



## Review

# Assessing the role of ghrelin and the enzyme ghrelin O-acyltransferase (GOAT) system in food reward, food motivation, and binge eating behavior

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Exendin-4 (PubChem CID: 16157882)  
Ro60-0175 (PubChem CID: 3045227)  
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NorBNI (PubChem CID: 5480230)  
WIN55,212-2 (PubChem CID: 5311501)  
Tetrahydrocannabinol (PubChem CID: 16078)  
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## ABSTRACT

The peripheral peptide hormone ghrelin is a powerful stimulator of food intake, which leads to body weight gain and adiposity in both rodents and humans. The hormone, thus, increases the vulnerability to obesity and binge eating behavior. Several studies have revealed that ghrelin's functions are due to its interaction with the growth hormone secretagogue receptor type 1a (GHSR1a) in the hypothalamic area; besides, ghrelin also promotes the reinforcing properties of hedonic food, acting at extra-hypothalamic sites and interacting with dopaminergic, cannabinoid, opioid, and orexin signaling. The hormone is primarily present in two forms in the plasma and the enzyme ghrelin O-acyltransferase (GOAT) allows the acylation reaction which causes the transformation of des-acyl-ghrelin (DAG) to the active form acyl-ghrelin (AG). DAG has been demonstrated to show antagonist properties; it is metabolically active, and counteracts the effects of AG on glucose metabolism and lipolysis, and reduces food consumption, body weight, and hedonic feeding response. Both peptides seem to influence the hypothalamic-pituitary-adrenal (HPA) axis and the corticosterone/cortisol level that drive the urge to eat under stressful conditions. These findings suggest that DAG and inhibition of GOAT may be targets for obesity and bingeing-related eating disorders and that AG/DAG ratio may be an important potential biomarker to assess the risk of developing maladaptive eating behaviors.

**Abbreviations:** GHSR1a, growth hormone secretagogue receptor type 1a; GOAT, ghrelin O-acyltransferase; DAG, des-acyl-ghrelin; AG, acyl-ghrelin; HPA, hypothalamic-pituitary-adrenal; GH, growth hormone; GPCR, G protein-coupled receptor; ARC, Arcuate nucleus of the hypothalamus; VTA, ventral tegmental area; NTS, Nucleus of the tractus solitarius; NPY, Neuropeptide Y; AgRP, Agouti-related peptide; ICV, intracerebroventricular; POMC, Pro-opiomelanocortin; MC4R, melanocortin-4 receptor; PVN, paraventricular nucleus of the hypothalamus; HPF, highly palatable foods; BE, binge eating; BN, Bulimia nervosa; AN, Anorexia nervosa; BED, Binge eating disorder; NAc, Nucleus accumbens; PFC, Prefrontal cortex; CPP, conditioned place preference; NMDA, N-methyl-D-aspartate; HFD, high-fat-diet; KO, knockout; IP, intraperitoneal; GLP-1, glucagon-like peptide 1; 5-HT, 5-hydroxytryptamine; MOR, mu-opioid receptor; DOR, delta-opioid receptor; KOR, kappa-opioid receptor; shRNA, short-hairpin RNAs; AEA, anandamide; 2-AG, 2-arachidonoylglycerol; CB1R, cannabinoid receptor type 1; CB2R, cannabinoid receptor type 2; WT, Wild type; AMPK, AMP-activated protein kinase activity; OX1R, orexin-1 receptor; OX2R, orexin-2 receptor; LHA, lateral hypothalamic area; VHP, ventral hippocampus; MBOATs, membrane-bound O-acyltransferases; MCT, medium-chain triglycerides; HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotrophic hormone; DAT-GHSR, GHSR expression limited to dopamine neurons; FAA, food anticipatory activity; fMRI, functional magnetic resonance imaging; BMI, body mass index; BES, Binge Eating Scale; TSST, Trier Social Stress Test.

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## 1. Introduction

In 1999, using an orphan-receptor strategy, the 28 amino acid octanoylated peptide ghrelin was purified from the rat stomach and recognized as the endogenous ligand of the growth hormone (GH) secretagogue receptor, a G protein-coupled receptor (GPCR), known as GHSR1a [1]. Both rat and human ghrelin are characterized by a length of 28 amino-acids, differing only in two residues, and share the presence of a modification at Ser3 by n-octanoic acid, which is essential for hormonal activity [1,2]. Ghrelin is the first known example of a bioactive peptide modified by an acylation reaction performed by the enzyme ghrelin O-acyltransferase (GOAT) [3,4]. Two principal forms of ghrelin peptide are observed in tissues and in plasma: the active n-octanoyl-modified form and the non-modified form, known as des-acyl-ghrelin (DAG), which lacks the ability to activate GHSR1a [1,5,6]. The main site of ghrelin production is the stomach; in situ hybridization and immunohistochemistry analysis revealed that ghrelin is synthesized by distinct endocrine cells of the gastric oxyntic glands, specifically the X/A-like cells, which contain round, compact, and electron-dense granules filled with ghrelin [7–10]. After the production, ghrelin is secreted in the bloodstream and reaches the anterior pituitary gland, where it stimulates the release of GH in a dose-dependent manner [1,8]. Interestingly, ghrelin is also synthesized in the small and large intestines and centrally by the neurons of the arcuate nucleus of the hypothalamus (ARC), even though the number of ghrelin-positive cells in this brain region is limited [1,5,8,9].

Ghrelin exerts its functions by binding and activating GHSR1a, a typical GPCR with 7 transmembrane domains, coupled to a  $G_{\alpha_{q/11}}$  protein, and promotes mobilization of calcium from intracellular stores, mediated by the activation of phospholipase C [11–13]. In the brain, GHSR1a is highly expressed in the pituitary gland and in the hypothalamus, specifically in the ARC, a crucial region for the neuroendocrine and appetite-stimulating properties of ghrelin [12,14], even though the expression of this receptor was also determined in other hypothalamic nuclei (anterior hypothalamic, suprachiasmatic, anteroventral preoptic, paraventricular, and tuberomammillary nuclei) [12,15] and at extra-hypothalamic sites, such as the dentate gyrus, the CA2 and CA3 regions of the hippocampus, the substantia nigra, the ventral tegmental area (VTA), and the dorsal and median raphe nuclei [15,16], indicating a role in various physiological functions over the regulation of GH secretion.

Soon after the discovery, ghrelin was demonstrated to be a powerful appetite-stimulating hormone, with the ability to promote feeding and adiposity and to regulate energy metabolism in humans and rodents [17–22]. Furthermore, ghrelin acts as a key regulator of meal initiation, considering that its blood levels follow a circadian fluctuation, aligned with mealtimes, with the highest concentrations found pre-prandially, while decreasing after meals [23–25]. Peripheral ghrelin can affect feeding behavior in different ways, since it is able to cross the blood-brain barrier by passive diffusion through the fenestrated capillaries of the median eminence, close to the ARC [26]. Alternatively, it can activate GHSR1a in the vagus nerve terminals, transmitting information to the nucleus of the tractus solitarius (NTS), which indirectly communicates with the hypothalamus [27]. The effects of ghrelin on food intake have been attributed to the activation of central GHSR1a, particularly in the ARC, where the potent appetite-stimulating peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) appear to mediate the orexigenic effects of ghrelin, considering that in this region, intracerebroventricular (ICV) injections of ghrelin were able to increase c-FOS expression on NPY-positive neurons and enhance the levels of NPY and AgRP mRNA transcripts [20,21,28]. More specifically, in the ARC, GHSR1a is predominantly expressed on NPY and AgRP neurons, which are activated and depolarized by ghrelin, conversely to pro-opiomelanocortin (POMC) neurons, which are hyperpolarized, due to an increase in GABA inhibitory post-synaptic currents, which results in potent orexigenic signals induced by ghrelin [18,29]. In addition,

another potential mechanism that explains how ghrelin exerts an orexigenic response is mediated by the upregulation of the enzyme prolyl carboxypeptidase, responsible for the inhibition of the hypothalamic peptide  $\alpha$ -MSH [30], which is then unable to activate the melanocortin-4 receptor (MC4R) in the paraventricular nucleus of the hypothalamus (PVN), consequently promoting satiety signals [31,32].

Intriguingly, as will be discussed in the next sections, the role of the ghrelin system and the enzyme GOAT in the regulation of food intake is not merely limited to the homeostatic aspect, which is principally related to its action in the hypothalamus, the main feeding center. It is also involved in the non-homeostatic aspect, consistent with the expression of ghrelin receptors even at extra-hypothalamic sites, interactions with other neurotransmitters, and stress responsiveness, which are implicated in feeding behavior. Therefore, in this review, we highlight how ghrelin may increase food motivation and reward, leading to the consumption of highly palatable foods (HPF), regardless of the metabolic demand and energy status [33–36], probably playing a role in aberrant feeding patterns, such as binge eating (BE) behavior. Notably, the BE episode is a main feature of eating disorders, particularly in bulimia nervosa (BN), BE/purging subtype of anorexia nervosa (AN), and binge eating disorder (BED). In contrast to other reviews that covered this topic [37–40], our aim was firstly to explore the extensive association between ghrelin, dietary behavior, food intake, and reward, focusing on the interaction with several neurotransmitters, beyond the point of view of obesity or energy metabolism. Subsequently, the potential use of GOAT inhibitors as a new pharmacological approach is discussed, as they exert an anorexigenic effect, limiting the production of ghrelin and simultaneously enhancing the level of DAG. Consequently, this innovative therapeutic strategy could be considered as a future perspective against obesity and bingeing-related eating disorders. Finally, the role of the ghrelin system in BE behavior and its relationship with the hypothalamic–pituitary–adrenal (HPA) axis are described.

## 2. The neural mechanisms underlying the role of ghrelin system in food reward

### 2.1. Ghrelin and the mesolimbic dopamine system

The influence of ghrelin signaling on the non-homeostatic aspect of feeding is consistent with the presence of GHSR1a not only in the hypothalamic nuclei, but also in reward-related brain areas, principally on dopaminergic VTA neurons [15,41,42]. These neurons send projections to the nucleus accumbens (NAc), prefrontal cortex (PFC), and other brain areas (including the hippocampus and hypothalamus), and are activated in response to both natural rewards such as food, and artificial rewards such as alcohol or drugs of abuse [43–46]. In particular, similar to drugs of abuse, microdialysis studies in animals revealed that the consumption of HPF (rich in sugar and/or fat), activating VTA dopaminergic neurons, triggers dopamine release in the NAc, which is correlated to their reinforcing properties [47,48].

Ghrelin was found to target and activate GHSR1a expressed in the VTA and promote synaptic input reorganization of VTA dopaminergic cells, triggering dopamine release and turnover in the NAc [41,49]. Thus, the action of ghrelin on dopamine neuronal activity suggests its ability to influence the brain reward system. Moreover, ghrelin modulates phasic dopamine release and NAc neural activity in response to motivationally relevant stimuli, such as food-predictive cues, which then drive and facilitate food-directed behaviors [50,51]. Intriguingly, the locomotor-stimulatory and dopamine-enhancing effects induced by ghrelin appear to be mediated by cholinergic transmission, since they were blocked by mecamylamine (a non-selective nicotinic acetylcholine receptor antagonist). Moreover, the direct stimulation of the GHSR1a in the laterodorsal tegmental area was able to activate the mesolimbic dopamine system, sending cholinergic projections to the VTA [49,52–55]. Furthermore, pre-treatment with mecamylamine completely blocked the increased food intake elicited by intra-VTA injections of

ghrelin in both satiated mice and rats and prevented the exogenous ghrelin-induced conditioned place preference (CPP) [56,57]. Thus, ghrelin may activate GHSR1a expressed on cholinergic neurons of the laterodorsal tegmental area, promoting the release of acetylcholine in the VTA, which in turn stimulates nicotinic acetylcholine receptors and consequently leads to the release of accumbal dopamine [53,54,56]. Since the acetylcholine-dopamine pathway is critically associated with the hedonic and reinforcing aspects of artificial and natural rewards [58, 59], the ability of ghrelin to activate this neural network suggests that it could increase the incentive salience of signals related to motivated behaviors, including drug and food seeking, and represents a potential target for addictive behaviors, such as compulsive-like eating and drug dependence.

Ghrelin-induced activation of the mesolimbic dopamine system also appears to involve other neural mechanisms, including glutamatergic transmission, considering that ghrelin requires excitatory glutamatergic input to increase the electrical activity of dopaminergic neurons in the VTA [41] and that the N-methyl-D-aspartate (NMDA) receptor antagonist D-AP5 (directly injected in the VTA) abolished ghrelin-associated locomotor stimulation and accumbal dopamine release [60].

## 2.2. The effects of ghrelin on food reward and motivation

Considering the previously discussed ability of ghrelin to influence mesolimbic dopamine signaling, several studies investigated whether this peptide could affect the intake and motivation to obtain highly palatable and rewarding foods using different experimental paradigms and conditions.

Systemic ghrelin seems to enhance consumption and preference for sweet taste food, regardless of the caloric content, indicating that feeding responses evoked by ghrelin could be partially related to reward seeking [57,61]. Accordingly, in a free-choice paradigm between the standard and HPF, intra-VTA injections of ghrelin increased the consumption of more rewarding food, without altering standard chow intake [34]. In addition, ghrelin, both centrally and peripherally administered, enhances the reward value of a high-fat diet (HFD) and chocolate pellets, under the CPP, a paradigm in which the animal learns to associate reward from food with a given environment [34,36]. Interestingly, the consumption of the more rewarding food in the free-choice paradigm and the CPP for the HPF are both significantly suppressed by genetic ablation and pharmacological blockade of GHSR1a [34,36,57,61], and the GHSR1a knockout (KO) mice also failed to present the accumbal dopamine release elicited by exposure to the HPF [34]. The absence of GHSR1a also revealed to be protective against stress-induced hedonic eating, since mice lacking this receptor failed to exhibit CPP and increased intake of HFD, after being exposed to a stressful procedure [62], a factor that is known to promote the overconsumption of HPF in both humans and rodents [63–68].

Ghrelin influenced not only the preference for HPF, but also enhanced the motivational effort required by the animals to obtain the food reward, as assessed by the operant conditioning paradigms.

Indeed, mice tested in an operant conditioning protocol to obtain HFD pellets, under a progressive ratio schedule of reinforcement, showed a higher breakpoint (determined as the last progressive ratio which an animal successfully completed to receive a reinforcement) after receiving peripheral ghrelin injections, when compared to the saline-treated counterpart [36].

Similar results were obtained in rats, observing that both intraperitoneal (IP, 0.33 mg/kg) and ICV (0.5 and 1 µg) ghrelin administrations increased all the measures of operant behavior (the active lever presses and number of sugar pellets earned) of the animals, in the satiated state [69]. ICV ghrelin infusion (0.1 and 1 nM) also enhanced the motivational effort of rats to obtain a 5% sucrose solution in the same experimental paradigm, without influencing the perception of food palatability, as determined via lickometry [70]. Furthermore, Skibicka et al. investigated the role of the endogenous ghrelin system in the

motivation for HPF. Sucrose self-administration was performed in overnight-food-restricted rats to ensure high levels of circulating ghrelin. In this experiment, IP injection of the GHSR1a antagonist JMV2959 at 3 mg/kg or ICV injections at 5 and 10 µg significantly decreased the operant response to sucrose pellets, confirming the hypothesis that central ghrelin signaling promotes the incentive values of different rewards, such as food [69]. Recently, Bake et al. also demonstrated that ICV ghrelin delivery in rats not only increases the motivation to obtain sucrose pellets in a progressive ratio operant responding paradigm, but this effect was also extended to the less rewarding chow pellets, further supporting the powerful ability of ghrelin to enhance the motivational effort of animals in the context of food intake, independent of caloric content and palatability [71].

Several studies have highlighted that the capacity of ghrelin to promote incentive-motivated behaviors is mediated by the VTA by selective injections in this brain region. Accordingly, King et al. used mini-osmotic pumps to chronically infuse ghrelin into the VTA of male rats for 14 days and found that both satiated and restricted animals, treated with 10 nM/day ghrelin, increased active lever presses for chocolate-flavored pellets, whereas treatment with the ghrelin receptor antagonist [D-Lys3]-GHRP-6 (200 nM/day) blocked the increase in the effort made by the rats to obtain the same pellets, despite being food deprived [72].

Direct infusion of 0.33 µg and 1.0 µg of acylated ghrelin in the VTA enhanced the operant response for sucrose pellets in male rats, which was conversely suppressed after microinjection of the GHSR1a antagonist JMV2959 at 10 µg dose; interestingly, delivery of both ghrelin and JMV2959 in the NAc shell did not alter food motivated behaviors of rats, confirming that the VTA, instead of the NAc, is a primary target of ghrelin influence in the motivation to obtain HPF [73]. However, although the NAc does not seem primarily involved, this area appears as a necessary downstream mediator of the effect of ghrelin on food reward, since the D1-like receptor antagonist SCH-23390 (0.3 µg) and the D2-like receptor antagonist eticlopride (1.0 µg), when directly infused in the NAc shell, interrupted the food-motivated behaviors of sucrose pellets elicited by intra-VTA ghrelin, without any influence on ghrelin-induced hyperphagia for standard chow [74]. This suggests the existence of divergent neurocircuitries that mediate the effect of ghrelin on eating behaviors, one involved in the non-homeostatic rewarding aspect, which requires dopaminergic signaling in the mesolimbic system, and the other involved in the homeostatic regulation of food intake. However, ghrelin signaling in the VTA acts as a primary target in modulating both reward-based eating and fasting-induced hyperphagia, as assessed in a restricted feeding protocol performed in rats, in which intra-VTA injections (1, 2, and 4 µg) of ghrelin promote overconsumption of an HFD in either calorically sated and 21-h food-restricted animals, effects attenuated by pre-treatment with the GHSR1a antagonist [D-Lys3]-GHRP-6 [75].

Finally, a recent report provided evidence that the anorexigenic gut hormone glucagon-like peptide 1 (GLP-1) and the monoamine neurotransmitter 5-hydroxytryptamine (5-HT), which is known to reduce food intake and profoundly influence food reward-related behaviors [76–80], crucially impact the ghrelin-associated behavioral response and appetitive motivation within the VTA [81], in which GLP-1 and 5-HT<sub>2c</sub> receptors are highly expressed [82,83]. Indeed, pre-treatment via both systemic and intra-VTA administration of the GLP-1 receptor agonist exendin-4 and the 5-HT<sub>2c</sub> receptor agonist Ro60-0175 significantly attenuated the elevated operant response to sucrose pellets induced by the VTA-injected ghrelin (300 pmol) [81].

## 2.3. Participation of opioids and endocannabinoids signaling in ghrelin's effects on food reward

The central ghrelin system seems to be involved in the opioid-associated changes in the mesolimbic dopaminergic system related to reward processing, and microdialysis studies revealed that pre-treatment with the GHSR1a antagonist JMV2959 was able to blunt the

augmentation of extracellular dopamine concentration in the NAc, following morphine and fentanyl injection [84–87], to reduce their associated CPP and behavioral stimulation [84–87] and attenuate heroin seeking in chronically food-restricted rats, when directly infused into the VTA [88]. Moreover, subchronic treatment with JMV2959 significantly increased the levels of the opioid peptides Met-enkephalin-Arg6Phe7, dynorphin B, and Leu-enkephalin-Arg6 in reward-associated regions, such as the VTA, hippocampus, and striatum [84], supporting that the ghrelin system regulates reinforcement processes via the modulation of opioid transmission in different brain areas.

In the context of food intake, the endogenous opioid system, through the mu-opioid receptor (MOR), delta-opioid receptor (DOR), and kappa-opioid receptor (KOR), is not only deeply involved in the regulation of appetite and metabolism, but also plays an important role in the hedonic and rewarding aspects of eating processes [89–91]. MOR agonists injected in the VTA are powerful stimulators of food intake, and the activation of MOR is necessary for the release of dopamine in the NAc in response to palatable food ingestion [92,93].

Furthermore, it was highlighted that the opioid system, principally through the MOR, behaves as a critical downstream mediator of the effect of ghrelin on free feeding and food reward. Indeed, despite in 2005 Naleid et al. reported that the MOR-preferring antagonist naltrexone did not block ghrelin-induced food intake, either injected in the VTA or in the NAc at a dose of 25 µg [94], a subsequent study revealed that pre-treatment with naltrexone (50 µg), infused in the lateral ventricle, was able to suppress both the free-feeding of chow and the enhanced operant responding to sucrose pellets induced by ghrelin [95]. However, when the same MOR antagonist was microinjected in the VTA at a dose of 25 µg, it selectively blocked the increased motivation for HPF seen after intra-VTA ghrelin administration, without affecting standard chow consumption [95]. This indicates a direct interaction between ghrelin and opioids in the VTA, as demonstrated by the observation that central ghrelin infusion results in elevated MOR expression in this brain region [95].

Interestingly, the MOR does not appear to be the unique opioid receptor involved in ghrelin's influence on food intake and reward, but also the KOR, which colocalizes with GHSR1a in both the hypothalamic area and in the VTA [96]. Indeed, central pharmacological blockade of KOR using the selective antagonist NorBNI, significantly decreased ghrelin-induced food intake, which was accompanied by a reduced expression of transcription factors and hypothalamic neuropeptides (AgRP and NPY) that are implicated in the orexigenic activity of ghrelin [96]. To investigate the specific neuronal population involved in the interaction of KOR with ghrelin, genetic silencing of KOR, in the ARC or the VTA, was performed in rats, using an adeno-associated viral vector encoding rat KOR short-hairpin RNAs (shRNA). The inactivation of KOR in the ARC attenuated the increased food intake caused by ICV ghrelin, but this was not replicated with genetic silencing in the VTA, suggesting that the KOR neuronal population in this brain region is not required for the orexigenic effect of ghrelin [96]. However, in the same year, Kawahara et al. reported a different finding: peripheral ghrelin administration might switch the dominant opioid receptor pathway for highly rewarding foods from MOR to KOR, which in turn results in a suppressed activity of the mesolimbic dopamine transmission, as assessed through the microdialysis technique [97,98].

Taking into account the discussed interaction between opioids and the ghrelin system and the role that endocannabinoids play in the reinforcing properties of opioids [99,100], it was investigated whether ghrelin signaling might influence the opioid-induced changes in mesolimbic anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which are the best characterized endocannabinoids [101,102]. The results of two studies by Sustkova-Fiserova et al. revealed that pre-treatment with the GHSR1a antagonist JMV2959 reversed the morphine- and fentanyl-associated increase in AEA and intensified the decrease in 2-AG in the NAc shell, effects abolished by the co-administration of JMV2959 with ghrelin [103,104]. Ghrelin receptor antagonism also reduced the

intravenous self-administration, tendency to relapse, and behavioral stimulation induced by WIN55,212-2 (a cannabinoid receptor type 1 [CB1R] agonist), as well as reversed the dopamine and endocannabinoid (AEA, 2-AG) release in the NAc shell and the accumbal GABA decrease [105,106] and, additionally, diminished the CPP and behavioral stimulation produced by the partial CB1R agonist tetrahydrocannabinol [105].

The endocannabinoid system, principally via the activation of the CB1R, is not only deeply involved in the regulation of food intake, producing orexigenic stimuli at the hypothalamic level, but also increases motivation to consume food by interacting with mesolimbic reward pathways. Therefore, targeting CB1R is considered a validated pharmacological tool to treat altered feeding behaviors and obesity [107,108]. However, despite the existence of a clear functional interconnection between endocannabinoids and ghrelin, which seems to occur via CB1Rs and independently from CB2Rs [109], only a few studies have investigated whether CB1R antagonism might be a useful strategy to attenuate ghrelin-stimulated food intake. The first evidence was reported in a study by Tucci et al., in which the orexigenic effect of intra-PVN ghrelin (100 pmol) in rats was prevented by the peripheral administration of the CB1R antagonist SR141716 (rimonabant, 1 mg/kg), while such a compound did not alter feeding behavior per se [110]. This result was subsequently confirmed by observing that IP-injected rimonabant prevented the increased food consumption elicited by subcutaneous administration of the GHSR1a agonist hexarelin [111].

Furthermore, ICV ghrelin stimulates food intake in wild type (WT) mice, but not in CB1R KO animals, and CB1R antagonism blocks the ability of ghrelin to increase the hypothalamic AMP-activated protein kinase activity (AMPK) and expression of 2-AG and suppresses excitatory synaptic inputs in the PVN, which are correlated with the induction of feeding behavior [112]. On the other hand, intact ghrelin signaling is required for the cannabinoid influence on AMPK activity, as the AMPK effects of CB1R agonism were abolished in central and peripheral tissues of GHSR1a KO animals [113].

Moreover, in order to investigate the contribution of peripheral CB1Rs on ghrelin-induced food consumption, an ultra-low dose of rimonabant (0.03 mg/kg), chosen to avoid central effects, and the selective peripheral CB1R antagonist LH-21 were able to counteract the hyperphagia stimulated by the ICV administered ghrelin, supporting that the interplay between ghrelin and endocannabinoid systems in the regulation of appetite might occur at a peripheral level [114]. Specifically, CB1Rs located in the neuroendocrine cells of the stomach regulate gastric ghrelin secretion and modulate ghrelin-induced food intake via a mechanism that requires intact vagal communication [115]. These findings could also have important therapeutic implications, since blockade of central CB1Rs, even though effective in reducing food intake, was commonly found to promote psychiatric side effects, such as anxiety and depression [116], while targeting the peripheral endocannabinoid system could reduce the possibility of occurrence of these aversive factors.

Finally, with regard to CB1R antagonism on ghrelin-induced activation of the mesolimbic system, a recent study by Kalafateli et al. showed that systemic and intra-VTA administrations of rimonabant were able to prevent locomotor stimulation and NAc dopamine release induced by centrally injected ghrelin, while they did not attenuate the increased food intake elicited by ghrelin, suggesting that the ability of ghrelin to activate the mesolimbic dopamine system depends on CB1Rs, but these are not involved in the regulation of feeding behavior [117]. However, this study had a limitation in performing the experiments in fed mice, without investigating the effect of ghrelin and rimonabant administrations under a motivated state, observed in the food restriction status [117].



#### 2.4. The interaction of ghrelin system with orexin neurotransmission

Orexin-A and orexin-B represent a family of peptides derived from the proteolytic processing of a common precursor (pre-pro-orexin) and are the endogenous ligands of two GPCRs, recognized as the orexin-1 (OX1R) and orexin-2 receptors (OX2R) [118]. Orexin-producing neurons are predominantly localized in the lateral hypothalamic area (LHA), generally identified as the main feeding center, and project widely to several brain regions, such as the hypothalamic nuclei and the brainstem [119,120], where they profoundly influence a great number of functions, including the sleep/wake cycle and the regulation of food intake [121,122]. Specifically, the orexin system, mainly through OX1R, is implicated in controlling both homeostatic and reward-based feeding, and antagonism at OX1R suppresses food intake [123], high-fat pellet self-administration [124], and compulsive-like eating [125].

Intriguingly, the LHA-orexin system is one of the most important brain sites in mediating the orexigenic properties of ghrelin, since both ICV and intra-LHA injections of this peptide have been demonstrated to promote c-FOS expression, and activation of orexin-expressing neurons in this region [126–129] and the ghrelin-induced food intake is attenuated by genetic deletion of orexins [129], treatment with antibodies against orexin-A and orexin-B [129], and administration of an OXRs antagonist [128].

Additionally, the action of ghrelin on food reward requires intact orexin signaling, as demonstrated in a study by Perello et al., who found that ghrelin-induced acquisition of CPP and increased breakpoint in the operant conditioning paradigm for HFD pellets, are suppressed in orexin-deficient mice and in WT mice pre-treated with the OX1R antagonist SB-334867 (10 µg/g BW), at a dose that alone does not influence the intake of freely available food [36]. These effects were accompanied by higher c-FOS expression in the LHA orexin neurons of mice treated with ghrelin, as determined by dual-label immunohistochemistry [36]. However, the exact neural circuitry underlying the interaction of ghrelin and orexins in food reward is not completely understood, but the authors proposed that ghrelin might activate GHSR1a expressed on orexin-containing neurons of the LHA [130,131], which in turn send projections to the VTA [132] and consequently activate the mesolimbic dopaminergic system, findings that are in accordance with the crucial role that orexins play in the reward-based processes related to the drugs of abuse and HPF overeating [133,134]. A subsequent investigation highlighted that, when directly injected into the lateral hypothalamus of rats, ghrelin potentiated the phasic dopamine spikes in the NAc evoked during sugar pellet retrieval, and this effect was recapitulated following intra-VTA administered orexin-A [50]. In addition, selective blockade of VTA OX1Rs with SB-334867 attenuated the ability of ghrelin to increase the consumption of rewarding food in ad libitum fed rats, revealing that ghrelin influences food intake and the associated reinforcement processes in an orexin-A-dependent manner [50].

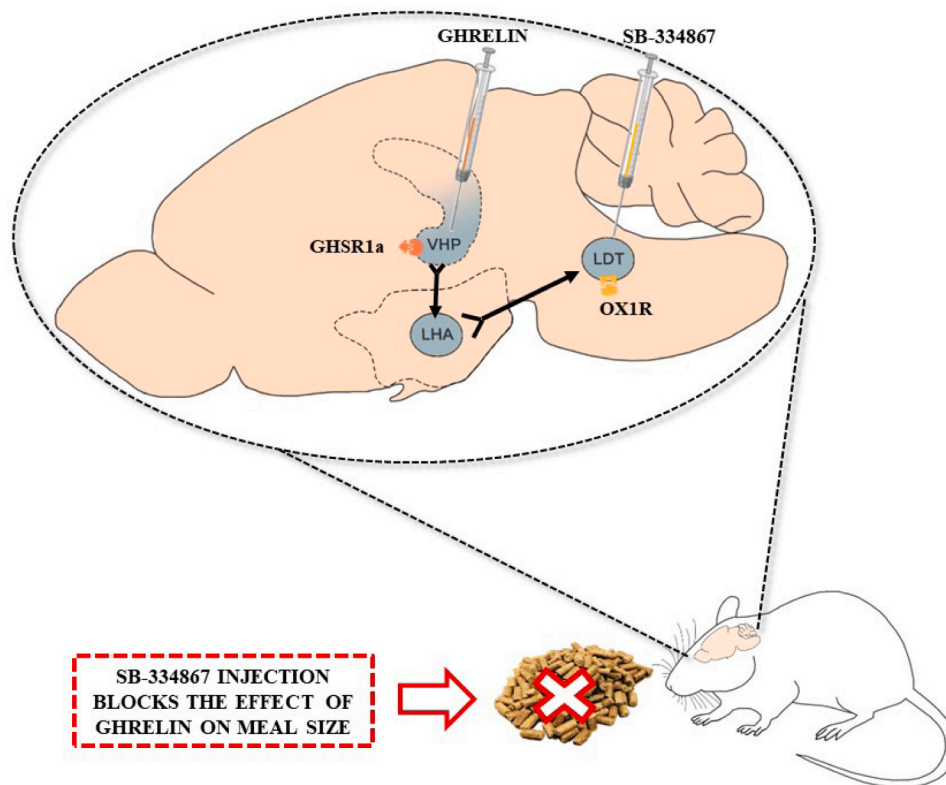
Recently, it was also observed that the activity of ghrelin signaling in the LHA might present sexual dimorphism: despite ghrelin, administered directly in the LHA, was found to promote food intake and food-motivated behaviors for sucrose in both male and female rodents, the acute pharmacological blockade of GHSR1a in the same brain region reduced food intake, body weight, and food motivation only in female rodents [135]. Moreover, the latter revealed a higher expression of GHSR1a mRNA (about 30%) in the LHA compared to male rats, and they also increased orexin gene expression in response to intra-LHA administered ghrelin [135].

Finally, a novel neural network linking ghrelin and orexin signaling was proposed by Hsu et al., revealing that GHSR1a-expressing neurons of the ventral hippocampus (VHP), which are necessary for conditioned feeding behaviors, provide direct inputs to the dorsal perifornical LHA-orexin neurons, which act as downstream mediators of ghrelin-induced feeding [136]. This is further supported by the significantly increased expression of c-FOS in orexin-expressing neurons of the dorsal perifornical LHA, after ghrelin administration in the VHP, and the

suppression of VHP ghrelin-induced hyperphagia, through central injection of an OX1R antagonist [136]. Furthermore, the LHA-orexin neurons that receive inputs from the VHP were demonstrated to send projections to the laterodorsal tegmental nucleus in the hindbrain, and blockade of OX1R in this region abolished the increased meal size observed with VHP injection of ghrelin, supporting the existence of a multi-order circuit linking hippocampal ghrelin neurotransmission and LHA orexin signaling to the laterodorsal tegmental nucleus, which is involved in promoting meal size and ingestive behaviors [137] (as shown in Fig. 1). These interesting results, combined with previous findings revealing that VHP ghrelin signaling promotes food intake, operant response to sucrose, cue-induced feeding, and dopaminergic activity in the NAc [138], should lead to future studies, in order to investigate whether the ghrelin system in the hippocampus and orexin neurotransmission might interact in the context of reward-based eating behaviors and aberrant feeding patterns.

#### 3. The enzyme GOAT and the implications in food intake

GOAT belongs to a family of membrane-bound O-acyltransferases (MBOATs) and is the only enzyme capable of mediating the acylation of ghrelin to produce acyl-ghrelin (AG), linking a medium fatty acid chain, typically octanoate, to a serine residue. This leads to the active form of the hormone, which is completely absent in mice lacking GOAT, emphasizing the essential importance of this key enzyme [3,4,139]. GOAT modulates food intake and food reward, and in rodents, the mRNA of this enzyme has been found in the peripheral system, such as the gut, stomach, and in the central nervous system, principally in the hypothalamus [4,5,140,141], accordingly with the expression of ghrelin, whose activation occurs primarily in the gastric tissue [3,4,141,142]. Elevated GOAT mRNA expression was observed in the hypothalamus, as well as in the stomach fundus, during both short- and long-term dietary restriction, with higher plasma levels of active ghrelin specially in 3-weeks food-restricted animals [143–146]. Thus, GOAT mRNA is modulated in the rat hypothalamus and stomach, following metabolic stress, and increased ghrelin levels may affect the undernutrition condition and result in adaptation to alterations in energy balance and body weight homeostasis [143,144]. However, a different evidence was found, revealing higher gastric expression of GOAT gene under ad libitum conditions, which significantly decreased during fasting conditions [139,147]. Moreover, in another study, after 48-h of fasting or partial food deprivation, GOAT mRNA levels remained fairly stable compared to ad libitum rats until there was an excessive weight loss which led to increased levels of both the enzyme and the corresponding produced peptide [142]. Ghrelin mRNA resembled GOAT expression, but AG concentrations in blood did not change over the course of fasting [139]. Ghrelin circulates in two major forms: AG and DAG, with the latter lacking octanoylation at serine 3 and represents the most abundant molecule of circulating hormone, even though DAG does not bind GHSR1a [5,148]. In fact, DAG was found to bind and act in the ARC cells in a GHSR-independent manner and was able to reduce, through ICV administration in mice, the orexigenic effect of peripherally injected ghrelin, hypothesizing that both peptides could act on different ARC cells, thus differentiating the response to food consumption [149]. After IP or ICV administration, DAG also seemed to induce inhibitory effects on feeding and delay gastric emptying in both sated and food-deprived mice, in contrast to the activities of AG, and peripheral injection of DAG increased c-FOS expression in the PVN and ARC, highlighting actions in these areas, but not in NTS [150]. Interestingly, the orexigenic properties of AG were blocked by IP co-administration with DAG (AG 13 µg/kg and DAG 64 and 127 µg/kg, inactive when injected alone) in ad libitum fed rats [151] and significantly diminished the neuronal activity in the ARC; when injected alone, they showed increased neuronal activity in the same brain region [151]. Moreover, Davis et al. investigated the involvement of GOAT in food motivation and in the hedonic aspect of feeding and, interestingly, a reduced hedonic feeding response was



**Fig. 1.** The neural network linking ghrelin signaling in the VHP, LHA-orexin neurons, and the laterodorsal tegmental nucleus. Ghrelin activates the GHSR1a expressed in the VHP, which in turns provides direct inputs to the to the dorsal perifornical LHA-orexin producing neurons. These neurons send projections to the OX1R-expressing neurons of the laterodorsal tegmental nucleus, in the hindbrain, which are critical mediators for the ability of ghrelin to increase meal size, since the OX1R antagonist SB-334867 blocks the increased meal size stimulated by ghrelin injection in the VHP [137]. GHSR1a: Growth Hormone Secretagogue Receptor type 1a, LDT: Laterodorsal Tegmental nucleus, LHA: Lateral Hypothalamic Area, OX1R: Orexin-1 Receptor, VHP: Ventral Hippocampus.

found in mice GOAT KO compared to WT littermates. The latter have consumed more HFD, after a period of caloric restriction and refeeding with standard food and, in addition, under ad libitum conditions, WT and GOAT KO mice showed similar rates of operant responding. Conversely, GOAT KO mice did not increase their levels of responding after 24-h of fasting, suggesting that AG is involved in the regulation of hedonic-based feeding behaviors [152]. The current study also revealed that, unlike GOAT KO, WT mice exposed to one week of a diet rich in medium-chain triglycerides (MCT) increased body weight, indicating a possible role of GOAT in the regulation of body weight and fat mass [152]. These findings were supported by Kouno et al., in which GOAT KO mice demonstrated attenuated voluntary sucrose consumption and a reduction in HFD and HFD plus high-sucrose diet intake compared to WT, together with a discrete reduction in body weight and food ingestion under the MCT diet [153,154]. GOAT KO mice fed with MCT plus high-sucrose diet showed a prominent reduction in feed efficiency, food intake, and body weight [154], considering the MCT diet responsible for the increment of the AG levels [155], and the activation of the ghrelin/GOAT system was impaired in the absence of GOAT in GOAT KO mice. Meanwhile, in WT mice, the MCT diet contributes to food intake and weight gain [154]. These findings support the hypothesis previously expressed by Kirchner et al., according to which acylation and activation of ghrelin are strictly influenced by the type of food ingested and fatty acid composition [139], assessing dietary lipids are used for ghrelin acylation [155], and transgenic mice are not capable of producing large amounts of AG when fed with regular chow. Additionally, GOAT KO mice under daily consumption of carbohydrates consumed less glucose and maltodextrin solution compared to WT mice, and the same mice fed with a diet rich in maltodextrin, corn starch, and HFD exhibited decreased food intake associated with less weight gain compared to WT mice [156]. Moreover, in WT mice, both AG and DAG were administered, showing how AG enhanced the major consumption of glucose and maltodextrin solutions, while DAG did not increase consumption [156]. GOAT KO rodents also showed altered salty taste perception, and the genomic ablation of GOAT or ghrelin in mice exhibited a significant

reduction in taste sensitivity to appetitive lipid stimuli, suggesting that AG may be strongly involved in lipid taste perception. Considering how fatty food can easily lead to obesity and loss of control over eating in BE episodes, these results are crucial for understanding the possible relationship between gustatory perception of dietary lipids and the modulatory role in taste sensitivity of ghrelin [157]. Recently, stable expression of GOAT was observed in HFD-induced obese mice: mRNA levels of this enzyme remained unchanged compared to mice fed a standard diet, highlighting how it may not be a crucial factor for the decreased ghrelin secretion in obesity [158]. Davis et al. evaluated the transcriptional levels of orexin and found diminished OX1R expression in the NAc in GOAT-KO mice, without changes in the hypothalamus, suggesting that a decreased orexin signal can affect eating behavior [152]. Moreover, Sirohi et al. analyzing rats under an HFD-limited regimen with ad libitum chow and found elevated AG plasma levels 1-h prior to the access to the fat diet, accompanied by a voluntary pre-prandial caloric restriction as an adaptation to maximize the amount of fat to ingest, which could be the probable cause for the AG profile (since rats were not in a negative metabolic state). In the same rats, an increased genetic expression of GHSR and pre-pro-orexin in the hypothalamus, and elevated OX2R mRNA in the medial PFC and not in the VTA were observed: alterations in the orexin cortical signaling may be related to ghrelin activity and could drive motivation and binge-like intake for rewarding food [159]. Additionally, Perello et al. did not observe the effects of ghrelin on HFD reward in orexin-deficient mice and in WT mice treated with an OX1R antagonist [36], hypothesizing that ghrelin exerts its rewarding effects through activation of the hypothalamic orexin system [36]. Given the role of orexin in BE behavior, characterized by compulsive overeating of HPF in a discrete period of time [160] and selective OX1R antagonism blocked BE for HPF without affecting standard food intake [125], it would be interesting to investigate the ghrelin system and the inhibition of GOAT on food motivation, feeding reward, and eating behavior in hypothalamic and extra-hypothalamic sites and their possible correlations.

### 3.1. GOAT-inhibitors as a therapeutic strategy

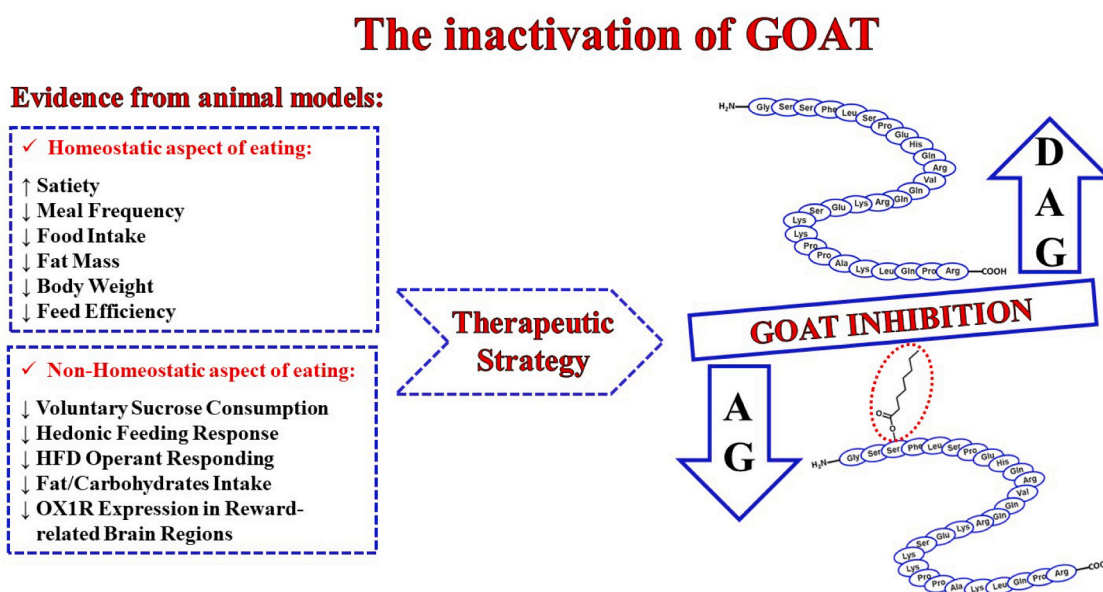
Since the discovery of various regulatory activities of ghrelin, several compounds have been synthesized to antagonize the functions of this hormone. Among these different strategies, the GHSR1a antagonists, analogs of DAG, and inhibitors of GOAT were emphasized; they have been analyzed and tested with different outcomes [161,162]. Recently, research has focused on the inhibition of the enzyme GOAT, since the blockage has a dualistic effect, preventing the activity of ghrelin and potentially increasing the concentration of the DAG counterpart with its related actions (Fig. 2). The primary aim is, until now, focused toward limiting the hyperphagic responses evoked by ghrelin, the development of obesity and the consequences of this status, with the possible future exploration and efficacy in the BED. IP injection with GO-CoA-Tat, a GOAT-inhibitor, in WT mice fed with the MCT diet, reduced plasma AG but not DAG levels and prevented the body weight gain compared to the vehicle counterpart. Moreover, through quantitative magnetic resonance, it was observed that rodents treated with GO-CoA-Tat presented significantly lower fat mass, but not lean mass. The compound was also tested in ghrelin KO animals and revealed that, under the same conditions, GO-CoA-Tat did not show a significant impact [163]. Additionally, GO-CoA-Tat in WT mice seems to exert an anorexigenic effect due to its ability to reduce the meal frequency and AG levels, but not the meal size or DAG profile, and increase satiety compared to the vehicle [164]. A further study showed that the above-mentioned inhibitor does not alter GOAT mRNA and reduces plasma levels of adrenocorticotrophic hormone (ACTH), aldosterone, and corticosterone concentrations with respect to control rodents, thus acting on the HPA axis function in the rat [165]. These insights show the potential use of GOAT-inhibitors as therapeutic targets, which is promising not only in obesity, but also in disorders that exhibit a tendency to consume high-calorie food and accumulate adipose tissue.

## 4. The role of ghrelin system in BE behavior from preclinical and clinical studies

### 4.1. Preclinical studies

Considering the role of ghrelin and GOAT in driving food intake and

enhancing food reward, it was investigated the potential involvement of the ghrelin system in the neurobiological mechanisms underlying BE behavior, since the impulsivity and reward sensitivity present in eating disorders can also be increased by ghrelin plasma levels or activity in the VTA [166,167]. BE has also been related to a dysfunction of ghrelin signaling [168], and a potential new pathway for the involvement of ghrelin in the neurobiology of BE is represented by the zona incerta GABA neurons, excited by ghrelin. Optogenetic stimulation of zona incerta GABA neurons can promote the characteristics of binge-like eating; in mice, an increased preference for high-fat and sweet items over normal food was demonstrated, probably due to the attenuated satiety feedback [169]. The effects and levels of ghrelin have been explored in different animal models, including rodents fed with HFD, or those using limited access to the HPF schedule with or without alternation of periods of caloric restriction. The feeding paradigm of Bello et al. demonstrated that intermittent acute food restriction followed by 2-h of access to HPF, twice-per-week, induced a binge-like phenotype without an increase in the body weight, as conversely revealed by the ad libitum HPF access (obese phenotype), and the total ghrelin levels are different between these two groups, with a higher level of this hormone in the binge-group, which contributed to the elevated consumption inside the discrete time interval [170]. The limited access to HPF, avoiding periods of restrictions, allowed anyway the manifestation of BE episodes; however, in the study of Bake et al., the profile of circulating ghrelin was not altered [171]. Moreover, in a study by Valdivia et al. in mice, 2-h HFD consumption over a 4-day period promoted an escalation of HFD intake, which is associated with the loss of control over eating, typical of BE episodes. Interestingly, ghrelin signaling is essential for the escalation of HFD intake, considering that it was not observed in GHSR1a-KO mice [172]. This finding was also supported by a subsequent study by King et al., using a limited access model of binge-like eating, in which mice were offered daily or intermittent (3 days per week) access to HFD for 2-h, in addition to ad libitum standard chow. It was observed that GHSR1a KO mice reduced the HFD intake, compared to the WT littermates, under the intermittent schedule, but not during the daily access, indicating a crucial role of ghrelin receptors in maintaining binge-like eating under certain experimental conditions. Interestingly, the lack of ghrelin receptors was accompanied by a reduced activation of dopaminergic neurons in the NAc, as revealed by c-FOS



**Fig. 2.** The effects of GOAT inactivation on homeostatic and non-homeostatic aspect of eating and on AG/DAG balance. The inhibition of the enzyme GOAT prevents the acylation reaction at Ser3 increasing the concentration of the DAG and impairing the activity of the AG, potentially reducing its related actions on food intake. AG: Acyl-Ghrelin, DAG: Des-Acyl-Ghrelin, GOAT: Ghrelin O-Acyltransferase, HFD: High Fat Diet, OX1R: Orexin-1 Receptor.



immunohistochemistry, confirming the interaction of ghrelin signaling with dopaminergic transmission in the mesolimbic system [173]. Indeed, in GHSR-deficient mice, an attenuated binge-like HPF intake was observed in contrast to WT mice [174], while satiated mice with GHSR expression limited to dopamine neurons (DAT-GHSR) enhanced BE behavior and food anticipatory activity (FAA) similar to WT mice, in contrast to GHSR-deficient mice [175]. Moreover, DAT-GHSR mice did not increase food intake in response to both IP and ICV injections of ghrelin, suggesting that the mechanisms underlying ghrelin control regarding the reward-related aspects of eating behaviors can be completely distinct from those mechanisms that regulate homeostatic feeding [175]. FAA, which is reduced in the absence of the ghrelin receptor and increased in the restricted feeding paradigm [176,177], was investigated in rats. They were fed with an HPF schedule, specifically chocolate, developing FAA in the hours preceding the access to palatable meal (15 min), revealing a positive correlation between plasma ghrelin levels, chocolate intake, and anticipatory locomotor activity in rats [178]. Thus, these findings highlight that anticipation of HPF in ad libitum animals may reflect the reward-motivated behavior component that drives to eat, whereas in restricted rodents, the principal factor is hunger, and ghrelin signaling is implicated in both conditions. In fact, ghrelin can stimulate the intake a palatable meal, enhancing the rewarding properties of chocolate, as demonstrated by the increased FAA when ghrelin was ICV injected. Unexpectedly, in a recent study by Bake et al., an acute ICV or intra-VTA injection of ghrelin in rats under the BE paradigm, prior to access to HFD, increased regular chow intake, while decreasing HFD consumption, which was normally preferred under the same conditions. This result suggests that ghrelin can shift dietary choice towards chow, even in animals that are strongly motivated to consume HFD. However, in contrast to acute treatment, chronic ICV ghrelin injections promote HFD consumption in the same feeding schedule, while having no impact on regular chow intake, which is interpreted as a hyperghrelinemic state of the animal following chronic treatment, which favors the consumption of energy-dense foods [179]. Moreover, in rodents with free access to HFD or high-carbohydrate diet, ICV injection of ghrelin showed a pronounced preference for HFD, and in two subgroups of rats, with a clear preference among these diets, the ICV administration of ghrelin again showed a predilection for the ingestion of fat over a high-carbohydrate diet [180].

#### 4.2. Clinical studies

Clinical studies using functional magnetic resonance imaging (fMRI) revealed an increased neural response in brain reward-related areas, such as the amygdala, striatum, orbitofrontal cortex, and hippocampus and not the hypothalamus, in healthy volunteers while viewing food pictures, after administration of ghrelin. The hormone may contribute to hedonic feeding, enhancing the incentive and hedonic responses to food-related cues [19,181]. Moreover, a reduction in plasma ghrelin was observed in both non-obese and obese women with BED and obese non-BE women compared to healthy and normal-weight women with BN. Variables as body weight, body mass index (BMI), and plasma concentrations of glucose did not affect the differences in plasma ghrelin levels in the several subjects examined, as well as the frequency and the severity of compensatory behavior in bulimic women and the frequency and severity of bingeing in BED. The diminished ghrelin profile was probably due to counteracting the excess energy and the necessity to reduce appetite [182]. Similar findings were obtained considering the lower fasting ghrelin level in overweight/obese binge eaters than in obese non-binge eaters, without correlation with body fat, since body composition did not differ among the groups, suggesting that BE behavior may downregulate ghrelin signaling [183]. Indeed, BE behavior can be engaged in the absence of hunger when ghrelin is present at low levels. Women with AN had significantly elevated plasma ghrelin concentrations compared to women with BN, BED, and women without eating disorders [184]. Conversely, other studies found that

plasma ghrelin in anorexic and bulimic patients with habitual BE/purge behavior was higher than that in anorexic and bulimic patients without purging behavior, despite similar nutritional parameters, suggesting that the plasma ghrelin profile could be potentially influenced by the number of BE/purge cycles and uncontrolled eating [185–187]. Moreover, examining plasma levels of ghrelin in obese adolescents with or without BE, it was shown no significant difference in ghrelin levels between the groups [188].

However, despite the non-discrimination between AG and DAG, several studies hypothesized the antagonistic function of DAG towards AG, considering its regulatory role in the consumption of food [150,189] and the imbalance of AG/DAG, which is responsible for the development of obesity and BE behavior. In obese individuals, the mean fasting concentrations of AG and DAG were significantly lower than in non-obese subjects, and this normal-weight group presented post-prandial AG levels which were significantly decreased, while DAG levels were unchanged. On the other hand, obese individuals showed decreased levels of DAG and unchanged AG levels [190]. Furthermore, in obese patients, GOAT is reduced and ghrelin levels are increased in gastric tissue. Specifically, ghrelin mRNA expression was elevated in the stomach and ghrelin-positive cells were detected, prompting a major energy consumption in the obese status. Conversely, a reduction in the quantity of GOAT-positive cells (whereas transcripts encoding GOAT were not significantly different) might be an adaptation to the abundance of ghrelin in obese compared to normal-weight subjects [191]. In contrast, in obese individuals with or without BED, significantly lower fasting AG levels were found in obese binge eaters compared to obese individuals without BED [192–194], probably due to a downregulation induced by overeating. However, different post-prandial changes were detected; ghrelin declined less in the BED group [192,194] or did not diverge significantly in the two groups [193]; meanwhile, the AG/DAG ratio decreased in obese non-binge eaters [193]. Recently, associations were found between low levels of DAG and the binge eating scale (BES), although the difference was not statistically significant. Furthermore, a negative statistically significant relationship was found between AG and BES, and the authors suggested that a DAG deficiency with normal levels of ghrelin could reflect the obesity status (negative correlations of DAG have been obtained with body weight and BMI), whereas low total ghrelin levels may be a marker of BED [148]. Moreover, when compulsive subjects with BED were compared to the AN patients, they displayed, as in the above results, significantly lower levels of both AG and DAG [195], supporting the hypothesis that acylation of ghrelin may be modulated by the lipids on the diet [155]. Indeed, during the limited caloric intake, the levels of ghrelin secretion may increase to stimulate hunger, developing resistance to AG, and prolonging starvation. On the other hand, low total ghrelin, a downregulation probably due to habitual overeating, could promote BE behavior/episode, even though more elucidation needs to be done for the possible effect of disbalance of the AG/DAG ratio.

### 5. Ghrelin system and the relation with HPA axis and aberrant feeding behaviors

#### 5.1. Ghrelin system and the relation with HPA axis in rodents

Ghrelin and DAG are deeply involved in stress-responsiveness and in the regulation of the HPA axis [196–202] and interestingly, stress, negative emotional states, and rewarding properties of food and hunger are important features that can trigger BE episodes [203–210]. Indeed, *Ghr*<sup>-/-</sup> mice were more anxious after an acute restraint stress than WT mice, with potent neuronal activation in the PVN and medial nucleus of the amygdala. Meanwhile, exogenous ghrelin reversed this effect, and the absence of ghrelin displayed a dysfunctional glucocorticoid negative feedback [197]. Numerous studies have revealed that acute stress increases peptide levels and regulates the HPA axis in both humans and rodents, and that DAG showed anxiolytic activity under stress conditions



accompanied by reduced corticosterone concentration. The effect observed with AG could be partially caused by DAG, even though DAG influence on the HPA axis was not correlated with the c-FOS activation in the PVN, suggesting an additional brain area involving the CRH receptor 2 [196,197,199,211,212]. In accordance with the development of BE behavior under adverse circumstances [68,205,206], the increase in ghrelin levels due to the stress conditions evidenced as this hormone could potentially lead to the episode of BE, not only for the stimulation of hunger or the urge to eat per se, but also in mediating reward, resulting in food craving, in order to alleviate negative feelings [197,213,214]. Thus, GOAT could be a promising target in modulating dysregulated appetite, and accordingly, emerging findings showed that GOAT KO mice, lacking AG but with elevated DAG, displayed a decrease in anxiety-like behavior and showed no difference in the corticosterone levels compared to WT mice; acute injections with DAG reduced anxiety-related behavior in Ghr KO mice, highlighting an anxiolytic role [215].

Collectively, the ability of AG to affect these factors highlights the complexity of ghrelin signaling and the possible implications of targeting this system to ameliorate not only the excessive feeding per se, but also the behavioral and motivational aspects that drive compulsive-like food consumption.

### 5.2. Ghrelin system and the relation with HPA axis and aberrant feeding behaviors in humans

The possibility of an increase in plasma ghrelin levels in response to psychological stress, as reported in rodents, was investigated in patients with BED. The study by Rouach et al. found an intermediate value of increased ghrelin levels in patients with BED, when compared to normal weight and obese subjects during and following the Trier Social Stress Test (TSST), a psychosocial protocol in which patients are exposed to a public speech in front of an evaluative audience [216]. The data revealed an interaction between cortisol and ghrelin responses, since there was a positive correlation between the rise of plasma ghrelin and the serum cortisol response. Moreover, a higher urge for uncontrolled eating was detected in the BED group, than in the other two groups, even if at the end of the test, it had also occurred in other subjects [217]. The same result was obtained by using another type of physiological laboratory stressor, the Cold Pressor Test, in overweight women with night eating status, which revealed increased cortisol, ghrelin, and hunger ratings [218]. Similarly, in lean and overweight/obese individuals, a differential impact of food cues and exposure to stress was observed on plasma ghrelin levels, leading to an increased ghrelin response only in the overweight/obese group and not in the lean counterpart. In the same group, the stress-induced increase in ghrelin plasma level was also significantly associated with higher HFP craving and consumption, as assessed by the Food Craving Scale and Food Snack Test, proposing that weight-dependent changes in ghrelin signaling might promote the ingestion of HPF, particularly under stressful conditions [219]. Another study showed high levels of AG only in patients with the BE variant of AN after acute stress, compared to BN and controls [220]. Even in the absence of psychological stress, a positive correlation between ghrelin and cortisol concentrations was detected in patients with BED, suggesting the stimulatory effects of ghrelin on the HPA axis at the hypothalamic level, and this relationship is affected by the nutritional status of the subjects [184]. In young healthy subjects, after an overnight fast, a single bolus injection of 100 µg of ghrelin led to pronounced ACTH and cortisol concentrations and a rapid stimulation of appetite, with vivid imagination of their preferred meal, especially salty or sweet food, compared to placebo controls [221]. Similarly, an increase in ACTH and cortisol levels was also observed with the acute intravenous administration of ghrelin at a dose of 1.0 mg/kg in AN, BN, and obese patients [222–224].

Healthy human volunteers have been tested to assess the effect of endogenously- or exogenously-induced hypercortisolism that led to

parallel growth of cortisol/AG concentrations, whereas elevated ACTH plasma levels did not stimulate ghrelin secretion [225].

Collectively, the results indicate the interrelationship between ghrelin and the HPA axis and the involvement of the HPA axis in the ghrelin response to psychological stress. Further studies were also conducted in the field of emotional eating, which is strongly associated with BED and with which it shares some crucial characteristics (such as the urge to eat after adverse events and negative feelings), examining the response following a TSST. In these studies [226,227], a more pronounced cortisol concentration in emotional than in non-emotional eaters was revealed and lower baseline ghrelin levels increased after the laboratory test in the same subjects. The anticipation of this stressor elicited cortisol responses and led, in the emotional eaters, to a greater food ingestion compared to the control, and ghrelin, which increased moderately in response to the stress, did not decline rapidly after a meal in emotional eaters as compared to non-emotional controls. To date, the blunted postprandial decreased level of ghrelin may prolong feeding signaling and reduce satiety response in both emotional and BE individuals [226,227].

## 6. Conclusion

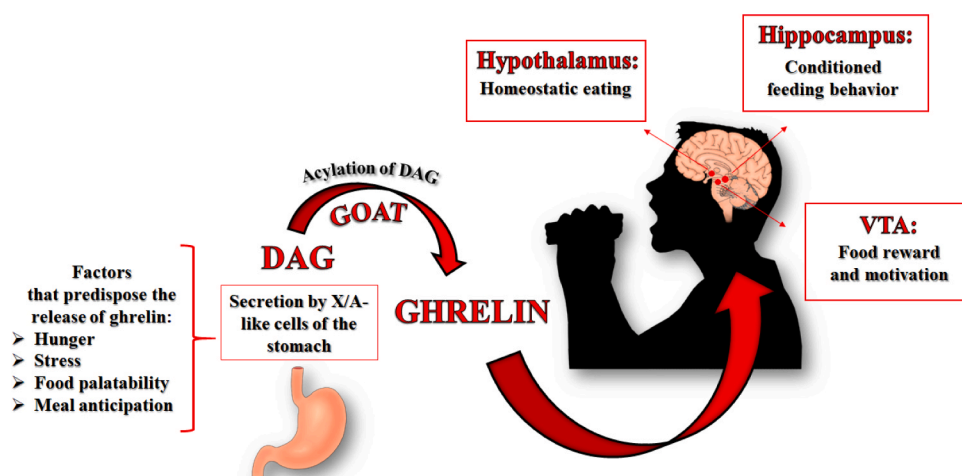
The ghrelin system and the enzyme GOAT, which mediates the acylation reaction, necessary for the production of this hormone in its active form AG, are increasingly involved in the complex modulation of food regulation, hunger stimulation, food choice, and obesity. They could potentially play a key role in BED, triggering reinforcement mechanisms associated with food reward and impulsive behaviors. Indeed, the ghrelin system is not only implicated in homeostatic feeding, but also acts in the mesolimbic system, as a promoter of food reward and craving, and in the hippocampus, associated with the learned and cognitive aspects of eating (Fig. 3). Interestingly, ghrelin performs orexigenic activity via interactions with several neurotransmitters, such as dopamine, opioids, endocannabinoids, and orexins, which are crucial mediators of food intake and aberrant feeding patterns. Preliminary encouraging anti-obesity results were shown by GOAT inhibitors and by the increase of DAG plasma levels, which revealed antagonist properties towards the orexigenic AG. DAG, being unable to activate GHSR1a, could potentially attenuate the activity of neural circuits that promote overeating of HPF and, additionally, reduce the ingestion of food even under aversive stressful conditions, affecting the HPA axis, body weight, and the growth of adipose tissue in both rodents and humans, factors not only behind the bingeing-related eating disorder, but also in obesity and related diseases. Thus, these effects suggest that the ghrelin-GOAT-GHSR1a system may be a target for reducing hedonic and non-homeostatic feeding.

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## CRediT authorship contribution statement

**Emanuela Micioni Di Bonaventura:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization (contributed equally to this review). **Luca Botticelli:** Conceptualization, Writing – original draft, Writing – review & editing, Visualization (contributed equally to this review). **Fabio Del Bello:** Writing – review & editing, Visualization. **Gianfabio Giorgioni:** Writing – review & editing, Visualization. **Alessandro Piergentili:** Writing – review & editing, Visualization. **Wilma Quaglia:** Writing – review & editing, Visualization. **Carlo Cifani:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition, Project administration, Visualization. **Maria Vittoria Micioni Di Bonaventura:** Conceptualization, Writing – review & editing, Supervision, Visualization. All authors have read and agreed to



**Fig. 3.** Summary of the principal factors that promote the release of ghrelin and the main brain areas involved in its orexigenic response. Ghrelin is secreted by the stomach in response to multiple factors and, after the conversion by the enzyme GOAT, it reaches the central nervous system, where it interacts with its receptor in brain areas involved in several aspects of eating behavior, such as the hypothalamus, VTA, and hippocampus. DAG: Des-Acyl-ghrelin, GOAT: Ghrelin O-Acyltransferase, VTA: Ventral Tegmental Area.

the published version of the manuscript.

## Conflict of interest

All authors declare that there are no conflicts of interest associated with the content of this paper.

## References

- [1] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, Ghrelin is a growth-hormone-releasing acylated peptide from stomach, *Nature* 402 (6762) (1999) 656–660.
- [2] M. Kojima, H. Hosoda, H. Matsuo, K. Kangawa, Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor, *Trends Endocrinol. Metab.* 12 (3) (2001) 118–122.
- [3] J. Yang, M.S. Brown, G. Liang, N.V. Grishin, J.L. Goldstein, Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone, *Cell* 132 (3) (2008) 387–396.
- [4] J.A. Gutierrez, P.J. Solenberg, D.R. Perkins, J.A. Willency, M.D. Knierman, Z. Jin, D.R. Wither, S. Luo, J.E. Onyia, J.E. Hale, Ghrelin octanoylation mediated by an orphan lipid transferase, *Proc. Natl. Acad. Sci. USA* 105 (17) (2008) 6320–6325.
- [5] H. Hosoda, M. Kojima, H. Matsuo, K. Kangawa, Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue, *Biochem. Biophys. Res. Commun.* 279 (3) (2000) 909–913.
- [6] M. Kojima, H. Hosoda, K. Kangawa, Clinical endocrinology and metabolism. Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach, *Best practice & research. Clin. Endocrinol. Metab.* 18 (4) (2004) 517–530.
- [7] H. Ariyasu, K. Takaya, T. Tagami, Y. Ogawa, K. Hosoda, T. Akamizu, M. Suda, T. Koh, K. Natsui, S. Toyooka, G. Shirakami, T. Usui, A. Shimatsu, K. Doi, H. Hosoda, M. Kojima, K. Kangawa, K. Nakao, Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans, *J. Clin. Endocrinol. Metab.* 86 (10) (2001) 4753–4758.
- [8] Y. Date, M. Kojima, H. Hosoda, A. Sawaguchi, M.S. Mondal, T. Suganuma, S. Matsukura, K. Kangawa, M. Nakazato, Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans, *Endocrinology* 141 (11) (2000) 4255–4261.
- [9] C. Dornonville de la Cour, M. Bjorkqvist, A.K. Sandvik, I. Bakke, C.M. Zhao, D. Chen, R. Hakanson, A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control, *Regul. Pept.* 99 (2–3) (2001) 141–150.
- [10] I. Sakata, K. Nakamura, M. Yamazaki, M. Matsubara, Y. Hayashi, K. Kangawa, T. Sakai, Ghrelin-producing cells exist as two types of cells, closed- and opened-type cells, in the rat gastrointestinal tract, *Peptides* 23 (3) (2002) 531–536.
- [11] J.P. Camina, Cell biology of the ghrelin receptor, *J. Neuroendocrinol.* 18 (1) (2006) 65–76.
- [12] A.D. Howard, S.D. Feighner, D.F. Cully, J.P. Arena, P.A. Liberato, C. I. Rosenblum, M. Hamelin, D.L. Hreniuk, O.C. Palyha, J. Anderson, P.S. Parass, C. Diaz, M. Chou, K.K. Liu, K.K. McKee, S.S. Pong, L.Y. Chung, A. Elbrecht, M. Dashkevich, R. Heavens, M. Rigby, D.J. Sirinathsinghji, D.C. Dean, D. G. Melillo, A.A. Patchett, R. Nargund, P.R. Griffin, J.A. DeMartino, S.K. Gupta, J. M. Schaeffer, R.G. Smith, L.H. Van der Ploeg, A receptor in pituitary and hypothalamus that functions in growth hormone release, *Science* 273 (5277) (1996) 974–977.
- [13] K.K. McKee, O.C. Palyha, S.D. Feighner, D.L. Hreniuk, C.P. Tan, M.S. Phillips, R. G. Smith, L.H. Van der Ploeg, A.D. Howard, Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors, *Mol. Endocrinol.* 11 (4) (1997) 415–423.
- [14] G.S. Tannenbaum, M. Lapointe, A. Beaudet, A.D. Howard, Expression of growth hormone secretagogue-receptors by growth hormone-releasing hormone neurons in the mediobasal hypothalamus, *Endocrinology* 139 (10) (1998) 4420–4423.
- [15] X.M. Guan, H. Yu, O.C. Palyha, K.K. McKee, S.D. Feighner, D.J. Sirinathsinghji, R. G. Smith, L.H. Van der Ploeg, A.D. Howard, Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues, *Brain Res. Mol. Brain Res.* 48 (1) (1997) 23–29.
- [16] M. Katayama, H. Nogami, J. Nishiyama, T. Kawase, K. Kawamura, Developmentally and regionally regulated expression of growth hormone secretagogue receptor mRNA in rat brain and pituitary gland, *Neuroendocrinology* 72 (6) (2000) 333–340.
- [17] A.M. Wren, C.J. Small, C.R. Abbott, W.S. Dhillo, L.J. Seal, M.A. Cohen, R. L. Batterham, S. Taheri, S.A. Stanley, M.A. Ghatel, S.R. Bloom, Ghrelin causes hyperphagia and obesity in rats, *Diabetes* 50 (11) (2001) 2540–2547.
- [18] M.A. Cowley, R.G. Smith, S. Diano, M. Tschop, N. Pronchuk, K.L. Grove, C. J. Strasburger, M. Bidlingmaier, M. Esterman, M.L. Heiman, L.M. Garcia-Segura, E.A. Nilini, P. Mendez, M.J. Low, P. Sotonyi, J.M. Friedman, H. Liu, S. Pinto, W. F. Colmers, R.D. Cone, T.L. Horvath, The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis, *Neuron* 37 (4) (2003) 649–661.
- [19] S. Malik, F. McGlone, D. Bedrossian, A. Dagher, Ghrelin modulates brain activity in areas that control appetitive behavior, *Cell Metab.* 7 (5) (2008) 400–409.
- [20] J. Kamegai, H. Tamura, T. Shimizu, S. Ishii, H. Sugihara, I. Wakabayashi, Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats, *Diabetes* 50 (11) (2001) 2438–2443.
- [21] M. Nakazato, N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, S. Matsukura, A role for ghrelin in the central regulation of feeding, *Nature* 409 (6817) (2001) 194–198.
- [22] A.M. Wren, L.J. Seal, M.A. Cohen, A.E. Brynes, G.S. Frost, K.G. Murphy, W. S. Dhillo, M.A. Ghatel, S.R. Bloom, Ghrelin enhances appetite and increases food intake in humans, *J. Clin. Endocrinol. Metab.* 86 (12) (2001) 5992.
- [23] D.E. Cummings, J.Q. Purnell, R.S. Frayo, K. Schimidova, B.E. Wisse, D.S. Weigle, A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans, *Diabetes* 50 (8) (2001) 1714–1719.
- [24] K. Howick, B.T. Griffin, J.F. Cryan, H. Schellekens, From belly to brain: targeting the ghrelin receptor in appetite and food intake regulation, *Int. J. Mol. Sci.* 18 (2) (2017).
- [25] D.L. Drazen, T.P. Vahl, D.A. D'Alessio, R.J. Seeley, S.C. Woods, Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status, *Endocrinology* 147 (1) (2006) 23–30.
- [26] M. Schaeffer, F. Langlet, C. Lafont, F. Molino, D.J. Hodson, T. Roux, L. Lamarque, P. Verdier, E. Bourrier, B. Dehouck, J.L. Baneris, J. Martinez, P.F. Mery, J. Marie, E. Trinquet, J.A. Fehrentz, V. Prevot, P. Mollard, Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons, *Proc. Natl. Acad. Sci. USA* 110 (4) (2013) 1512–1517.
- [27] Y. Date, T. Shimbara, S. Koda, K. Toshinari, T. Ida, N. Murakami, M. Miyazato, K. Kokame, Y. Ishizuka, Y. Ishida, H. Kageyama, S. Shioda, K. Kangawa, M. Nakazato, Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus, *Cell Metab.* 4 (4) (2006) 323–331.
- [28] M. Shintani, Y. Ogawa, K. Ebihara, M. Aizawa-Abe, F. Miyazawa, K. Takaya, T. Hayashi, G. Inoue, K. Hosoda, M. Kojima, K. Kangawa, K. Nakao, Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway, *Diabetes* 50 (2) (2001) 227–232.

- [29] S.R. Chen, H. Chen, J.J. Zhou, G. Pradhan, Y. Sun, H.L. Pan, D.P. Li, Ghrelin receptors mediate ghrelin-induced excitation of agouti-related protein/neuropeptide Y but not pro-opiomelanocortin neurons, *J. Neurochem.* 142 (4) (2017) 512–520.
- [30] J. Kwon Jeong, J. Dae Kim, S. Diano, Ghrelin regulates hypothalamic prolyl carboxypeptidase expression in mice, *Mol. Metab.* 2 (1) (2013) 23–30.
- [31] E. Micioni Di Bonaventura, L. Botticelli, D. Tomassoni, S.K. Tayebati, M. V. Micioni Di Bonaventura, C. Cifani, The melanocortin system behind the dysfunctional eating behaviors, *Nutrients* 12 (11) (2020).
- [32] S.H. Ju, G.B. Cho, J.W. Sohn, Understanding melanocortin-4 receptor control of neuronal circuits: Toward novel therapeutics for obesity syndrome, *Pharmacol. Res.* 129 (2018) 10–19.
- [33] S.L. Dickson, E. Eggecioglu, S. Landgren, K.P. Skibicka, J.A. Engel, E. Jerlhag, The role of the central ghrelin system in reward from food and chemical drugs, *Mol. Cell. Endocrinol.* 340 (1) (2011) 80–87.
- [34] E. Eggecioglu, E. Jerlhag, N. Salome, K.P. Skibicka, D. Haage, Y.M. Bohlooly, D. Andersson, M. Bjursell, D. Perrissoud, J.A. Engel, S.L. Dickson, Ghrelin increases intake of rewarding food in rodents, *Addict. Biol.* 15 (3) (2010) 304–311.
- [35] M. Perello, S.L. Dickson, Ghrelin signalling on food reward: a salient link between the gut and the mesolimbic system, *J. Neuroendocrinol.* 27 (6) (2015) 424–434.
- [36] M. Perello, I. Sakata, S. Birnbaum, J.C. Chuang, S. Osborne-Lawrence, S. A. Rovinsky, J. Woloszyn, M. Yanagisawa, M. Lutter, J.M. Zigman, Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner, *Biol. Psychiatry* 67 (9) (2010) 880–886.
- [37] P. Alvarez-Castro, L. Pena, F. Cordido, Ghrelin in obesity, physiological and pharmacological considerations, *Mini Rev. Med. Chem.* 13 (4) (2013) 541–552.
- [38] C.T. Lim, B. Kola, M. Korbonits, The ghrelin/GOAT/GHS-R system and energy metabolism, *Rev. Endocr. Metab. Disord.* 12 (3) (2011) 173–186.
- [39] O. Al Massadi, M. Lopez, M. Tschop, C. Dieguez, R. Nogueiras, Current understanding of the hypothalamic ghrelin pathways inducing appetite and adiposity, *Trends Neurosci.* 40 (3) (2017) 167–180.
- [40] O. Al Massadi, R. Nogueiras, C. Dieguez, J.A. Girault, Ghrelin and food reward, *Neuropharmacology* 148 (2019) 131–138.
- [41] A. Abizaid, Z.W. Liu, Z.B. Andrews, M. Shanabrough, E. Borok, J.D. Elsworth, R. H. Roth, M.W. Sleeman, M.R. Picciotto, M.H. Tschop, X.B. Gao, T.L. Horvath, Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite, *J. Clin. Investig.* 116 (12) (2006) 3229–3239.
- [42] J.M. Zigman, J.E. Jones, C.E. Lee, C.B. Saper, J.K. Elmquist, Expression of ghrelin receptor mRNA in the rat and the mouse brain, *J. Comp. Neurol.* 494 (3) (2006) 528–548.
- [43] P.J. Kenny, Common cellular and molecular mechanisms in obesity and drug addiction, *Nat. Rev. Neurosci.* 12 (11) (2011) 638–651.
- [44] N.D. Volkow, G.J. Wang, J.S. Fowler, D. Tomasi, Addiction circuitry in the human brain, *Annu. Rev. Pharmacol. Toxicol.* 52 (2012) 321–336.
- [45] L. Botticelli, E. Micioni Di Bonaventura, F. Del Bello, G. Giorgioni, A. Piergentili, A. Romano, W. Quaglia, C. Cifani, M.V. Micioni Di Bonaventura, Underlying susceptibility to eating disorders and drug abuse: genetic and pharmacological aspects of dopamine D4 receptors, *Nutrients* 12 (8) (2020).
- [46] S.G. Nair, B.M. Navarre, C. Cifani, C.L. Pickens, J.M. Bossert, Y. Shaham, Role of dorsal medial prefrontal cortex dopamine D1-family receptors in relapse to high-fat food seeking induced by the anxiogenic drug yohimbine, *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 36 (2) (2011) 497–510.
- [47] N.M. Avena, P. Rada, B.G. Hoebel, Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake, *Neurosci. Biobehav. Rev.* 32 (1) (2008) 20–39.
- [48] V. Bassareo, G. Di Chiara, Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments, *Neuroscience* 89 (3) (1999) 637–641.
- [49] E. Jerlhag, E. Eggecioglu, S.L. Dickson, A. Douhan, L. Svensson, J.A. Engel, Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens, *Addict. Biol.* 12 (1) (2007) 6–16.
- [50] J.J. Cone, J.E. McCutcheon, M.F. Roitman, Ghrelin acts as an interface between physiological state and phasic dopamine signaling, *J. Neurosci.: Off. J. Soc. Neurosci.* 34 (14) (2014) 4905–4913.
- [51] J.J. Cone, J.D. Roitman, M.F. Roitman, Ghrelin regulates phasic dopamine and nucleus accumbens signaling evoked by food-predictive stimuli, *J. Neurochem.* 133 (6) (2015) 844–856.
- [52] E. Jerlhag, E. Eggecioglu, S.L. Dickson, M. Andersson, L. Svensson, J.A. Engel, Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward, *Addict. Biol.* 11 (1) (2006) 45–54.
- [53] E. Jerlhag, E. Eggecioglu, S.L. Dickson, L. Svensson, J.A. Engel, Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors are involved in mediating the ghrelin-induced locomotor stimulation and dopamine overflow in nucleus accumbens, *Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol.* 18 (7) (2008) 508–518.
- [54] E. Jerlhag, A.C. Janson, S. Waters, J.A. Engel, Concomitant release of ventral tegmental acetylcholine and accumbal dopamine by ghrelin in rats, *PLoS One* 7 (11) (2012), e49557.
- [55] P. Mu, Y.H. Huang, Cholinergic system in sleep regulation of emotion and motivation, *Pharmacol. Res.* 143 (2019) 113–118.
- [56] S.L. Dickson, E. Hrabovszky, C. Hansson, E. Jerlhag, M. Alvarez-Crespo, K. P. Skibicka, C.S. Molnar, Z. Liposits, J.A. Engel, E. Eggecioglu, Blockade of central nicotinic acetylcholine receptor signaling attenuate ghrelin-induced food intake in rodents, *Neuroscience* 171 (4) (2010) 1180–1186.
- [57] E. Disse, A.L. Bussier, N. Deblon, P.T. Pfluger, M.H. Tschop, M. Laville, F. Rohner-Jeanrenaud, Systemic ghrelin and reward: effect of cholinergic blockade, *Physiol. Behav.* 102 (5) (2011) 481–484.
- [58] P.V. Rada, G.P. Mark, J.J. Yeomans, B.G. Hoebel, Acetylcholine release in ventral tegmental area by hypothalamic self-stimulation, eating, and drinking, *Pharmacol. Biochem. Behav.* 65 (3) (2000) 375–379.
- [59] J.S. Yeomans, A. Mathur, M. Tampakeras, Rewarding brain stimulation: role of tegmental cholinergic neurons that activate dopamine neurons, *Behav. Neurosci.* 107 (6) (1993) 1077–1087.
- [60] E. Jerlhag, E. Eggecioglu, S.L. Dickson, J.A. Engel, Glutamatergic regulation of ghrelin-induced activation of the mesolimbic dopamine system, *Addict. Biol.* 16 (1) (2011) 82–91.
- [61] E. Disse, A.L. Bussier, C. Veyrat-Durebex, N. Deblon, P.T. Pfluger, M.H. Tschop, M. Laville, F. Rohner-Jeanrenaud, Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content, *Physiol. Behav.* 101 (2) (2010) 277–281.
- [62] J.C. Chuang, M. Perello, I. Sakata, S. Osborne-Lawrence, J.M. Savitt, M. Lutter, J. M. Zigman, Ghrelin mediates stress-induced food-reward behavior in mice, *J. Clin. Investig.* 121 (7) (2011) 2684–2692.
- [63] C. Cifani, C. Polidori, S. Melotto, R. Ciccocioppo, M. Massi, A preclinical model of binge eating elicited by yo-yo dieting and stressful exposure to food: effect of sibutramine, fluoxetine, topiramate, and midazolam, *Psychopharmacology* 204 (1) (2009) 113–125.
- [64] W.F. Coutinho, R.O. Moreira, C. Spagnol, J.C. Appolinario, Does binge eating disorder alter cortisol secretion in obese women? *Eat. Behav.* 8 (1) (2007) 59–64.
- [65] E. Epel, R. Lapidus, B. McEwen, K. Brownell, Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior, *Psychoneuroendocrinology* 26 (1) (2001) 37–49.
- [66] M.E. Gluck, A. Geliebter, J. Hung, E. Yahav, Cortisol, hunger, and desire to binge eat following a cold stress test in obese women with binge eating disorder, *Psychosom. Med.* 66 (6) (2004) 876–881.
- [67] M.V. Micioni Di Bonaventura, R. Ciccocioppo, A. Romano, J.M. Bossert, K.C. Rice, M. Ubaldi, R. Laurent St, S. Gaetani, M. Massi, Y. Shaham, C. Cifani, Role of bed nucleus of the stria terminalis corticotrophin-releasing factor receptors in frustration stress-induced binge-like palatable food consumption in female rats with a history of food restriction, *J. Neurosci.: Off. J. Soc. Neurosci.* 34 (34) (2014) 11316–11324.
- [68] M.V. Micioni Di Bonaventura, E. Micioni Di Bonaventura, C. Polidori, C. Cifani, Preclinical models of stress and environmental influences on binge eating, in: G.K. W. Frank, L.A. Berner (Eds.), *Binge Eating: A Transdiagnostic Psychopathology*, Springer International Publishing, Cham, 2020, pp. 85–101.
- [69] K.P. Skibicka, C. Hansson, E. Eggecioglu, S.L. Dickson, Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression, *Addict. Biol.* 17 (1) (2012) 95–107.
- [70] J. Overduin, D.P. Figlewicz, J. Bennett-Jay, S. Kittleson, D.E. Cummings, Ghrelin increases the motivation to eat, but does not alter food palatability, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 303 (3) (2012) R259–R269.
- [71] T. Bake, C.E. Edvardsson, C.J. Cummings, S.L. Dickson, Ghrelin's effects on food motivation in rats are not limited to palatable foods, *J. Neuroendocrinol.* 31 (7) (2019), 12665.
- [72] S.J. King, A.M. Isaacs, E. O'Farrell, A. Abizaid, Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area, *Horm. Behav.* 60 (5) (2011) 572–580.
- [73] K.P. Skibicka, C. Hansson, M. Alvarez-Crespo, P.A. Friberg, S.L. Dickson, Ghrelin directly targets the ventral tegmental area to increase food motivation, *Neuroscience* 180 (2011) 129–137.
- [74] K.P. Skibicka, R.H. Shirazi, C. Rabasa-Papio, M. Alvarez-Crespo, C. Neuber, H. Vogel, S.L. Dickson, Divergent circuitry underlying food reward and intake effects of ghrelin: dopaminergic VTA-accumbens projection mediates ghrelin's effect on food reward but not food intake, *Neuropharmacology* 73 (2013) 274–283.
- [75] X.J. Wei, B. Sun, K. Chen, B. Lv, X. Luo, J.Q. Yan, Ghrelin signaling in the ventral tegmental area mediates both reward-based feeding and fasting-induced hyperphagia on high-fat diet, *Neuroscience* 300 (2015) 53–62.
- [76] A.E. Price, N.C. Anastasio, S.J. Stutz, J.D. Hommel, K.A. Cunningham, Serotonin 5-HT<sub>2C</sub> receptor activation suppresses binge intake and the reinforcing and motivational properties of high-fat food, *Front. Pharmacol.* 9 (2018) 821.
- [77] S. Sirohi, J.D. Schurdak, R.J. Seeley, S.C. Benoit, J.F. Davis, Central & peripheral glucagon-like peptide-1 receptor signaling differentially regulate addictive behaviors, *Physiol. Behav.* 161 (2016) 140–144.
- [78] P. Xu, Y. He, X. Cao, L. Valencia-Torres, X. Yan, K. Saito, C. Wang, Y. Yang, A. Hinton, Jr, L. Zhu, G. Shu, M.G. Myers, Jr, Q. Wu, Q. Tong, L.K. Heisler, Y. Xu, Activation of serotonin 2C receptors in dopamine neurons inhibits binge-like eating in mice, *Biol. Psychiatry* 81 (9) (2017) 737–747.
- [79] Y. Yang, P.P. Choi, W.W. Smith, W. Xu, D. Ma, Z.A. Corder, N.C. Liang, T. H. Moran, Exendin-4 reduces food intake via the PI3K/AKT signaling pathway in the hypothalamus, *Sci. Rep.* 7 (1) (2017) 6936.
- [80] T. Rodrigues, P. Borges, L. Mar, D. Marques, M. Albano, H. Eickhoff, C. Carrelo, B. Almeida, S. Pires, M. Abrantes, B. Martins, C. Uriarte, F. Botelho, P. Gomes, S. Silva, R. Seica, P. Matafome, GLP-1 improves adipose tissue glyoxalase activity



- and capillarization improving insulin sensitivity in type 2 diabetes, *Pharmacol. Res.* 161 (2020), 105198.
- [81] E. Howell, H.M. Baumgartner, L.J. Zallar, J.A. Selva, L. Engel, P.J. Currie, Glucagon-like peptide-1 (GLP-1) and 5-hydroxytryptamine 2c (5-HT<sub>2c</sub>) receptor agonists in the ventral tegmental area (VTA) inhibit ghrelin-stimulated appetitive reward, *Int. J. Mol. Sci.* 20 (4) (2019).
- [82] M.J. Bubar, S.J. Stutz, K.A. Cunningham, 5-HT(2C) receptors localize to dopamine and GABA neurons in the rat mesoaccumbens pathway, *PLoS One* 6 (6) (2011), 20508.
- [83] I. Merchenthaler, M. Lane, P. Shughrue, Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system, *J. Comp. Neurol.* 403 (2) (1999) 261–280.
- [84] J.A. Engel, I. Nylander, E. Jerlhag, A ghrelin receptor (GHS-R1A) antagonist attenuates the rewarding properties of morphine and increases opioid peptide levels in reward areas in mice, *Eur. Neuropsychopharmacol.* J. Eur. Coll. Neuropsychopharmacol. 25 (12) (2015) 2364–2371.
- [85] P. Jerabek, T. Havlickova, N. Puskina, C. Charalambous, M. Lapka, P. Kacer, M. Sustkova-Fiserova, Ghrelin receptor antagonism of morphine-induced conditioned place preference and behavioral and accumbens dopaminergic sensitization in rats, *Neurochem. Int.* 110 (2017) 101–113.
- [86] M. Sustkova-Fiserova, P. Jerabek, T. Havlickova, P. Kacer, M. Krsiak, Ghrelin receptor antagonism of morphine-induced accumbens dopamine release and behavioral stimulation in rats, *Psychopharmacology* 231 (14) (2014) 2899–2908.
- [87] M. Sustkova-Fiserova, N. Puskina, T. Havlickova, M. Lapka, K. Syslova, V. Pohorala, C. Charalambous, Ghrelin receptor antagonism of fentanyl-induced conditioned place preference, intravenous self-administration, and dopamine release in the nucleus accumbens in rats, *Addict. Biol.* 25 (6) (2020), 12845.
- [88] T.M. D'Cunha, A. Chisholm, C. Hryhorczuk, S. Fulton, U. Shalev, A role for leptin and ghrelin in the augmentation of heroin seeking induced by chronic food restriction, *Psychopharmacology* 237 (3) (2020) 787–800.
- [89] R.J. Bodnar, Endogenous opioid modulation of food intake and body weight: Implications for opioid influences upon motivation and addiction, *Peptides* 116 (2019) 42–62.
- [90] B.A. Gosnell, A.S. Levine, Reward systems and food intake: role of opioids, *Int. J. Obes.* 33 (Suppl. 2) (2009) S54–S58.
- [91] S. Pecina, K.S. Smith, Hedonic and motivational roles of opioids in food reward: implications for overeating disorders, *Pharmacol. Biochem. Behav.* 97 (1) (2010) 34–46.
- [92] J.A. Echo, N. Lamonte, T.F. Ackerman, R.J. Bodnar, Alterations in food intake elicited by GABA and opioid agonists and antagonists administered into the ventral tegmental area region of rats, *Physiol. Behav.* 76 (1) (2002) 107–116.
- [93] G. Tanda, G. Di Chiara, A dopamine-mu1 opioid link in the rat ventral tegmentum shared by palatable food (Fonzies) and non-psychostimulant drugs of abuse, *Eur. J. Neurosci.* 10 (3) (1998) 1179–1187.
- [94] A.M. Naleid, M.K. Grace, D.E. Cummings, A.S. Levine, Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens, *Peptides* 26 (11) (2005) 2274–2279.
- [95] K.P. Skibicka, R.H. Shirazi, C. Hansson, S.L. Dickson, Ghrelin interacts with neuropeptide Y Y1 and opioid receptors to increase food reward, *Endocrinology* 153 (3) (2012) 1194–1205.
- [96] A. Romero-Pico, M.J. Vazquez, D. Gonzalez-Touceda, C. Folgueira, K.P. Skibicka, M. Alvarez-Crespo, M.A. Van Gestel, D.A. Velasquez, C. Schwarzer, H. Herzog, M. Lopez, R.A. Adan, S.L. Dickson, C. Dieguez, R. Nogueiras, Hypothalamic kappa-opioid receptor modulates the orexigenic effect of ghrelin, *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 38 (7) (2013) 1296–1307.
- [97] A.W. Bruijnzeel, kappa-Opioid receptor signaling and brain reward function, *Brain Res. Rev.* 62 (1) (2009) 127–146.
- [98] Y. Kawahara, F. Kaneko, M. Yamada, Y. Kishikawa, H. Kawahara, A. Nishi, Food reward-sensitive interaction of ghrelin and opioid receptor pathways in mesolimbic dopamine system, *Neuropharmacology* 67 (2013) 395–402.
- [99] A. Rashidy-Pour, P. Pahlevani, A. Vaziri, P. Shaigani, L. Zarepour, A.A. Vafaei, A. Haghparsat, Involvement of CB1 receptors in the ventral tegmental area in the potentiation of morphine rewarding properties in acquisition but not expression in the conditioned place preference model, *Behav. Brain Res.* 247 (2013) 259–267.
- [100] H. Zhang, A.A. Lipinski, E. Liktors-Busa, A.F. Smith, A. Moutal, R. Khanna, P. R. Langlais, T.M. Largent-Milnes, T.W. Vanderah, The effects of repeated morphine treatment on the endogenous cannabinoid system in the ventral tegmental area, *Front. Pharmacol.* 12 (2021), 632757.
- [101] T.M. Gomes, D. Dias da Silva, H. Carmo, F. Carvalho, J.P. Silva, Epigenetics and the endocannabinoid system signaling: an intricate interplay modulating neurodevelopment, *Pharmacol. Res.* 162 (2020), 105237.
- [102] G. Morris, K. Walder, S. Kloiber, P. Amming, M. Berk, C.C. Bortolasci, M. Maes, B.K. Puri, A.F. Carvalho, The endocannabinoidome in neuropsychiatry: opportunities and potential risks, *Pharmacol. Res.* 170 (2021), 105729.
- [103] M. Sustkova-Fiserova, C. Charalambous, T. Havlickova, M. Lapka, P. Jerabek, N. Puskina, K. Syslova, Alterations in rat accumbens endocannabinoid and GABA content during fentanyl treatment: the role of ghrelin, *Int. J. Mol. Sci.* 18 (11) (2017).
- [104] M. Sustkova-Fiserova, P. Jerabek, T. Havlickova, K. Syslova, P. Kacer, Ghrelin and endocannabinoids participation in morphine-induced effects in the rat nucleus accumbens, *Psychopharmacology* 233 (3) (2016) 469–484.
- [105] C. Charalambous, T. Havlickova, M. Lapka, N. Puskina, R. Slamberova, M. Kuchar, M. Sustkova-Fiserova, Cannabinoid-induced conditioned place preference, intravenous self-administration, and behavioral stimulation influenced by ghrelin receptor antagonism in rats, *Int. J. Mol. Sci.* 22 (5) (2021).
- [106] C. Charalambous, M. Lapka, T. Havlickova, K. Syslova, M. Sustkova-Fiserova, Alterations in rat accumbens dopamine, endocannabinoids and GABA content during WIN55,212-2 treatment: the role of ghrelin, *Int. J. Mol. Sci.* 22 (1) (2020).
- [107] C. D'Addario, M.V. Micioni Di Bonaventura, M. Pucci, A. Romano, S. Gaetani, R. Ciccocioppo, C. Cifani, M. Maccarrone, Endocannabinoid signaling and food addiction, *Neurosci. Biobehav. Rev.* 47 (2014) 203–224.
- [108] M. Pucci, E. Zaplati, M.V. Micioni Di Bonaventura, E. Micioni Di Bonaventura, P. De Cristofaro, M. Maccarrone, C. Cifani, C. D'Addario, On the role of central type-1 cannabinoid receptor gene regulation in food intake and eating behaviors, *Int. J. Mol. Sci.* 22 (1) (2021).
- [109] C.H. Ting, C.W. Chi, C.P. Li, C.Y. Chen, Differential modulation of endogenous cannabinoid CB1 and CB2 receptors in spontaneous and splice variants of ghrelin-induced food intake in conscious rats, *Nutrition* 31 (1) (2015) 230–235.
- [110] S.A. Tucci, E.K. Rogers, M. Korbonits, T.C. Kirkham, The cannabinoid CB1 receptor antagonist SR141716 blocks the orexigenic effects of intrahypothalamic ghrelin, *Br. J. Pharmacol.* 143 (5) (2004) 520–523.
- [111] A.E. Rigamonti, C. Giordani, S.G. Bonomo, S.G. Cella, E.E. Muller, Early tolerance to the hypophagic effect of the cannabinoid receptor antagonist SR141716 does not impede blockade of an orexigenic stimulus, *Eur. J. Pharmacol.* 542 (2006) 116–120.
- [112] B. Kola, I. Farkas, M. Christ-Crain, G. Wittmann, F. Lolli, F. Amin, J. Harvey-White, Z. Liposits, G. Kunos, A.B. Grossman, C. Fekete, M. Korbonits, The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system, *PLoS One* 3 (3) (2008), 1797.
- [113] C.T. Lim, B. Kola, D. Feltrin, D. Perez-Tilve, M.H. Tschop, A.B. Grossman, M. Korbonits, Ghrelin and cannabinoid receptors regulate the orexigenic effect to affect cellular energy metabolism, *Mol. Cell. Endocrinol.* 365 (2) (2013) 303–308.
- [114] F. Alen, I. Crespo, M.T. Ramirez-Lopez, N. Jagerovic, P. Goya, F.R. de Fonseca, R. G. de Heras, L. Orio, Ghrelin-induced orexigenic effect in rats depends on the metabolic status and is counteracted by peripheral CB1 receptor antagonism, *PLoS One* 8 (4) (2013), 60918.
- [115] L.L. Senin, O. Al-Massadi, C. Folgueira, C. Castela, M. Pardo, S. Barja-Fernandez, A. Roca-Rivada, M. Amil, A.B. Crujeiras, T. Garcia-Caballero, E. Gabellieri, R. Leis, C. Dieguez, U. Pagotto, F.F. Casanueva, L.M. Seoane, The gastric CB1 receptor modulates ghrelin production through the mTOR pathway to regulate food intake, *PLoS One* 8 (11) (2013), 80339.
- [116] F.A. Moreira, J.A. Crippa, The psychiatric side-effects of rimonabant, *Rev. Bras. Psiquiatr.* 31 (2) (2009) 145–153.
- [117] A.L. Kalafateli, D. Vallof, J.W. Jorulf, M. Heilig, E. Jerlhag, A cannabinoid receptor antagonist attenuates ghrelin-induced activation of the mesolimbic dopamine system in mice, *Physiol. Behav.* 184 (2018) 211–219.
- [118] T. Sakurai, A. Amemiya, M. Ishii, I. Matsuzaki, R.M. Chemelli, H. Tanaka, S. C. Williams, J.A. Richardson, G.P. Kozlowski, S. Wilson, J.R. Arch, R. E. Buckingham, A.C. Haynes, A.S. Carr, R.S. Annan, D.E. McNulty, W.S. Liu, J. A. Terrett, N.A. Elshourbagy, D.J. Bergsma, M. Yanagisawa, Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior, *Cell* 92 (4) (1998) 573–585.
- [119] Y. Date, Y. Ueta, H. Yamashita, H. Yamaguchi, S. Matsukura, K. Kangawa, T. Sakurai, M. Yanagisawa, M. Nakazato, Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems, *Proc. Natl. Acad. Sci. USA* 96 (2) (1999) 748–753.
- [120] C. Peyron, D.K. Tighe, A.N. van den Pol, L. de Lecea, H.C. Heller, J.G. Sutcliffe, T. S. Kilduff, Neurons containing hypocretin (orexin) project to multiple neuronal systems, *J. Neurosci.: Off. J. Soc. Neurosci.* 18 (23) (1998) 9996–10015.
- [121] S. Nishino, M. Okuro, N. Kotorii, E. Aneagawa, Y. Ishimaru, M. Matsumura, T. Kanbayashi, Hypocretin/orexin and narcolepsy: new basic and clinical insights, *Acta Physiol.* 198 (3) (2010) 209–222.
- [122] T. Sakurai, Roles of orexins and orexin receptors in central regulation of feeding behavior and energy homeostasis, *CNS Neurol. Disord. Drug Targets* 5 (3) (2006) 313–325.
- [123] A.C. Haynes, B. Jackson, H. Chapman, M. Tadayyon, A. Johns, R.A. Porter, J. R. Arch, A selective orexin-1 receptor antagonist reduces food consumption in male and female rats, *Regul. Pept.* 96 (1–2) (2000) 45–51.
- [124] S.G. Nair, S.A. Golden, Y. Shaham, Differential effects of the hypocretin 1 receptor antagonist SB 334867 on high-fat food self-administration and reinstatement of food seeking in rats, *Br. J. Pharmacol.* 154 (2) (2008) 406–416.
- [125] L. Piccoli, M.V. Micioni Di Bonaventura, C. Cifani, V.J. Costantini, M. Massagrande, D. Montanari, P. Martinelli, M. Antolini, R. Ciccocioppo, M. Massi, E. Merlo-Pich, R. Di Fabio, M. Corsi, Role of orexin-1 receptor mechanisms on compulsive food consumption in a model of binge eating in female rats, *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 37 (9) (2012) 1999–2011.
- [126] C.B. Lawrence, A.C. Snape, F.M. Baudoin, S.M. Luckman, Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers, *Endocrinology* 143 (1) (2002) 155–162.
- [127] P.K. Olszewski, D. Li, M.K. Grace, C.J. Billington, C.M. Kotz, A.S. Levine, Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus, *Peptides* 24 (4) (2003) 597–602.
- [128] M. So, H. Hashimoto, R. Saito, Y. Yamamoto, Y. Motojima, H. Ueno, S. Sonoda, M. Yoshimura, T. Maruyama, K. Kusuhara, Y. Ueta, Inhibition of ghrelin-induced feeding in rats by pretreatment with a novel dual orexin receptor antagonist, *J. Physiol. Sci.: JPS* 68 (2) (2018) 129–136.
- [129] K. Toshinai, Y. Date, N. Murakami, M. Shimada, M.S. Mondal, T. Shimbara, J. L. Guan, Q.P. Wang, H. Funahashi, T. Sakurai, S. Shioda, S. Matsukura,

- K. Kangawa, M. Nakazato, Ghrelin-induced food intake is mediated via the orexin pathway, *Endocrinology* 144 (4) (2003) 1506–1512.
- [130] V. Mitchell, S. Bouret, J.C. Beauvillain, A. Schilling, M. Perret, C. Kordon, J. Epelbaum, Comparative distribution of mRNA encoding the growth hormone secretagogue-receptor (GHS-R) in *Microcebus murinus* (Primate, lemurian) and rat forebrain and pituitary, *J. Comp. Neurol.* 429 (3) (2001) 469–489.
- [131] A. Yamanaka, C.T. Beuckmann, J.T. Willie, J. Hara, N. Tsujino, M. Mieda, M. Tominaga, K. Yagami, F. Sugiyama, K. Goto, M. Yanagisawa, T. Sakurai, Hypothalamic orexin neurons regulate arousal according to energy balance in mice, *Neuron* 38 (5) (2003) 701–713.
- [132] K.A. Richardson, G. Aston-Jones, Lateral hypothalamic orexin/hypocretin neurons that project to ventral tegmental area are differentially activated with morphine preference, *The, J. Neurosci.: Off. J. Soc. Neurosci.* 32 (11) (2012) 3809–3817.
- [133] G. Aston-Jones, R.J. Smith, G.C. Sartor, D.E. Moorman, L. Massi, P. Tahsili-Fahadan, K.A. Richardson, Lateral hypothalamic orexin/hypocretin neurons: a role in reward-seeking and addiction, *Brain Res.* 1314 (2010) 74–90.
- [134] A.M. Cason, R.J. Smith, P. Tahsili-Fahadan, D.E. Moorman, G.C. Sartor, G. Aston-Jones, Role of orexin/hypocretin in reward-seeking and addiction: implications for obesity, *Physiol. Behav.* 100 (5) (2010) 419–428.
- [135] L. Lopez-Ferreras, J.E. Richard, R.H. Anderberg, F.H. Nilsson, K. Olandersson, S.E. Kanoski, K.P. Skibicka, Ghrelin's control of food reward and body weight in the lateral hypothalamic area is sexually dimorphic, *Physiol. Behav.* 176 (2017) 40–49.
- [136] T.M. Hsu, J.D. Hahn, V.R. Konanur, E.E. Noble, A.N. Suarez, J. Thai, E. M. Nakamoto, S.E. Kanoski, Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways, *eLife* 4 (2015).
- [137] A.N. Suarez, C.M. Liu, A.M. Cortella, E.E. Noble, S.E. Kanoski, Ghrelin and orexin interact to increase meal size through a descending hippocampus to hindbrain signaling pathway, *Biol. Psychiatry* 87 (11) (2020) 1001–1011.
- [138] S.E. Kanoski, S.M. Fortin, K.M. Ricks, H.J. Grill, Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling, *Biol. Psychiatry* 73 (9) (2013) 915–923.
- [139] H. Kirchner, J.A. Gutierrez, P.J. Solenberg, P.T. Pfluger, T.A. Czyzyk, J. A. Willency, A. Schurmann, H.G. Joost, R.J. Jandacek, J.E. Hale, M.L. Heiman, M. H. Tschop, GOAT links dietary lipids with the endocrine control of energy balance, *Nat. Med.* 15 (7) (2009) 741–745.
- [140] M. Kojima, H. Ohgusu, Chapter 237 - ghrelin O-acyltransferase (GOAT), in: A. J. Kastin (Ed.), *Handbook of Biologically Active Peptides*, Second edition., Academic Press, Boston, 2013, pp. 1730–1735.
- [141] M.D. Gahete, J. Cordoba-Chacon, R. Salvatori, J.P. Castano, R.D. Kineman, R. M. Luque, Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach, *Mol. Cell. Endocrinol.* 317 (1–2) (2010) 154–160.
- [142] C.R. Gonzalez, M.J. Vazquez, M. Lopez, C. Dieguez, Influence of chronic undernutrition and leptin on GOAT mRNA levels in rat stomach mucosa, *J. Mol. Endocrinol.* 41 (6) (2008) 415–421.
- [143] O. Gualillo, J.E. Caminos, R. Nogueiras, L.M. Seoane, E. Arvat, E. Ghigo, F. F. Casanueva, C. Dieguez, Effect of food restriction on ghrelin in normal-cycling female rats and in pregnancy, *Obes. Res.* 10 (7) (2002) 682–687.
- [144] M. Wellman, A. Abizaid, Knockdown of central ghrelin O-acyltransferase by vivo-morpholino reduces body mass of rats fed a high-fat diet, *Peptides* 70 (2015) 17–22.
- [145] H. Yang, Y.H. Youm, C. Nakata, V.D. Dixit, Chronic caloric restriction induces forestomach hypertrophy with enhanced ghrelin levels during aging, *Peptides* 28 (10) (2007) 1931–1936.
- [146] R. Barazzoni, M. Zanetti, M. Stebel, G. Biolo, L. Cattin, G. Guarneri, Hyperleptinemia prevents increased plasma ghrelin concentration during short-term moderate caloric restriction in rats, *Gastroenterology* 124 (5) (2003) 1188–1192.
- [147] R.A. Reimer, A.D. Maurer, D.C. Lau, R.N. Auer, Long-term dietary restriction influences plasma ghrelin and GOAT mRNA level in rats, *Physiol. Behav.* 99 (5) (2010) 605–610.
- [148] S. Perna, D. Spadaccini, C. Gasparri, G. Peroni, V. Infantino, G. Iannello, A. Riva, G. Petrangolini, T.A. Alalwan, S. Al-Thawadi, M. Rondanelli, Association between des-acyl ghrelin at fasting and predictive index of muscle derangement, metabolic markers and eating disorders: a cross-sectional study in overweight and obese adults, *Nutr. Neurosci.* (2020) 1–7.
- [149] G. Fernandez, A. Cabral, M.P. Cornejo, P.N. De Francesco, G. Garcia-Romero, M. Reynaldo, M. Perello, Des-acyl ghrelin directly targets the arcuate nucleus in a ghrelin-receptor independent manner and impairs the orexigenic effect of ghrelin, *J. Neuroendocrinol.* 28 (2) (2016) 12349.
- [150] A. Asakawa, A. Inui, M. Fujimiya, R. Sakamaki, N. Shinfuku, Y. Ueta, M. M. Meguid, M. Kasuga, Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin, *Gut* 54 (1) (2005) 18–24.
- [151] T. Inhoff, H. Monnikes, S. Noetzel, A. Stengel, M. Goebel, Q.T. Dinh, A. Riedl, N. Bannert, A.S. Wisse, B. Wiedenmann, B.F. Klapp, Y. Tache, P. Kobelt, Desacyl ghrelin inhibits the orexigenic effect of peripherally injected ghrelin in rats, *Peptides* 29 (12) (2008) 2159–2168.
- [152] J.F. Davis, M. Perello, D.L. Choi, I.J. Magrisso, H. Kirchner, P.T. Pfluger, M. Tschop, J.M. Zigman, S.C. Benoit, GOAT induced ghrelin acylation regulates hedonic feeding, *Horm. Behav.* 62 (5) (2012) 598–604.
- [153] T. Kouno, N. Akiyama, T. Ito, T. Okuda, I. Nanchi, M. Notoya, S. Oka, H. Yukioka, Ghrelin O-acyltransferase knockout mice show resistance to obesity when fed high-sucrose diet, *J. Endocrinol.* 228 (2) (2016) 115–125.
- [154] T. Kouno, N. Akiyama, T. Ito, K. Fujieda, I. Nanchi, T. Okuda, M. Notoya, T. Iwasaki, H. Yukioka, The role of acylated-ghrelin in the regulation of sucrose intake, *Endocr. J.* 64 (Suppl.) (2017) S21–S23.
- [155] Y. Nishi, H. Hiejima, H. Hosoda, H. Kaiya, K. Mori, Y. Fukue, T. Yanase, H. Nawata, K. Kangawa, M. Kojima, Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin, *Endocrinology* 146 (5) (2005) 2255–2264.
- [156] T. Kouno, N. Akiyama, K. Fujieda, I. Nanchi, T. Okuda, T. Iwasaki, S. Oka, H. Yukioka, Reduced intake of carbohydrate prevents the development of obesity and impaired glucose metabolism in ghrelin O-acyltransferase knockout mice, *Peptides* 86 (2016) 145–152.
- [157] H. Cai, W.N. Cong, C.M. Daimon, R. Wang, M.H. Tschop, J. Sevigny, B. Martin, S. Maudsley, Altered lipid and salt taste responsiveness in ghrelin and GOAT null mice, *PLoS One* 8 (10) (2013), 76553.
- [158] M. Nunez-Salces, H. Li, S. Christie, A.J. Page, The effect of high-fat diet-induced obesity on the expression of nutrient chemosensors in the mouse stomach and the gastric ghrelin cell, *Nutrients* 12 (9) (2020).
- [159] S. Sirohi, A. Van Cleef, J.F. Davis, Patterned feeding induces neuroendocrine, behavioral and genetic changes that promote palatable food intake, *Int. J. Obes.* 41 (3) (2017) 412–419.
- [160] American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders: DSM-5*, American Psychiatric Association, Arlington, VA, 2013.
- [161] S. Allas, T. Abribat, Clinical perspectives for ghrelin-derived therapeutic products, *Endocr. Dev.* 25 (2013) 157–166.
- [162] M.A. Schalla, A. Stengel, Pharmacological modulation of ghrelin to induce weight loss: successes and challenges, *Curr. Diabetes Rep.* 19 (10) (2019) 102.
- [163] B.P. Barnett, Y. Hwang, M.S. Taylor, H. Kirchner, P.T. Pfluger, V. Bernard, Y. Y. Lin, E.M. Bowers, C. Mukherjee, W.J. Song, P.A. Longo, D.J. Leahy, M. A. Hussain, M.H. Tschop, J.D. Boeke, P.A. Cole, Glucose and weight control in mice with a designed ghrelin O-acyltransferase inhibitor, *Science* 330 (6011) (2010) 1689–1692.
- [164] P. Teuffel, L. Wang, P. Prinz, M. Goebel-Stengel, S. Scharner, P. Kobelt, T. Hofmann, M. Rose, B.F. Klapp, J.R. Reeve Jr., A. Stengel, Treatment with the ghrelin-O-acyltransferase (GOAT) inhibitor GO-CoA-Tat reduces food intake by reducing meal frequency in rats, *J. Physiol. Pharmacol.: Off. J. Pol. Physiol. Soc.* 66 (4) (2015) 493–503.
- [165] M. Rucinski, A. Ziolkowska, M. Szyszka, A. Hochol, L.K. Malendowicz, Evidence suggesting that ghrelin O-acyl transferase inhibitor acts at the hypothalamus to inhibit hypothalamo-pituitary-adrenocortical axis function in the rat, *Peptides* 35 (2) (2012) 149–159.
- [166] R.H. Anderberg, C. Hansson, M. Fenander, J.E. Richard, S.L. Dickson, H. Nissbrandt, F. Bergquist, K.P. Skibicka, The stomach-derived hormone ghrelin increases impulsive behavior, *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 41 (5) (2016) 1199–1209.
- [167] E. Ralevski, M. Shanabrough, J. Newcomb, E. Gandelman, R. Hayden, T. L. Horvath, I. Petrakis, Ghrelin is related to personality differences in reward sensitivity and impulsivity, *Alcohol Alcohol.* 53 (1) (2018) 52–56.
- [168] A. Geliebter, C.N. Ochner, R. Aviram-Friedman, Appetite-related gut peptides in obesity and binge eating disorder, *Am. J. Lifestyle Med.* 2 (4) (2008) 305–314.
- [169] X. Zhang, A.N. van den Pol, Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation, *Science* 356 (6340) (2017) 853–859.
- [170] N.T. Bello, A.S. Guarda, C.E. Terrillion, G.W. Redgrave, J.W. Coughlin, T. H. Moran, Repeated binge access to a palatable food alters feeding behavior, hormone profile, and hindbrain c-Fos responses to a test meal in adult male rats, *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 297 (3) (2009) R622–R631.
- [171] T. Bake, D.G. Morgan, J.G. Mercer, Feeding and metabolic consequences of scheduled consumption of large, binge-type meals of high fat diet in the Sprague-Dawley rat, *Physiol. Behav.* 128 (2014) 70–79.
- [172] S. Valdivia, M.P. Cornejo, M. Reynaldo, P.N. De Francesco, M. Perello, Escalation in high fat intake in a binge eating model differentially engages dopamine neurons of the ventral tegmental area and requires ghrelin signaling, *Psychoneuroendocrinology* 60 (2015) 206–216.
- [173] S.J. King, T. Rodrigues, A. Watts, E. Murray, A. Wilson, A. Abizaid, Investigation of a role for ghrelin signaling in binge-like feeding in mice under limited access to high-fat diet, *Neuroscience* 319 (2016) 233–245.
- [174] M.P. Cornejo, D. Castrogiovanni, H.B. Schioth, M. Reynaldo, J. Marie, J. A. Fehrentz, M. Perello, Growth hormone secretagogue receptor signalling affects high-fat intake independently of plasma levels of ghrelin and LEAP2, in a 4-day binge eating model, *J. Neuroendocrinol.* 31 (10) (2019), 12785.
- [175] M.P. Cornejo, F. Barrile, D. Cassano, J.P. Aguggia, G. Garcia Romero, M. Reynaldo, M.F. Andreoli, P.N. De Francesco, M. Perello, Growth hormone secretagogue receptor in dopamine neurons controls appetitive and consummatory behaviors towards high-fat diet in ad-libitum fed mice, *Psychoneuroendocrinology* 119 (2020), 104718.
- [176] L.A. Verhagen, E. Egicioglu, M.C. Luijendijk, J.J. Hillebrand, R.A. Adan, S. L. Dickson, Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity, *Eur. Neuropsychopharmacol.: J. Eur. Coll. Neuropsychopharmacol.* 21 (5) (2011) 384–392.
- [177] I.D. Blum, Z. Patterson, R. Khazall, E.W. Lamont, M.W. Sleeman, T.L. Horvath, A. Abizaid, Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice, *Neuroscience* 164 (2) (2009) 351–359.
- [178] M. Merkestein, M.A. Brans, M.C. Luijendijk, J.W. de Jong, E. Egicioglu, S. L. Dickson, R.A. Adan, Ghrelin mediates anticipation to a palatable meal in rats, *Obesity* 20 (5) (2012) 963–971.

- [179] T. Bake, K.T. Hellgren, S.L. Dickson, Acute ghrelin changes food preference from a high-fat diet to chow during binge-like eating in rodents, *J. Neuroendocrinol.* 29 (4) (2017).
- [180] T. Shimbara, M.S. Mondal, T. Kawagoe, K. Toshinai, S. Koda, H. Yamaguchi, Y. Date, M. Nakazato, Central administration of ghrelin preferentially enhances fat ingestion, *Neurosci. Lett.* 369 (1) (2004) 75–79.
- [181] A.P. Goldstone, C.G. Precht, S. Scholtz, A.D. Miras, N. Chhina, G. Durighel, S. S. Deliran, C. Beckmann, M.A. Ghatei, D.R. Ashby, A.D. Waldman, B.D. Gaylin, M.O. Thorner, G.S. Frost, S.R. Bloom, J.D. Bell, Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food, *Am. J. Clin. Nutr.* 99 (6) (2014) 1319–1330.
- [182] P. Monteleone, M. Fabrazzo, A. Tortorella, V. Martiadis, C. Serritella, M. Maj, Circulating ghrelin is decreased in non-obese and obese women with binge eating disorder as well as in obese non-binge eating women, but not in patients with bulimia nervosa, *Psychoneuroendocrinology* 30 (3) (2005) 243–250.
- [183] A. Geliebter, M.E. Gluck, S.A. Hashim, Plasma ghrelin concentrations are lower in binge-eating disorder, *J. Nutr.* 135 (5) (2005) 1326–1330.
- [184] A. Troisi, G. Di Lorenzo, I. Lega, M. Tesaro, A. Bertoli, R. Leo, M. Iantorno, C. Pecchioli, S. Rizza, M. Turriziani, R. Lauro, A. Siracusano, Plasma ghrelin in anorexia, bulimia, and binge-eating disorder: relations with eating patterns and circulating concentrations of cortisol and thyroid hormones, *Neuroendocrinology* 81 (4) (2005) 259–266.
- [185] M. Tanaka, T. Naruo, N. Nagai, N. Kuroki, T. Shiya, M. Nakazato, S. Matsukura, S. Nozoe, Habitual binge/purge behavior influences circulating ghrelin levels in eating disorders, *J. Psychiatr. Res.* 37 (1) (2003) 17–22.
- [186] M. Tanaka, T. Naruo, T. Muranaga, D. Yasuhara, T. Shiya, M. Nakazato, S. Matsukura, S. Nozoe, Increased fasting plasma ghrelin levels in patients with bulimia nervosa, *Eur. J. Endocrinol.* 146 (6) (2002) R1–R3.
- [187] M. Tanaka, T. Naruo, D. Yasuhara, Y. Tatebe, N. Nagai, T. Shiya, M. Nakazato, S. Matsukura, S. Nozoe, Fasting plasma ghrelin levels in subtypes of anorexia nervosa, *Psychoneuroendocrinology* 28 (7) (2003) 829–835.
- [188] K.B. Adamo, S.L. Wilson, Z.M. Ferraro, S. Hadjiyannakis, E. Doucet, G. S. Goldfield, Appetite sensations, appetite signaling proteins, and glucose in obese adolescents with subclinical binge eating disorder, *ISRN Obes.* 2014 (2014), 312826.
- [189] F. Broglio, C. Gottero, F. Prodham, C. Gauna, G. Muccioli, M. Papotti, T. Aribat, A. J. Van Der Lely, E. Ghigo, Non-acylated ghrelin counteracts the metabolic but not the neuroendocrine response to acylated ghrelin in humans, *J. Clin. Endocrinol. Metab.* 89 (6) (2004) 3062–3065.
- [190] J.A. Dardzinska, S. Malgorzewicz, L. Kaska, M. Proczko, T. Stefaniak, M. Stankiewicz, Z. Sledzinski, Fasting and postprandial acyl and desacyl ghrelin levels in obese and non-obese subjects, *Endokrynol. Pol.* 65 (5) (2014) 377–381.
- [191] Y. Ritze, A. Schollenberger, M. Hamze Sinno, N. Buhler, M. Bohle, G. Bardos, H. Sauer, I. Mack, P. Enck, S. Zipfel, T. Meile, A. Konigsrainer, M. Kramer, S. C. Bischoff, Gastric ghrelin, GOAT, leptin, and leptinR expression as well as peripheral serotonin are dysregulated in humans with obesity, *Neurogastroenterol. Motil.: Off. J. Eur. Gastrointest. Motil. Soc.* 28 (6) (2016) 806–815.
- [192] A. Geliebter, S.A. Hashim, M.E. Gluck, Appetite-related gut peptides, ghrelin, PYY, and GLP-1 in obese women with and without binge eating disorder (BED), *Physiol. Behav.* 94 (5) (2008) 696–699.
- [193] D. Hernandez, N. Mehta, A. Geliebter, Meal-related acyl and des-acyl ghrelin and other appetite-related hormones in people with obesity and binge eating, *Obesity* 27 (4) (2019) 629–635.
- [194] A. Geliebter, E.K. Yahav, M.E. Gluck, S.A. Hashim, Gastric capacity, test meal intake, and appetitive hormones in binge eating disorder, *Physiol. Behav.* 81 (5) (2004) 735–740.
- [195] M. Galmiche, N. Lucas, P. Dechelotte, C. Deroissart, M.L. Sollic, J. Rondeaux, S. Azhar, S. Grigioni, G. Colange, J. Delay, N. Achamrah, V. Folepe, L. Belmonte, A. Lamarre, A. Rimbart, T. Saillard, A. Petit, M. Quillard, M. Coeffier, A. Gillibert, G. Lambert, R. Legrand, M.P. Tavolacci, Plasma peptide concentrations and peptide-reactive immunoglobulins in patients with eating disorders at inclusion in the french EDILS cohort (eating disorders inventory and longitudinal survey), *Nutrients* 12 (2) (2020).
- [196] S.J. Spencer, T.L. Emmerzaal, T. Kozicz, Z.B. Andrews, Ghrelin's role in the hypothalamic-pituitary-adrenal axis stress response: implications for mood disorders, *Biol. Psychiatry* 78 (1) (2015) 19–27.
- [197] S.J. Spencer, L. Xu, M.A. Clarke, M. Lemus, A. Reichenbach, B. Geenen, T. Kozicz, Z.B. Andrews, Ghrelin regulates the hypothalamic-pituitary-adrenal axis and restricts anxiety after acute stress, *Biol. Psychiatry* 72 (6) (2012) 457–465.
- [198] H.J. Huang, X.C. Zhu, Q.Q. Han, Y.L. Wang, N. Yue, J. Wang, R. Yu, B. Li, G. C. Wu, Q. Liu, J. Yu, Ghrelin alleviates anxiety- and depression-like behaviors induced by chronic unpredictable mild stress in rodents, *Behav. Brain Res.* 326 (2017) 33–43.
- [199] R. Stark, V.V. Santos, B. Geenen, A. Cabral, T. Dinan, J.A. Bayliss, S.H. Lockie, A. Reichenbach, M.B. Lemus, M. Perello, S.J. Spencer, T. Kozicz, Z.B. Andrews, Des-acyl ghrelin and ghrelin O-acyltransferase regulate hypothalamic-pituitary-adrenal axis activation and anxiety in response to acute stress, *Endocrinology* 157 (10) (2016) 3946–3957.
- [200] A. Asakawa, A. Inui, T. Kaga, H. Yuzuriba, T. Nagata, M. Fujimiya, G. Katsuura, S. Makino, M.A. Fujino, M. Kasuga, A role of ghrelin in neuroendocrine and behavioral responses to stress in mice, *Neuroendocrinology* 74 (3) (2001) 143–147.
- [201] A. Stengel, L. Wang, Y. Tache, Stress-related alterations of acyl and desacyl ghrelin circulating levels: mechanisms and functional implications, *Peptides* 32 (11) (2011) 2208–2217.
- [202] S. Alboni, M.V. Micioni Di Bonaventura, C. Benatti, M.E. Giusepponi, N. Brunello, C. Cifani, Hypothalamic expression of inflammatory mediators in an animal model of binge eating, *Behav. Brain Res.* 320 (2017) 420–430.
- [203] M.V. Micioni Di Bonaventura, T.A. Lutz, A. Romano, M. Pucci, N. Geary, L. Asarian, C. Cifani, Estrogenic suppression of binge-like eating elicited by cyclic food restriction and frustrative-nonreward stress in female rats, *Int. J. Eat. Disord.* 50 (6) (2017) 624–635.
- [204] M.V. Micioni Di Bonaventura, M. Ubaldi, M.E. Giusepponi, K.C. Rice, M. Massi, R. Cicciocioppo, C. Cifani, Hypothalamic CRF1 receptor mechanisms are not sufficient to account for binge-like palatable food consumption in female rats, *Int. J. Eat. Disord.* 50 (10) (2017) 1194–1204.
- [205] R. Turton, R. Chami, J. Treasure, Emotional eating, binge eating and animal models of binge-type eating disorders, *Curr. Obes. Res.* 6 (2) (2017) 217–228.
- [206] A. Dingemans, U. Danner, M. Parks, Emotion regulation in binge eating disorder: a review, *Nutrients* 9 (11) (2017).
- [207] W. Nicholls, T.J. Devonport, M. Blake, The association between emotions and eating behaviour in an obese population with binge eating disorder, *Obes. Rev.: Off. J. Int. Assoc. Study Obes.* 17 (1) (2016) 30–42.
- [208] A. Kania, A. Szlaga, P. Sambak, A. Gugula, E. Blasiak, M.V. Micioni Di Bonaventura, M.A. Hossain, C. Cifani, G. Hess, A.L. Gundlach, A. Blasiak, RLN3/RXFP3 Signaling in the PVN inhibits magnocellular neurons via M-like current activation and contributes to binge eating behavior, *J. Neurosci.: Off. J. Soc. Neurosci.* 40 (28) (2020) 5362–5375.
- [209] M. Pucci, M.V. Micioni Di Bonaventura, M.E. Giusepponi, A. Romano, M. Filafferro, M. Maccarrone, R. Cicciocioppo, C. Cifani, C. D'Addario, Epigenetic regulation of nociceptin/orphanin FQ and corticotropin-releasing factor system genes in frustration stress-induced binge-like palatable food consumption, *Addict. Biol.* 21 (6) (2016) 1168–1185.
- [210] M.V. Micioni Di Bonaventura, G. Vitale, M. Massi, C. Cifani, Effect of hypericum perforatum extract in an experimental model of binge eating in female rats, *J. Obes.* 2012 (2012), 956137.
- [211] E. Kristensson, M. Sundqvist, M. Astin, M. Kjerling, H. Mattsson, C. Dornonville de la Cour, R. Hakanson, E. Lindstrom, Acute psychological stress raises plasma ghrelin in the rat, *Regul. Pept.* 134 (2006) 114–117.
- [212] C.Y. Chen, A. Inui, A. Asakawa, K. Fujino, I. Kato, C.C. Chen, N. Ueno, M. Fujimiya, Des-acyl ghrelin acts by CRF type 2 receptors to disrupt fasted stomach motility in conscious rats, *Gastroenterology* 129 (1) (2005) 8–25.
- [213] M.E. Gluck, E. Yahav, S.A. Hashim, A. Geliebter, Ghrelin levels after a cold pressor stress test in obese women with binge eating disorder, *Psychosom. Med.* 76 (1) (2014) 74–79.
- [214] H. Schellekens, B.C. Finger, T.G. Dinan, J.F. Cryan, Ghrelin signalling and obesity: at the interface of stress, mood and food reward, *Pharmacol. Ther.* 135 (3) (2012) 316–326.
- [215] P. Mahbod, E.P. Smith, M.E. Fitzgerald, R.L. Morano, B.A. Packard, S. Ghosal, J. R. Scheimann, D. Perez-Tilve, J.P. Herman, J. Tong, Desacyl ghrelin decreases anxiety-like behavior in male mice, *Endocrinology* 159 (1) (2018) 388–399.
- [216] M.A. Birkett, The Trier Social Stress Test protocol for inducing psychological stress, *J. Vis. Exp.* 56 (2011).
- [217] V. Rouach, M. Bloch, N. Rosenberg, S. Gilad, R. Limor, N. Stern, Y. Greenman, The acute ghrelin response to a psychological stress challenge does not predict the post-stress urge to eat, *Psychoneuroendocrinology* 32 (6) (2007) 693–702.
- [218] A. Geliebter, S. Carnell, M.E. Gluck, Cortisol and ghrelin concentrations following a cold pressor stress test in overweight individuals with and without night eating, *Int. J. Obes.* 37 (8) (2013) 1104–1108.
- [219] R. Sinha, P. Gu, R. Hart, J.B. Guarnaccia, Food craving, cortisol and ghrelin responses in modeling highly palatable snack intake in the laboratory, *Physiol. Behav.* 208 (2019), 112563.
- [220] M.L. Westwater, F. Mancini, J. Shapleske, J. Serfontein, M. Ernst, H. Ziauddeen, P.C. Fletcher, Dissociable hormonal profiles for psychopathology and stress in anorexia and bulimia nervosa, *Psychol. Med.* (2020) 1–11.
- [221] D.A. Schmid, K. Held, M. Ising, M. Uhr, J.C. Weikel, A. Steiger, Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls, *Neuropsychopharmacol.: Off. Publ. Am. Coll. Neuropsychopharmacol.* 30 (6) (2005) 1187–1192.
- [222] F. Broglio, L. Gianotti, S. Destefanis, S. Fassino, G. Abbate Daga, V. Mondelli, F. Lanfranco, C. Gottero, C. Gauna, L. Hofland, A.J. Van der Lely, E. Ghigo, The endocrine response to acute ghrelin administration is blunted in patients with anorexia nervosa, a ghrelin hypersecretory state, *Clin. Endocrinol.* 60 (5) (2004) 592–599.
- [223] S. Fassino, G.A. Daga, V. Mondelli, A. Piero, F. Broglio, A. Picu, R. Giordano, M. Baldi, E. Arvat, E. Ghigo, L. Gianotti, Hormonal and metabolic responses to acute ghrelin administration in patients with bulimia nervosa, *Psychoneuroendocrinology* 30 (6) (2005) 534–540.
- [224] F. Tassone, F. Broglio, S. Destefanis, S. Rovere, A. Benso, C. Gottero, F. Prodham, R. Rossetto, C. Gauna, A.J. Van der Lely, E. Ghigo, M. Maccario, Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity, *J. Clin. Endocrinol. Metab.* 88 (11) (2003) 5478–5483.
- [225] I. Azzam, S. Gilad, R. Limor, N. Stern, Y. Greenman, Ghrelin stimulation by hypothalamic-pituitary-adrenal axis activation depends on increasing cortisol levels, *Endocr. Connect.* 6 (8) (2017) 847–855.
- [226] K. Rasopow, A. Abizaid, K. Matheson, H. Anisman, Psychosocial stressor effects on cortisol and ghrelin in emotional and non-emotional eaters: influence of anger and shame, *Hormon. Behav.* 58 (4) (2010) 677–684.
- [227] K. Rasopow, A. Abizaid, K. Matheson, H. Anisman, Anticipation of a psychosocial stressor differentially influences ghrelin, cortisol and food intake among emotional and non-emotional eaters, *Appetite* 74 (2014) 35–43.