and M.N. Potenza, *Stress and eating behaviors.* Minerva endocrinologica, 2013. **38**(3): p. 255.
76. Voigt, J.-P. and H. Fink, *Serotonin controlling feeding and satiety.* Behavioural brain research, 2015. **277**: p. 14-31.
77. Wallin, M.S. and A. Rissanen, *Food and mood: relationship between food, serotonin and affective disorders.* Acta Psychiatrica Scandinavica, 1994. **89**: p. 36-40.
78. Cools, R., K. Nakamura, and N.D. Daw, *Serotonin and dopamine: unifying affective, activational, and decision functions.* Neuropsychopharmacology, 2011. **36**(1): p. 98.
79. Van de Giessen, E.M., *Neurobiological aspects of obesity: dopamine, serotonin, and imaging*. 2012.
80. Mickelsen, L.E., et al., *Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons.* Nature neuroscience, 2019. **22**(4): p. 642-656.
81. Kennedy, G., *The hypothalamic control of food intake in rats.* Proceedings of the Royal Society of London. Series B-Biological Sciences, 1950. **137**(889): p. 535-549.
82. Anand, B.K. and J.R. Brobeck, *Hypothalamic control of food intake in rats and cats.* The Yale journal of biology and medicine, 1951. **24**(2): p. 123.
83. Anand, B.K. and J.R. Brobeck, *Localization of a "feeding center" in the hypothalamus of the rat.* Proceedings of the society for experimental biology and Medicine, 1951. **77**(2): p. 323-325.
84. Olds, J., *A preliminary mapping of electrical reinforcing effects in the rat brain.* Journal of Comparative and Physiological Psychology, 1956. **49**(3): p. 281.
85. Olds, J. and P. Milner, *Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain.* Journal of comparative and physiological psychology, 1954. **47**(6): p. 419.
86. Hoebel, B.G. and P. Teitelbaum, *Hypothalamic control of feeding and self-stimulation.* Science, 1962. **135**(3501): p. 375-377.
87. Margules, D. and J. Olds, *Identical" feeding" and" rewarding" systems in the lateral hypothalamus of rats.* Science, 1962. **135**(3501): p. 374-375.
88. Bower, G.H. and N.E. Miller, *Rewarding and punishing effects from stimulating the same place in the rat's brain.* Journal of Comparative and Physiological Psychology, 1958. **51**(6): p. 669.
89. Mendelson, J. and W.J. Freed, *Do rats terminate hypothalamic stimulation only in order to turn it on again?* Behavioral Biology, 1973. **8**(5): p. 619-628.
90. Barbano, M.F., et al., *Feeding and reward are differentially induced by activating GABAergic lateral hypothalamic projections to VTA.* Journal of Neuroscience, 2016. **36**(10): p. 2975-2985.
91. Nieh, E.H., et al., *Inhibitory input from the lateral hypothalamus to the ventral tegmental area disinhibits dopamine neurons and promotes behavioral activation.* Neuron, 2016. **90**(6): p. 1286-1298.

92. Tyree, S.M. and L. de Lecea, *Lateral hypothalamic control of the ventral tegmental area: reward evaluation and the driving of motivated behavior.* Frontiers in systems neuroscience, 2017. **11**: p. 50.
93. Nieh, E.H., et al., *Decoding neural circuits that control compulsive sucrose seeking.* Cell, 2015. **160**(3): p. 528-541.
94. Morales, I., *Brain regulation of hunger and motivation: The case for integrating homeostatic and hedonic concepts and its implications for obesity and addiction.* Appetite, 2022. **177**: p. 106146.
95. Petzold, A., et al., *Complementary lateral hypothalamic populations resist hunger pressure to balance nutritional and social needs.* Cell Metabolism, 2023. **35**(3): p. 456-471. e6.
96. Elias, C.F., et al., *Chemically defined projections linking the mediobasal hypothalamus and the lateral hypothalamic area.* Journal of comparative neurology, 1998. **402**(4): p. 442-459.
97. Broberger, C., et al., *Hypocretin/orexin-and melanin-concentrating hormone-expressing cells form distinct populations in the rodent lateral hypothalamus: relationship to the neuropeptide Y and agouti gene-related protein systems.* Journal of Comparative Neurology, 1998. **402**(4): p. 460-474.
98. Wu, Z., et al., *GABAergic projections from lateral hypothalamus to paraventricular hypothalamic nucleus promote feeding.* Journal of Neuroscience, 2015. **35**(8): p. 3312-3318.
99. Heimer, L., et al., *Specificity in the projection patterns of accumbal core and shell in the rat.* Neuroscience, 1991. **41**(1): p. 89-125.
100. Mogenson, G., L. Swanson, and M. Wu, *Neural projections from nucleus accumbens to globus pallidus, substantia innominata, and lateral preoptic-lateral hypothalamic area: an anatomical and electrophysiological investigation in the rat.* Journal of Neuroscience, 1983. **3**(1): p. 189-202.
101. Reppucci, C.J. and G.D. Petrovich, *Organization of connections between the amygdala, medial prefrontal cortex, and lateral hypothalamus: a single and double retrograde tracing study in rats.* Brain Structure and Function, 2016. **221**: p. 2937-2962.
102. Rossi, M.A., et al., *Obesity remodels activity and transcriptional state of a lateral hypothalamic brake on feeding.* Science, 2019. **364**(6447): p. 1271-1274.
103. Garcia, A., et al., *Lateral Hypothalamic GABAergic neurons encode and potentiate sucrose's palatability.* Frontiers in neuroscience, 2021. **14**: p. 608047.
104. Adermark, L., et al., *Weight gain and neuroadaptations elicited by high fat diet depend on fatty acid composition.* Psychoneuroendocrinology, 2021. **126**: p. 105143.
105. Jennings, J.H., et al., *Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors.* Cell, 2015. **160**(3): p. 516-527.
106. Stamatakis, A.M., et al., *Lateral hypothalamic area glutamatergic neurons and their projections to the lateral habenula regulate feeding and reward.* Journal of Neuroscience, 2016. **36**(2): p. 302-311.
107. Jennings, J.H., et al., *The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding.* Science, 2013. **341**(6153): p. 1517-1521.
108. Navarro, M., et al., *Lateral hypothalamus GABAergic neurons modulate consummatory behaviors regardless of the caloric content or biological relevance of the consumed stimuli.* Neuropsychopharmacology, 2016. **41**(6): p. 1505-1512.
109. Inutsuka, A., et al., *Concurrent and robust regulation of feeding behaviors and metabolism by orexin neurons.* Neuropharmacology, 2014. **85**: p. 451-460.
110. Sartin, J., et al., *Effect of intracerebroventricular orexin-B on food intake in sheep.* Journal of animal science, 2001. **79**(6): p. 1573-1577.
111. Kokare, D.M., et al., *GABAA receptors mediate orexin-A induced stimulation of food intake.* Neuropharmacology, 2006. **50**(1): p. 16-24.
112. Dilsiz, P., et al., *MCH neuron activity is sufficient for reward and reinforces feeding.* Neuroendocrinology, 2020. **110**(3-4): p. 258-270.

113. Ludwig, D.S., et al., *Melanin-concentrating hormone overexpression in transgenic mice leads to obesity and insulin resistance.* The Journal of clinical investigation, 2001. **107**(3): p. 379-386.
114. Noble, E.E., et al., *Control of feeding behavior by cerebral ventricular volume transmission of melanin-concentrating hormone.* Cell metabolism, 2018. **28**(1): p. 55-68. e7.
115. Shimada, M., et al., *Mice lacking melanin-concentrating hormone are hypophagic and lean.* Nature, 1998. **396**(6712): p. 670-674.
116. Qualls-Creekmore, E., et al., *Galanin-expressing GABA neurons in the lateral hypothalamus modulate food reward and noncompulsive locomotion.* Journal of Neuroscience, 2017. **37**(25): p. 6053-6065.
117. Woodworth, H.L., et al., *Lateral hypothalamic neurotensin neurons orchestrate dual weight loss behaviors via distinct mechanisms.* Cell reports, 2017. **21**(11): p. 3116-3128.
118. Bianco, I.H. and S.W. Wilson, *The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain.* Philosophical Transactions of the Royal Society B: Biological Sciences, 2009. **364**(1519): p. 1005-1020.
119. Agetsuma, M., et al., *The habenula is crucial for experience-dependent modification of fear responses in zebrafish.* Nature neuroscience, 2010. **13**(11): p. 1354-1356.
120. Stephenson-Jones, M., et al., *Evolutionary conservation of the habenular nuclei and their circuitry controlling the dopamine and 5-hydroxytryptophan (5-HT) systems.* Proceedings of the National Academy of Sciences, 2012. **109**(3): p. E164-E173.
121. Marburg, O., *The structure and fiber connections of the human habenula.* Journal of Comparative Neurology, 1944. **80**(2): p. 211-233.
122. Aizawa, H., et al., *Molecular characterization of the subnuclei in rat habenula.* Journal of Comparative Neurology, 2012. **520**(18): p. 4051-4066.
123. Geisler, S., K.H. Andres, and R.W. Veh, *Morphologic and cytochemical criteria for the identification and delineation of individual subnuclei within the lateral habenular complex of the rat.* Journal of Comparative Neurology, 2003. **458**(1): p. 78-97.
124. Wagner, F., T. Stroh, and R.W. Veh, *Correlating habenular subnuclei in rat and mouse by using topographic, morphological, and cytochemical criteria.* Journal of Comparative Neurology, 2014. **522**(11): p. 2650-2662.
125. Weiss, T. and R. Veh, *Morphological and electrophysiological characteristics of neurons within identified subnuclei of the lateral habenula in rat brain slices.* Neuroscience, 2011. **172**: p. 74-93.
126. Andres, K.H., M.V. Düring, and R.W. Veh, *Subnuclear organization of the rat habenular complexes.* Journal of Comparative Neurology, 1999. **407**(1): p. 130-150.
127. Lecourtier, L. and P.H. Kelly, *A conductor hidden in the orchestra? Role of the habenular complex in monoamine transmission and cognition.* Neuroscience & Biobehavioral Reviews, 2007. **31**(5): p. 658-672.
128. Zhao, H. and B. Rusak, *Circadian firing-rate rhythms and light responses of rat habenular nucleus neurons in vivo and in vitro.* Neuroscience, 2005. **132**(2): p. 519-528.
129. Guilding, C., A. Hughes, and H. Piggins, *Circadian oscillators in the epithalamus.* Neuroscience, 2010. **169**(4): p. 1630-1639.
130. Baltazar, R.M., L.M. Coolen, and I.C. Webb, *Diurnal rhythms in neural activation in the mesolimbic reward system: critical role of the medial prefrontal cortex.* European Journal of Neuroscience, 2013. **38**(2): p. 2319-2327.
131. Hikosaka, O., et al., *Habenula: crossroad between the basal ganglia and the limbic system.* Journal of Neuroscience, 2008. **28**(46): p. 11825-11829.
132. Sutherland, R.J.J.N. and B. Reviews, *The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex.* 1982. **6**(1): p. 1-13.
133. Brown, P.L. and P.D. Shepard, *Functional evidence for a direct excitatory projection from the lateral habenula to the ventral tegmental area in the rat.* Journal of neurophysiology, 2016. **116**(3): p. 1161-1174.

134. Lammel, S., et al., *Input-specific control of reward and aversion in the ventral tegmental area.* Nature, 2012. **491**(7423): p. 212-217.

135. Jhou, T.C., et al., *The mesopontine rostromedial tegmental nucleus: a structure targeted by the lateral habenula that projects to the ventral tegmental area of Tsai and substantia nigra compacta.* Journal of Comparative Neurology, 2009. **513**(6): p. 566-596.

136. Hong, S., et al., *Negative reward signals from the lateral habenula to dopamine neurons are mediated by rostromedial tegmental nucleus in primates.* Journal of Neuroscience, 2011. **31**(32): p. 11457-11471.

137. Stamatakis, A.M. and G.D. Stuber, *Activation of lateral habenula inputs to the ventral midbrain promotes behavioral avoidance.* Nature neuroscience, 2012. **15**(8): p. 1105.

138. Namboodiri, V.M.K., J. Rodriguez-Romaguera, and G.D. Stuber, *The habenula.* Current Biology, 2016. **26**(19): p. R873-R877.

139. Herkenham, M. and W.J. Nauta, *Efferent connections of the habenular nuclei in the rat.* Journal of Comparative Neurology, 1979. **187**(1): p. 19-47.

140. Wang, R.Y. and G.K. Aghajanian, *Physiological evidence for habenula as major link between forebrain and midbrain raphe.* Science, 1977. **197**(4298): p. 89-91.

141. Quina, L.A., et al., *Efferent pathways of the mouse lateral habenula.* Journal of Comparative Neurology, 2015. **523**(1): p. 32-60.

142. Araki, M., P.L. McGeer, and H. Kimura, *The efferent projections of the rat lateral habenular nucleus revealed by the PHA-L anterograde tracing method.* Brain research, 1988. **441**(1-2): p. 319-330.

143. Behzadi, G., et al., *Afferents to the median raphe nucleus of the rat: retrograde cholera toxin and wheat germ conjugated horseradish peroxidase tracing, and selectived-[3H] aspartate labelling of possible excitatory amino acid inputs.* Neuroscience, 1990. **37**(1): p. 77-100.

144. Peyron, C., et al., *Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods.* Neuroscience, 1997. **82**(2): p. 443-468.

145. Sego, C., et al., *Lateral habenula and the rostromedial tegmental nucleus innervate neurochemically distinct subdivisions of the dorsal raphe nucleus in the rat.* Journal of Comparative Neurology, 2014. **522**(7): p. 1454-1484.

146. Vertes, R.P., W.J. Fortin, and A.M. Crane, *Projections of the median raphe nucleus in the rat.* Journal of comparative neurology, 1999. **407**(4): p. 555-582.

147. Xie, G., et al., *Serotonin modulates glutamatergic transmission to neurons in the lateral habenula.* Scientific reports, 2016. **6**(1): p. 1-11.

148. Shabel, S.J., et al., *Input to the lateral habenula from the basal ganglia is excitatory, aversive, and suppressed by serotonin.* Neuron, 2012. **74**(3): p. 475-481.

149. Bernard, R. and R.W. Veh, *Individual neurons in the rat lateral habenular complex project mostly to the dopaminergic ventral tegmental area or to the serotonergic raphe nuclei.* Journal of Comparative Neurology, 2012. **520**(11): p. 2545-2558.

150. Klemm, W.R., *Habenular and interpeduncularis nuclei: shared components in multiple-function networks.* Medical science monitor: international medical journal of experimental and clinical research, 2004. **10**(11): p. RA261-73.

151. Lazaridis, I., et al., *A hypothalamus-habenula circuit controls aversion.* Molecular psychiatry, 2019: p. 1.

152. Lecca, S., et al., *Aversive stimuli drive hypothalamus-to-habenula excitation to promote escape behavior.* Elife, 2017. **6**: p. e30697.

153. Golden, S.A., et al., *Basal forebrain projections to the lateral habenula modulate aggression reward.* Nature, 2016. **534**(7609): p. 688-692.

154. Hong, S. and O. Hikosaka, *The globus pallidus sends reward-related signals to the lateral habenula.* Neuron, 2008. **60**(4): p. 720-729.

155. Wallace, M.L., et al., *Genetically distinct parallel pathways in the entopeduncular nucleus for limbic and sensorimotor output of the basal ganglia.* Neuron, 2017. **94**(1): p. 138-152. e5.

156. Li, H., D. Pullmann, and T.C. Jhou, *Valence-encoding in the lateral habenula arises from the entopeduncular region.* Elife, 2019. **8**: p. e41223.

157. Knowland, D., et al., *Distinct ventral pallidal neural populations mediate separate symptoms of depression.* Cell, 2017. **170**(2): p. 284-297. e18.

158. Tooley, J., et al., *Glutamatergic ventral pallidal neurons modulate activity of the habenula–tegmental circuitry and constrain reward seeking.* Biological psychiatry, 2018. **83**(12): p. 1012-1023.

159. Lin, S., et al., *The ATP level in the medial prefrontal cortex regulates depressive-like behavior via the medial prefrontal cortex-lateral habenula pathway.* Biological Psychiatry, 2022. **92**(3): p. 179-192.

160. Zhou, W., et al., *A neural circuit for comorbid depressive symptoms in chronic pain.* Nature neuroscience, 2019. **22**(10): p. 1649-1658.

161. Companion, M.A., et al., *Lateral habenula-projecting central amygdala circuits expressing GABA and NPY Y1 receptor modulate binge-like ethanol intake in mice.* Addiction neuroscience, 2022. **3**: p. 100019.

162. Root, D.H., et al., *Role of glutamatergic projections from ventral tegmental area to lateral habenula in aversive conditioning.* Journal of Neuroscience, 2014. **34**(42): p. 13906-13910.

163. Stamatakis, A.M., et al., *A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward.* Neuron, 2013. **80**(4): p. 1039-1053.

164. Shabel, S.J., et al., *GABA/glutamate co-release controls habenula output and is modified by antidepressant treatment.* Science, 2014. **345**(6203): p. 1494-1498.

165. Meye, F.J., et al., *Shifted pallidal co-release of GABA and glutamate in habenula drives cocaine withdrawal and relapse.* Nature neuroscience, 2016. **19**(8): p. 1019-1024.

166. Barker, D.J., et al., *Lateral preoptic control of the lateral habenula through convergent glutamate and GABA transmission.* Cell reports, 2017. **21**(7): p. 1757-1769.

167. Shabel, S.J., et al., *Stress transforms lateral habenula reward responses into punishment signals.* Proceedings of the National Academy of Sciences, 2019. **116**(25): p. 12488-12493.

168. Nuno-Perez, A., et al., *Stress undermines reward-guided cognitive performance through synaptic depression in the lateral habenula.* Neuron, 2021. **109**(6): p. 947-956. e5.

169. Nuno-Perez, A., et al., *Lateral habenula gone awry in depression: bridging cellular adaptations with therapeutics.* Frontiers in neuroscience, 2018. **12**: p. 485.

170. Proulx, C.D., O. Hikosaka, and R. Malinow, *Reward processing by the lateral habenula in normal and depressive behaviors.* Nature neuroscience, 2014. **17**(9): p. 1146.

171. Yang, Y., et al., *Lateral habenula in the pathophysiology of depression.* Current opinion in neurobiology, 2018. **48**: p. 90-96.

172. Caldecott-Hazard, S., J. Mazziotta, and M. Phelps, *Cerebral correlates of depressed behavior in rats, visualized using 14C-2-deoxyglucose autoradiography.* Journal of Neuroscience, 1988. **8**(6): p. 1951-1961.

173. Mirrione, M.M., et al., *Increased metabolic activity in the septum and habenula during stress is linked to subsequent expression of learned helplessness behavior.* Frontiers in human neuroscience, 2014. **8**: p. 29.

174. Andalman, A.S., et al., *Neuronal dynamics regulating brain and behavioral state transitions.* Cell, 2019. **177**(4): p. 970-985. e20.

175. Yang, Y., et al., *Ketamine blocks bursting in the lateral habenula to rapidly relieve depression.* Nature, 2018. **554**(7692): p. 317.

176. Morris, J., et al., *Covariation of activity in habenula and dorsal raphe nuclei following tryptophan depletion.* Neuroimage, 1999. **10**(2): p. 163-172.

177. Strotmann, B., et al., *Mapping of the internal structure of human habenula with ex vivo MRI at 7T.* Frontiers in human neuroscience, 2013. **7**: p. 878.
178. Schmidt, F.M., et al., *Habenula volume increases with disease severity in unmedicated major depressive disorder as revealed by 7T MRI.* European archives of psychiatry and clinical neuroscience, 2017. **267**(2): p. 107-115.
179. Ely, B.A., et al., *Resting-state functional connectivity of the human habenula in healthy individuals: Associations with subclinical depression.* Human brain mapping, 2016. **37**(7): p. 2369-2384.
180. Sartorius, A., et al., *Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient.* Biological psychiatry, 2010. **67**(2): p. e9-e11.
181. Zhang, C., et al., *Habenula deep brain stimulation for refractory bipolar disorder.* Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation, 2019. **12**(5): p. 1298-1300.
182. Hu, H., Y. Cui, and Y. Yang, *Circuits and functions of the lateral habenula in health and in disease.* Nature Reviews Neuroscience, 2020. **21**(5): p. 277-295.
183. Gu, H.-W., et al., *Contribution of activating lateral hypothalamus-lateral habenula circuit to nerve trauma-induced neuropathic pain in mice.* Neurobiology of Disease, 2023: p. 106155.
184. Sheth, C., et al., *The lateral hypothalamus to lateral habenula projection, but not the ventral pallidum to lateral habenula projection, regulates voluntary ethanol consumption.* Behavioural brain research, 2017. **328**: p. 195-208.
185. Poller, W.C., et al., *A glutamatergic projection from the lateral hypothalamus targets VTA-projecting neurons in the lateral habenula of the rat.* Brain research, 2013. **1507**: p. 45-60.
186. Hennigan, K., K. D'Ardenne, and S.M. McClure, *Distinct midbrain and habenula pathways are involved in processing aversive events in humans.* Journal of Neuroscience, 2015. **35**(1): p. 198-208.
187. Kim, T.-K. and P.-L. Han, *Functional connectivity of basolateral amygdala neurons carrying orexin receptors and melanin-concentrating hormone receptors in regulating sociability and mood-related behaviors.* Experimental neurobiology, 2016. **25**(6): p. 307.
188. Fu, O., et al., *Hypothalamic neuronal circuits regulating hunger-induced taste modification.* Nature communications, 2019. **10**(1): p. 1-14.
189. Rossi, M.A., et al., *Transcriptional and functional divergence in lateral hypothalamic glutamate neurons projecting to the lateral habenula and ventral tegmental area.* Neuron, 2021. **109**(23): p. 3823-3837. e6.
190. Jhou, T.C. and P.J. Vento, *Bidirectional regulation of reward, punishment, and arousal by dopamine, the lateral habenula and the rostromedial tegmentum (RMTg).* Current Opinion in Behavioral Sciences, 2019. **26**: p. 90-96.
191. Carlson, H.N., B.A. Christensen, and W.E. Pratt, *Stimulation of mu opioid, but not GABAergic, receptors of the lateral habenula alters free feeding in rats.* Neuroscience Letters, 2022. **771**: p. 136417.
192. Smith, S.L., J.A. Harrold, and G. Williams, *Diet-induced obesity increases μ opioid receptor binding in specific regions of the rat brain.* Brain research, 2002. **953**(1-2): p. 215-222.
193. Swanson, J.L., et al., *Activation of basal forebrain-to-lateral habenula circuitry drives reflexive aversion and suppresses feeding behavior.* Scientific Reports, 2022. **12**(1): p. 22044.
194. Ip, C.K., et al., *Critical Role of Lateral Habenula Circuits in the Control of Stress-Induced Palatable Food Consumption.* Neuron, 2023. **111**: p. 1-18.
195. Maldonado, M., et al., *The habenula as a novel link between the homeostatic and hedonic pathways in cancer-associated weight loss: a pilot study.* Journal of cachexia, sarcopenia and muscle, 2018. **9**(3): p. 497-504.
196. Wang, J., et al., *Habenular connectivity predict weight loss and negative emotional-related eating behavior after laparoscopic sleeve gastrectomy.* Cerebral Cortex, 2022.
197. Wang, J., et al., *Habenular and mediodorsal thalamic connectivity predict persistent weight loss after laparoscopic sleeve gastrectomy.* Obesity, 2022. **30**(1): p. 172-182.
198. Peters, A., et al., *The neuroendocrine control of glucose allocation.* Experimental and clinical endocrinology & diabetes, 2002. **110**(05): p. 199-211.
199. Carnagarin, R., et al., *Autonomic regulation of glucose homeostasis: a specific role for sympathetic nervous system activation.* Current diabetes reports, 2018. **18**: p. 1-10.
200. Ito, M., M. Kadekaro, and L. Sokoloff, *Effects of lateral habenular lesions on local cerebral glucose utilization in the rat.* Brain research, 1985. **337**(2): p. 245-254.
201. Qu, P., et al., *Habenula lesions improve glucose metabolism in rats with type 2 diabetes by increasing insulin sensitivity and inhibiting gluconeogenesis.* BMJ Open Diabetes Research and Care, 2020. **8**(1): p. e001250.
202. Reitsma, M.B., et al., *Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: a systematic analysis from the Global Burden of Disease Study 2015.* The Lancet, 2017. **389**(10082): p. 1885-1906.
203. Willi, C., et al., *Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis.* Jama, 2007. **298**(22): p. 2654-2664.
204. Weedon, M., *The importance of TCF7L2.* Diabetic Medicine, 2007. **24**(10): p. 1062-1066.
205. del Bosque-Plata, L., et al., *The role of TCF7L2 in type 2 diabetes.* Diabetes, 2021. **70**(6): p. 1220-1228.
206. Zhou, Y., et al., *TCF7L2 is a master regulator of insulin production and processing.* Human molecular genetics, 2014. **23**(24): p. 6419-6431.
207. Cauchi, S. and P. Froguel, *TCF7L2 genetic defect and type 2 diabetes.* Current diabetes reports, 2008. **8**(2): p. 149.
208. Sandberg, H., et al., *The effect of smoking on serum somatotropin, immunoreactive insulin and blood glucose levels of young adult males.* Journal of Pharmacology and Experimental Therapeutics, 1973. **184**(3): p. 787-791.
209. Haggard, H.W. and L.A. Greenberg, *The effects of cigarette smoking upon the blood sugar.* Science, 1934. **79**(2042): p. 165-166.
210. Duncan, A., et al., *Habenular TCF7L2 links nicotine addiction to diabetes.* Nature, 2019. **574**(7778): p. 372-377.
211. Jha, M.K., et al., *Smoking status links habenular volume to glycated hemoglobin: Findings from the Human Connectome Project-Young Adult.* Psychoneuroendocrinology, 2021. **131**: p. 105321.
212. Ehrlich, I., et al., *Amygdala inhibitory circuits and the control of fear memory.* Neuron, 2009. **62**(6): p. 757-771.
213. Fadok, J.P., et al., *New perspectives on central amygdala function.* Current opinion in neurobiology, 2018. **49**: p. 141-147.
214. Gilpin, N.W., M.A. Herman, and M. Roberto, *The central amygdala as an integrative hub for anxiety and alcohol use disorders.* Biological psychiatry, 2015. **77**(10): p. 859-869.
215. Tovote, P., J.P. Fadok, and A. Lüthi, *Neuronal circuits for fear and anxiety.* Nature Reviews Neuroscience, 2015. **16**(6): p. 317-331.
216. Petrovich, G.D. and M. Gallagher, *Amygdala subsystems and control of feeding behavior by learned cues.* Annals of the New York Academy of Sciences, 2003. **985**(1): p. 251-262.
217. Merali, Z., et al., *Aversive and appetitive events evoke the release of corticotropin-releasing hormone and bombesin-like peptides at the central nucleus of the amygdala.* Journal of Neuroscience, 1998. **18**(12): p. 4758-4766.
218. Petrovich, G.D., N.S. Canteras, and L.W. Swanson, *Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems.* Brain research reviews, 2001. **38**(1-2): p. 247-289.
219. Zhang-Molina, C., M.B. Schmit, and H. Cai, *Neural circuit mechanism underlying the feeding controlled by insula-central amygdala pathway.* IScience, 2020. **23**(4).
220. Carter, M.E., et al., *Genetic identification of a neural circuit that suppresses appetite.* Nature, 2013. **503**(7474): p. 111-114.

221. Lee, H.J., D.S. Wheeler, and P.C. Holland, *Interactions between amygdala central nucleus and the ventral tegmental area in the acquisition of conditioned cue-directed behavior in rats.* European Journal of Neuroscience, 2011. **33**(10): p. 1876-1884.

222. Lee, H.J., et al., *Role of substantia nigra–amygdala connections in surprise-induced enhancement of attention.* Journal of Neuroscience, 2006. **26**(22): p. 6077-6081.

223. Warlow, S.M. and K.C. Berridge, *Incentive motivation:'wanting'roles of central amygdala circuitry.* Behavioural brain research, 2021. **411**: p. 113376.

224. Kong, M.-S. and L.S. Zweifel, *Central amygdala circuits in valence and salience processing.* Behavioural brain research, 2021. **410**: p. 113355.

225. Vígh, J., et al., *Bombesin injection into the central amygdala influences feeding behavior in the rat.* Peptides, 1999. **20**(4): p. 437-444.

226. Box, B.M. and G. Mogenson, *Alterations in ingestive behaviors after bilateral lesions of the amygdala in the rat.* Physiology & Behavior, 1975. **15**(6): p. 679-688.

227. Kim, J., et al., *Basolateral to central amygdala neural circuits for appetitive behaviors.* Neuron, 2017. **93**(6): p. 1464-1479. e5.

228. Douglass, A.M., et al., *Central amygdala circuits modulate food consumption through a positive-valence mechanism.* Nature neuroscience, 2017. **20**(10): p. 1384-1394.

229. Hardaway, J.A., et al., *Central amygdala prepronociceptin-expressing neurons mediate palatable food consumption and reward.* Neuron, 2019. **102**(5): p. 1037-1052. e7.

230. Parker, K.E., et al., *Central amygdala opioid transmission is necessary for increased high-fat intake following 24-h food deprivation, but not following intra-accumbens opioid administration.* Behavioural brain research, 2014. **260**: p. 131-138.

231. Cai, H., et al., *Central amygdala PKC-δ+ neurons mediate the influence of multiple anorexigenic signals.* Nature neuroscience, 2014. **17**(9): p. 1240-1248.

232. Campos, C.A., et al., *Parabrachial CGRP neurons control meal termination.* Cell metabolism, 2016. **23**(5): p. 811-820.

233. Iemolo, A., et al., *Pituitary adenylate cyclase-activating peptide in the central amygdala causes anorexia and body weight loss via the melanocortin and the TrkB systems.* Neuropsychopharmacology, 2015. **40**(8): p. 1846-1855.

234. Calu, D.J., et al., *Neural correlates of variations in event processing during learning in central nucleus of amygdala.* Neuron, 2010. **68**(5): p. 991-1001.

235. Holland, P.C., et al., *Amygdala circuitry in attentional and representational processes.* Trends in cognitive sciences, 1999. **3**(2): p. 65-73.

236. Robinson, M.J., S.M. Warlow, and K.C. Berridge, *Optogenetic excitation of central amygdala amplifies and narrows incentive motivation to pursue one reward above another.* Journal of Neuroscience, 2014. **34**(50): p. 16567-16580.

237. Mahler, S.V. and K.C. Berridge, *Which cue to "want?" Central amygdala opioid activation enhances and focuses incentive salience on a prepotent reward cue.* Journal of Neuroscience, 2009. **29**(20): p. 6500-6513.

238. Seo, D.-o., et al., *A GABAergic projection from the centromedial nuclei of the amygdala to ventromedial prefrontal cortex modulates reward behavior.* Journal of Neuroscience, 2016. **36**(42): p. 10831-10842.

239. Warlow, S.M., E.E. Naffziger, and K.C. Berridge, *The central amygdala recruits mesocorticolimbic circuitry for pursuit of reward or pain.* Nature communications, 2020. **11**(1): p. 2716.

240. Gallagher, M., P.W. Graham, and P.C. Holland, *The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior.* Journal of Neuroscience, 1990. **10**(6): p. 1906-1911.

241. McDannald, M., et al., *Amygdala central nucleus function is necessary for learning but not expression of conditioned visual orienting.* European Journal of Neuroscience, 2004. **20**(1): p. 240-248.

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1

242. Lee, H.J., et al., *Role of amygdalo-nigral circuitry in conditioning of a visual stimulus paired with food.* Journal of Neuroscience, 2005. **25**(15): p. 3881-3888.

243. Lee, H.J., M. Gallagher, and P.C. Holland, *The central amygdala projection to the substantia nigra reflects prediction error information in appetitive conditioning.* Learning & Memory, 2010. **17**(10): p. 531-538.

244. Van Essen, D.C., et al., *The WU-Minn human connectome project: an overview.* Neuroimage, 2013. **80**: p. 62-79.