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# Ghrelin and food reward

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## **1. Introduction**

Throughout history, physicians and thinkers realized the importance of a proper diet for the maintenance of good health. In support of it, recent medical literature often cites the sentence, “Let food be thy medicine and medicine be thy food”, wrongly attributed to Hippocrates (Cardenas, 2013). Ludwig Andreas Feuerbach “you are what you eat” is also often quoted in this context (Feuerbach, 1863/4). The success of these citations reflects the concern about food in today’s world in which overabundance of calories coupled to sedentary life style gives rise to the current obesity epidemic (Hill and Peters, 1998). Indeed, recent evidence shows a marked increase in obesity from 1975 to 2016 (NCD-RisC, 2017). This condition relates to a state of fat excess arising from high energy intake and low physical expenditure (Hill and Peters, 1998). Obesity, however, is a complex and multifactorial pathology involving genetic, biological, environmental, and behavioral factors. Contrasting with obesity, undernutrition or malnutrition in spite of food availability is a common problem in the context of cancer- or aging-related cachexia (Favaro-Moreira et al., 2016). It is also a condition in which feeding control is altered. Since the discovery of the orexigenic hormone ghrelin in 1999, there has been much focus on elucidating the pathways through which it operates, with the expectation that this knowledge could help the discovery of novel targets of therapeutic value for these conditions.

In this review we focus on ghrelin, which is one of the most potent orexigenic signal, and we critically assess its central effects and their relevance in the regulation of food reward-associated behaviors and energy balance.

## **2. Interaction between homeostatic and reward mechanisms in food intake**

It is well known that homeostatic neuronal circuits in the hypothalamus integrate peripheral information, such as metabolites and hormones, to modulate food intake and energy balance [reviews in (Al Massadi et al., 2017; Belgardt and Bruning, 2010)]. However feeding control is also regulated by brain structures pertaining to the reward system (Berthoud, 2011). The reward system was serendipitously discovered in rats by Olds and Milner who used its self-stimulation for operant training (Olds and Milner, 1954). The existence of similar responses was demonstrated in primates (Briese and Olds, 1964) and the underlying circuitry progressively dissected, including its links with the dopaminergic neurons and their targets (Corbett and Wise, 1980). From a “Darwinian

standpoint” it makes sense that reward mechanisms are recruited to increase motivation to eat when hungry and to continue eating when food is plenty to ensure storage for future times of shortage [see (Lockie and Andrews, 2013), for a discussion of this issue].

Interestingly, the neuronal circuits involved in homeostatic and reward mechanisms appear to be highly interlaced and recent reports emphasize their functional links. The mesolimbic dopaminergic system, including the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), plays a relevant role in the regulation of food seeking and motivation, while the dorsal striatum is implicated through its role in habitual/automatic behaviors. The ingestion of palatable food induces an increment in dopamine (DA) release from neurons of the VTA and the adjacent *substantia nigra* that project to the NAc and dorsal striatum (Bassareo and Di Chiara, 1999; Pfaus et al., 1995). Imaging experiments in humans confirmed the results obtained in animal models and showed the activation of the NAc, caudate nucleus, and putamen in response to reward stimuli including, food... or romantic love (Acevedo et al., 2012; Small et al., 2003). Although a major role of DA neurons is to code for errors in reward prediction, electrophysiological experiments in non-human primates showed that under uncertainty conditioning, their activity also reflects reward expectation (Fiorillo et al., 2003; Schultz et al., 1992). As discussed below, many studies indicate that ghrelin interacts in multiple ways with the reward system.

### **3. Ghrelin**

Ghrelin was purified from rat stomach about twenty years ago as a 28-amino acid octanoylated peptide and shown to be the endogenous ligand of the growth hormone (GH) secretagogue receptor (now termed GHSR1a, Howard et al., 1996; Kojima et al., 1999). GHSR1a is a 7-transmembrane receptor coupled to  $G_{\alpha q/11}$ , which activates phospholipase  $C\gamma$  and  $Ca^{2+}$  release from internal stores [see (Camina, 2006) for a review]. Beyond the initial results in relation with GH releasing effects (Kojima et al., 1999; Seoane et al., 2000; Takaya et al., 2000), most of the studies focused on ghrelin’s ability to regulate food-related behaviors and metabolism [reviewed in (Al Massadi et al., 2017; Perello and Dickson, 2015)]. Ghrelin is produced by gut endocrine cells mostly located in gastric oxyntic glands, in the fundus of the stomach (Date et al., 2000). Ghrelin production in the brain is controversial [see (Cabral et al., 2017) for a review]. Although ghrelin immunoreactivity was reported in hypothalamic neurons (Cowley et al., 2003; Kojima et al., 1999), other authors suggested this likely resulted from unspecific staining (Furness

et al., 2011). In addition, several ghrelin reporter mouse lines failed to reveal positive cells in the brain (Sakata et al., 2009a). Although sensitive techniques such as RT-PCR revealed some ghrelin transcripts in the brain, the significance of these results remains unclear (Cabral et al., 2017). Interestingly, ghrelin-O-acyltransferase (GOAT), the enzyme that catalyzes the octanoylation of ghrelin, is expressed (Gahete et al., 2010; Sakata et al., 2009b; Wellman and Abizaid, 2015) and active in specific brain regions (Murtuza and Isokawa, 2018), raising the possibility that local processing of ghrelin might exist, although the source of the putative unmodified ghrelin remains to be identified.

Peripheral ghrelin can act in the CNS by penetrating into the ventromedial part of the hypothalamus through the fenestrated capillaries of the median eminence close to the arcuate nucleus (ARC, Schaeffer et al., 2013, **Figure 1**). Ghrelin can also cross the blood-brain barrier in other regions such as the *area postrema* [see (Cabral et al., 2017)] or reach the cerebrospinal fluid through the choroid plexus and tanycytes (Uriarte et al., 2018). Another route by which peripheral ghrelin could impact on brain function is the activation of GHSR1a in the vagus nerve terminals and transmission of information to the nucleus of the *tractus solitarius* (NTS), which is indirectly connected to the hypothalamus, providing a potential pathway to regulate food intake or other behaviors (Date et al., 2006, **Figure 1**). It should also be pointed out that ghrelin-independent activity of GHSR1a may be relevant in some cells, either through its constitutive activity or as a result of heterodimerization and potential sensitivity to other ligands (Schellekens et al., 2013). The contribution of these mechanisms to the actions attributed to ghrelin remains however to be determined.

With respect to ghrelin regulation, the levels of this hormone are high when nutrient availability is low, as during fasting, and decrease when energy supply is sufficient, as happens after consumption of a meal [for review see (Al Massadi et al., 2014)]. Ghrelin levels in the circulation are inversely correlated with the body mass index, and are up-regulated in under-nourished states, such as anorexia nervosa, and down-regulated in states of positive energy balance, like obesity (Mequinion et al., 2013; Otto et al., 2001; Tschop et al., 2001). Ghrelin is considered to act as a meal initiation cue and its levels rise just before eating in rodents and humans (Cummings et al., 2001; Drazen et al., 2006). It is also involved in anticipatory locomotor activity (Blum et al., 2009). Indeed, reports using fixed meal patterns suggest that ghrelin is increased preprandially, as part of an anticipatory and learned response corresponding to the animal expectation to eat, and this increase is independent of the state of fasting or feeding (Drazen et al.,

2006; Merkestein et al., 2012). Ghrelin secretion in the stomach can also be regulated through exposition to food-associated stimuli and the consequent activation of the vagal efferent fibers of the NTS (Arosio et al., 2004; Seoane et al., 2007; Sugino et al., 2002). Therefore, factors such as anticipatory response, cephalic or oropharyngeal stimulation, and food-related sensory stimuli appear to contribute to the regulation of ghrelin [see (Al Massadi et al., 2014)].

#### **4. Role of ghrelin in reward and addiction**

In addition to its well-known effects on energy homeostasis regulation at the hypothalamic level [reviews in (Al Massadi et al., 2017; Muller et al., 2015)], ghrelin also has the ability to increase food motivation acting on hypothalamic and extra-hypothalamic areas implicated in motivational and incentive behavior, -VTA and NAc- (Naleid et al., 2005; Skibicka et al., 2011), in learning and memory -hippocampus and amygdala- (Alvarez-Crespo et al., 2012; Diano et al., 2006; Kanoski et al., 2013; Palotai et al., 2013), and in control/decision making -prefrontal cortex- (Kroemer et al., 2015; Parent et al., 2015).

Ghrelin modulates the reward system (Egecioglu et al., 2010; Perello et al., 2010). It enhances the time mice spend in a place previously associated with highly palatable food and this effect is abrogated in GHSR1a-null mice (Egecioglu et al., 2010; Perello et al., 2010; Skibicka et al., 2011; Skibicka et al., 2012a). However this effect varies depending of the route and the doses used and if the conditioning sessions are done in the presence or absence of food (Schele et al., 2017). For example, peripheral injection of a high dose of ghrelin in the absence of food causes conditioned place aversion (CPA, Lockie et al., 2015). However with low dose or if the conditioning sessions were made in the presence of food, ghrelin induces conditioned place preference (CPP, Jerlhag, 2008; Lockie et al., 2015). In like manner, central administration of ghrelin in the presence of food induces CPA (Schele et al., 2017). Intracerebroventricular (i.c.v.) or peripheral ghrelin injections increase the intake of highly palatable food (Denis et al., 2015; Perello et al., 2010). Ghrelin injection into the VTA enhances cue-induced reinstatement of operant responses for palatable food pellets (St-Onge et al., 2016), while injection into the ventral hippocampus also enhances cue-potentiated feeding in food-sated animals (Kanoski et al., 2013). Conversely, genetic or pharmacological inhibition of ghrelin signaling abrogates the potentiated feeding after presentation of a conditioned stimulus (Walker et al., 2012).

Brain imaging in rats showed that intravenous ghrelin administration activates brain areas that controls homeostatic feeding and components of the mesolimbic reward circuitry including the prefrontal cortex, NAc, and septum (Sarvari et al., 2014). In human subjects exposed to palatable food pictures, ghrelin activates reward-related areas including amygdala, ventral striatum, anterior insula, orbitofrontal cortex and hippocampus (Goldstone et al., 2014; Malik et al., 2008). Ghrelin also activates the olfactory area of the piriform cortex, and increases olfactory sensitivity (Malik et al., 2008; Tong et al., 2011). During presentation of food pictures, circulating ghrelin levels are positively correlated with the brain response to these pictures (Kroemer et al., 2013). Interestingly, brain activation in response to food pictures differs depending on the genotype at an “obesity-risk” locus associated with dysregulated circulating ghrelin levels (Karra et al., 2013).

Other findings suggest that appetitive and food seeking behaviors may be driven by negative emotions. For example, starvation-sensitive agouti-related peptide (AgRP) neurons, which are activated by ghrelin (see below), provide a negative-valence teaching signal (Betley et al., 2015). Importantly, although ghrelin induces food seeking behavior in rodents and humans, it was shown in most studies that this peptide causes CPA in rodents (Lockie et al., 2015; Schele et al., 2017). Thus, by itself ghrelin appears to be aversive rather than rewarding whereas it increases the motivation to seek and consume food. Along these lines, it was proposed that ghrelin mediates eating behaviors associated with stress or depression since chronic stress-increased intake and CPP for high fat food were prevented in mice devoid of GHSR1a in catecholaminergic neurons (Chuang et al., 2011).

Beside these studies related to food, several groups examined the role of ghrelin in the actions of psychostimulants. Modulation of the ghrelin system and food restriction alter locomotor sensitization, DA release in the NAc, and CPP induced by cocaine, amphetamine, and nicotine (Abizaid et al., 2011; Clifford et al., 2012; Clifford et al., 2011; Davis et al., 2007; Jerlhag et al., 2010; Jerlhag and Engel, 2011; Wellman et al., 2011; Wellman et al., 2005; Zheng et al., 2013). Moreover, GHSR1a deletion diminishes the reinforcement for intracranial self-stimulation with an electrode implanted in the lateral hypothalamic area (LHA, Wellman et al., 2012).

All these observations show that ghrelin has a potent modulatory role on the reward system and that it increases motivation for food and can also modulate responses to addictive stimuli.

## 5. Ghrelin and the mesolimbic dopamine pathway

Many studies indicate that DA is the main mediator of the actions of ghrelin on the reward system. DA is implicated in reward-associated behaviors and the mesolimbic pathway is a key component of food motivational aspects (Palmiter, 2007). Ghrelin activates the mesolimbic DA pathway (Abizaid et al., 2006; Jerlhag et al., 2006; Jerlhag et al., 2007). Ghrelin administration into the VTA induces feeding (Abizaid et al., 2006; Jerlhag et al., 2006; Naleid et al., 2005; Skibicka et al., 2011) and enhances palatable food intake (Egecioglu et al., 2010; Perello et al., 2010). The orexigenic action of ghrelin or food deprivation is blunted by the administration of a ghrelin antagonist in the VTA (Abizaid et al., 2006; Denis et al., 2015). Furthermore, ghrelin administered in the VTA increases the effort that rats make to obtain a food reward (Skibicka et al., 2011). Highly palatable food-related behaviors are impaired in VTA-lesioned rats or after ghrelin receptors blockade in the VTA, but not in the NAc, suggesting the role of ghrelin action in the VTA (Abizaid et al., 2006; Denis et al., 2015; Egecioglu et al., 2010; Skibicka et al., 2011). Interestingly, the positive effects of ghrelin on food intake are seen only when one type of food is presented. In contrast, ghrelin can change the preference from high fat food to chow in a binge eating protocol or a free choice paradigm, suggesting its implication in food choice (Bake et al., 2017; Schele et al., 2016). This change in food preference was reproduced by an injection of ghrelin in the VTA (Bake et al., 2017). These results suggest a specific role of the VTA for ghrelin actions on both food motivation and choice.

Ghrelin potentiates DA release in the NAc induced by food or food predictive cues, an effect that seems to depend on orexin in the VTA (Cone et al., 2014; Cone et al., 2015). Food cues increase VTA DA neurons firing in food-restricted rats but ghrelin injected i.p. does not modify this effect (van der Plasse et al., 2015). In support of the role of VTA to NAc DA pathway, the injection of an antagonist of either D1 or D2 DA receptor in the NAc blocked the actions of ghrelin in the VTA on food reward behavior but not chow intake (Skibicka et al., 2013).

Ghrelin administration into the VTA increases DA neurons activity (Abizaid et al., 2006; Jerlhag et al., 2006; Jerlhag et al., 2007). Ghrelin action on DA neurons is mediated, at least in part, by cholinergic neurons since ghrelin increases acetylcholine release in the VTA, thereby activating DA neurons and inducing DA release in the NAc (Jerlhag et al., 2012, **Figure 2**). This suggested acetylcholine-DA link could also be involved in ghrelin actions on locomotor activity (Jerlhag et al., 2006). In addition, ghrelin



may act directly on DA neurons since GHSR1a is expressed in about half of them in the VTA (Abizaid et al., 2006; Guan et al., 1997; Zigman et al., 2006).

Although most actions of ghrelin on DA neurons seem to originate in the VTA, GHSR1a also appears to co-localize with DA D1 receptors in several brain areas where they were they may form heterodimers (Jiang et al., 2006). GHSR1a were also proposed to heteromerize with DA D2 receptors in hypothalamic neurons and thereby regulate appetite *in vivo* (Kern et al., 2012). Few studies, however, addressed the possible direct effects of ghrelin in the NAc. Ghrelin injected into the NAc moderately increases food intake (Naleid et al., 2005) and potentiates cocaine-induced locomotor activity (Jang et al., 2013).

In conclusion, many studies highlight the importance of the VTA-NAc dopaminergic pathway in the action of ghrelin on food seeking and reward-associated behaviors. The results suggest that most effects of ghrelin originate in the VTA, at least in part through modulation of cholinergic neurons, although some direct effects in the NAc cannot be ruled out.

## **6. Ghrelin interactions with the opioid system**

The endogenous opioid system is an important regulator of appetite and metabolism (Bodnar, 2017; Nogueiras et al., 2012). In fact, several pharmacological studies have demonstrated that agonists of the three opioid receptors ( $\mu$ ,  $\kappa$ , and  $\delta$ ) increase food intake, whereas antagonists of these receptors decrease food intake (Bodnar, 2016, 2018). Apart from these orexigenic effects, the opioid system regulate food reward and motivation. Opioids act on the mesolimbic DA system inducing DA release through activation of  $\mu$ -opioid receptors in the VTA (Spanagel et al., 1992) and  $\mu$ - and/or  $\delta$ -opioid receptors in the NAc (Hirose et al., 2005; Yoshida et al., 1999), whereas  $\kappa$ -opioid receptors in the NAc decrease DA release (Spanagel et al., 1992). Both ghrelin and opioids act in the same areas, including the hypothalamus and the VTA, to regulate homeostatic feeding and food reward behaviors respectively (Ikeda et al., 2015; Romero-Pico et al., 2013; Skibicka et al., 2012b). In support of their functional interaction, ghrelin-induced chow intake and motivated behavior toward sucrose are blunted by the opioid receptor antagonist naltrexone (Skibicka et al., 2012b). Conversely, sub-chronic administration of ghrelin receptor antagonist was reported to increase the levels of Leu-enkephalin-Arg(6) in the NAc, of Met-enkephalin-Arg(6)Phe(7) in the VTA, and of dynorphin B in the hippocampus, providing evidence of cross-talk between ghrelin

signaling and opioid system (Engel et al., 2015). Finally, ghrelin seems to be involved in the effects of opiates since pharmacological inhibition of ghrelin signaling diminishes morphine-induced locomotor activity, DA release in the NAc and increase in stereotypic behaviors in rodents (Sustkova-Fiserova et al., 2014). These results provide evidence for a high degree of interaction between ghrelin and the opioid system.

## **7. Ghrelin and endocannabinoids**

Several interactions between ghrelin and the endocannabinoid system have been reported at the periphery and in brain areas regulating food intake and energy balance, indicating a crosstalk between these two systems (Kola et al., 2008; Senin et al., 2013). Endocannabinoids have a strong implication in the control of feeding as indicated by their ability to increase consumption of palatable liquids and foods in satiated animals and motivated behaviors [see (Kirkham, 2009) for a review]. The cannabinoid CB1 receptor (CB1R) is mostly expressed on axon terminals (Herkenham et al., 1991). Cannabinoid agonists stimulate VTA DA neurons activity leading to increased release of DA within the NAc shell (French, 1997; Solinas et al., 2006), whereas a CB1R antagonist blunts the mesolimbic DA release elicited by the presentation of novel palatable foods (Melis et al., 2007; Oleson et al., 2012). Recently, the functional interaction between ghrelin and endocannabinoids was supported by studies showing that the peripheral, intra-VTA or intra-NAc administration of a ghrelin antagonist blocks the rise in anandamide and decrease in 2-arachidonoylglycerol levels induced in the NAc by fentanyl treatment (Sustkova-Fiserova et al., 2017). Conversely, the ghrelin-dependent DA release in the NAc and the consequent activation of locomotor activity appear to depend on cannabinoid signaling in the VTA. This hypothesis is based on studies in mice in which the systemic administration of a CB1R antagonist blunted the increase in DA and in locomotor activity induced by i.c.v. injection of ghrelin (Kalafateli et al., 2018). These effects were replicated when both CB1R antagonist and ghrelin were administered into the VTA (Kalafateli et al., 2018). These results point to the VTA as an important locus of interaction between ghrelin and the endocannabinoids.

## **8. Crosstalk between food reward and homeostatic mechanisms**

### **8.1 *Neuropeptides implicated in the actions of ghrelin***

Ghrelin induces feeding by activating GHSR1a (Sun et al., 2004), which is expressed in the ARC (Guan et al., 1997), a nucleus containing AgRP- and NPY-positive neurons

(Luquet et al., 2005). AgRP is an antagonist at melanocortin receptors [for review see (Dieguez et al., 2011)], whose endogenous agonist is  $\alpha$ -MSH. Genetic deletion of both NPY and AgRP is required to block ghrelin-induced feeding (Chen et al., 2004). Moreover, feeding induction by ghrelin is partially recovered in GHSR1a knockout mice by selective re-expression of this receptor in NPY/AgRP neurons (Wang et al., 2014). Therefore, it is widely accepted that ghrelin necessitates an intact ARC melanocortin system to induce feeding. The group of Serge Luquet re-evaluated this paradigm and showed that, although NPY/AgRP-ablated mice fed on a standard diet did not respond to ghrelin, the orexigenic effect of ghrelin was restored when these mice were fed a high fat/high sucrose diet (Denis et al., 2015). These results suggest that the ghrelin-dependent induction of food intake in the absence of NPY/AgRP neurons is nutrient-sensitive. In other words, when NPY/AgRP neurons activity is impaired, ghrelin can still increase the motivation to feed via other circuits, such as those involving DA neurons (Denis et al., 2015). These findings highlight the complexity of feeding behavior and mechanisms of action of ghrelin, as well as the importance of food palatability.

### 8.2. Orexin in the LHA in the action of ghrelin on the reward system

The lateral hypothalamus area (LHA) is implicated in the homeostatic and motivational control of food intake [see (Stuber and Wise, 2016) for a review]. Both chronic and acute highly palatable food ingestion activates neuronal activity in the LHA (de Macedo et al., 2016; Valdivia et al., 2015), a region where the orexin neurons are localized. Orexin was suggested to mediate the communication between the LHA and the mesolimbic DA neurotransmission (Korotkova et al., 2003; Nieh et al., 2015, **Figure 2**). In support of this hypothesis it was shown that ghrelin injection targets orexin neurons in the LHA to induce feeding (Hsu et al., 2015; Lawrence et al., 2002; Olszewski et al., 2003; Perello et al., 2010; Yamanaka et al., 2003). Moreover, the pretreatment of VTA with an orexin receptor antagonist blunts the ghrelin-induced food intake (Cone et al., 2014). Ghrelin signaling in the LHA is indispensable for food reward behaviors such as food-seeking behavior, motivation to eat evaluated by operant conditioning for sucrose, and CPP for high fat diet (Lopez-Ferreras et al., 2017; Perello et al., 2010). Finally, pharmacological, virogenetic or genetic blockade of the orexin pathway blunts the effects of ghrelin on the reward system (Lopez-Ferreras et al., 2017; Perello et al., 2010) and ghrelin necessitates an intact orexin pathway to induce these food-related behaviors (Perello et al., 2010). In conclusion, ghrelin could regulate food reward behaviors not only through a local action

in mesolimbic areas, but also, indirectly, by its actions on the ARC melanocortin system or on the LHA orexin neurons through neuronal projections to the VTA.

### 8.3. Ghrelin actions in food reward depend on metabolic status

The motivation to obtain and seek food is influenced by the metabolic state, which modulates the perceived value of a reinforcement (Lockie and Andrews, 2013). Since, as discussed above, in starvation ghrelin increases food seeking and motivation, ghrelin tends to protect the organism from states of prolonged negative energy balance by increasing the perceived value of food. Conversely, in states of positive energy balance such as obesity, ghrelin becomes less effective in the induction of those food-related behaviors. In diet-induced obese (DIO) mice, although ghrelin injection into the VTA increases food intake, ghrelin ability to produce CPP for food is highly compromised (Lockie et al., 2015). The ability of ghrelin to increase the motivation to obtain food after operant conditioning response is impaired in DIO mice (Finger et al., 2012). In contrast, cocaine still induces CPP in DIO mice, indicating that psychostimulants and ghrelin act at different levels to condition behavior (Lockie et al., 2015). These data suggest that the resistance to ghrelin action on food reward-associated behaviors in obese rodents does not take place in the VTA but involves upstream structure(s) in which the AgRP neurons were suggested to have an important role (Lockie et al., 2015).

Another condition in which ghrelin may be relevant is malnutrition observed in cachexia induced by cancer or in elderly patients under institutional care (25-60% prevalence of malnutrition) or hospitalization (35-65 % prevalence, Favaro-Moreira et al., 2016). Given the fact that ghrelin is one of the most potent orexigenic signal, targeting the ghrelin system could be an interesting therapeutic strategy in subjects undergoing a negative energy balance. Indeed the use of ghrelin agonists in cancer-induced cachexia appears to be promising (Garcia et al., 2015) [and see (Su et al., 2016) for a meta-analysis]. These agonists have also been proposed in other disease states such as anorexia nervosa (Mequinion et al., 2013; Perello and Zigman, 2012), with mildly encouraging preliminary results (Fazeli et al., 2018).

## **9. Concluding remarks**

The mesolimbic system, including the VTA and the NAc, is the main final pathway for most ghrelin's actions on food reward-associated behaviors. Control of DA plays a key role in ghrelin actions on the reward system, which also involve endogenous opioid

peptides and endocannabinoids. This interdependence shows the complexity of feeding control, in which multiple neurotransmitters are coordinated to achieve a proper behavioral and physiological equilibrium. In physiological conditions, the respective contribution of direct actions of ghrelin in the VTA and indirect effects through various afferents, including from the hypothalamus, is still an open question. In light of recent data indicating the existence of different DA neurons subtypes (Poulin et al., 2018), it will be very interesting to identify on which of these neurons ghrelin exerts direct or indirect effects.

It is important to underline that although ghrelin increases the motivation to eat even in fed state, its effects on food intake and reward are blunted in obese animal models (Zigman et al., 2016). This emphasizes also the interconnection between homeostatic regulation of food intake and reward. The recent discovery of an endogenous GHSR1a antagonist implicated in metabolic control (Ge et al., 2018), suggests new approaches for the study of ghrelin system and food reward (Al-Massadi et al., 2018). A better mechanistic understanding of ghrelin signaling in the regulation of food reward behaviors could be crucial for the elucidation of the origin and pathophysiology of food-related diseases including obesity, bulimia, anorexia nervosa or binge-eating disorder.

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**Conflict of interest**

The authors declare that they have no conflicts of interest in the authorship or publication of this work.

## References

- Abizaid, A., Liu, Z. W., Andrews, Z. B., Shanabrough, M., Borok, E., Elsworth, J. D., Roth, R. H., Sleeman, M. W., Picciotto, M. R., Tschop, M. H., Gao, X. B., Horvath, T. L., 2006. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 116, 3229-3239.
- Abizaid, A., Mineur, Y. S., Roth, R. H., Elsworth, J. D., Sleeman, M. W., Picciotto, M. R., Horvath, T. L., 2011. Reduced locomotor responses to cocaine in ghrelin-deficient mice. *Neuroscience* 192, 500-506.
- Acevedo, B. P., Aron, A., Fisher, H. E., Brown, L. L., 2012. Neural correlates of long-term intense romantic love. *Soc Cogn Affect Neurosci* 7, 145-159.
- Al-Massadi, O., Muller, T., Tschop, M., Dieguez, C., Nogueiras, R., 2018. Ghrelin and LEAP-2: Rivals in Energy Metabolism. *Trends Pharmacol Sci* 39, 685-694.
- Al Massadi, O., Lear, P. V., Muller, T. D., Lopez, M., Dieguez, C., Tschop, M. H., Nogueiras, R., 2014. Review of novel aspects of the regulation of ghrelin secretion. *Curr Drug Metab* 15, 398-413.
- Al Massadi, O., Lopez, M., Tschop, M., Dieguez, C., Nogueiras, R., 2017. Current Understanding of the Hypothalamic Ghrelin Pathways Inducing Appetite and Adiposity. *Trends Neurosci* 40, 167-180.
- Alvarez-Crespo, M., Skibicka, K. P., Farkas, I., Molnar, C. S., Egecioglu, E., Hrabovszky, E., Liposits, Z., Dickson, S. L., 2012. The amygdala as a neurobiological target for ghrelin in rats: neuroanatomical, electrophysiological and behavioral evidence. *PLoS One* 7, e46321.
- Arosio, M., Ronchi, C. L., Beck-Peccoz, P., Gebbia, C., Giavoli, C., Cappiello, V., Conte, D., Peracchi, M., 2004. Effects of modified sham feeding on ghrelin levels in healthy human subjects. *J Clin Endocrinol Metab* 89, 5101-5104.
- Bake, T., Hellgren, K. T., Dickson, S. L., 2017. Acute ghrelin changes food preference from a high-fat diet to chow during binge-like eating in rodents. *J Neuroendocrinol* 29.
- Bassareo, V., Di Chiara, G., 1999. Modulation of feeding-induced activation of mesolimbic dopamine transmission by appetitive stimuli and its relation to motivational state. *Eur J Neurosci* 11, 4389-4397.
- Belgardt, B. F., Bruning, J. C., 2010. CNS leptin and insulin action in the control of energy homeostasis. *Ann N Y Acad Sci* 1212, 97-113.
- Berthoud, H. R., 2011. Metabolic and hedonic drives in the neural control of appetite: who is the boss? *Curr Opin Neurobiol* 21, 888-896.
- Betley, J. N., Xu, S., Cao, Z. F. H., Gong, R., Magnus, C. J., Yu, Y., Sternson, S. M., 2015. Neurons for hunger and thirst transmit a negative-valence teaching signal. *Nature* 521, 180-185.
- Blum, I. D., Patterson, Z., Khazall, R., Lamont, E. W., Sleeman, M. W., Horvath, T. L., Abizaid, A., 2009. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience* 164, 351-359.
- Bodnar, R. J., 2016. Endogenous opiates and behavior: 2014. *Peptides* 75, 18-70.
- Bodnar, R. J., 2017. Endogenous Opiates and Behavior: 2015. *Peptides* 88, 126-188.
- Bodnar, R. J., 2018. Endogenous Opiates and Behavior: 2016. *Peptides* 101, 167-212.
- Briese, E., Olds, J., 1964. Reinforcing Brain Stimulation and Memory in Monkeys. *Exp Neurol* 10, 493-508.
- Cabral, A., Lopez Soto, E. J., Epelbaum, J., Perello, M., 2017. Is Ghrelin Synthesized in the Central Nervous System? *Int J Mol Sci* 18.
- Camina, J. P., 2006. Cell biology of the ghrelin receptor. *J Neuroendocrinol* 18, 65-76.
- Cardenas, 2013. Let not thy food be confused with thy medicine: The Hippocratic misquotation. *e-SPEN Journal* 8, e260ee262.
- Clifford, P. S., Rodriguez, J., Schul, D., Hughes, S., Kniffin, T., Hart, N., Eitan, S., Brunel, L., Fehrentz, J. A., Martinez, J., Wellman, P. J., 2012. Attenuation of cocaine-induced locomotor

sensitization in rats sustaining genetic or pharmacologic antagonism of ghrelin receptors. *Addict Biol* 17, 956-963.

Clifford, S., Zeckler, R. A., Buckman, S., Thompson, J., Hart, N., Wellman, P. J., Smith, R. G., 2011. Impact of food restriction and cocaine on locomotion in ghrelin- and ghrelin-receptor knockout mice. *Addict Biol* 16, 386-392.

Cone, J. J., McCutcheon, J. E., Roitman, M. F., 2014. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *J Neurosci* 34, 4905-4913.

Cone, J. J., Roitman, J. D., Roitman, M. F., 2015. Ghrelin regulates phasic dopamine and nucleus accumbens signaling evoked by food-predictive stimuli. *J Neurochem* 133, 844-856.

Corbett, D., Wise, R. A., 1980. Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping study. *Brain Res* 185, 1-15.

Cowley, M. A., Smith, R. G., Diano, S., Tschop, M., Pronchuk, N., Grove, K. L., Strasburger, C. J., Bidlingmaier, M., Esterman, M., Heiman, M. L., Garcia-Segura, L. M., Nillni, E. A., Mendez, P., Low, M. J., Sotonyi, P., Friedman, J. M., Liu, H., Pinto, S., Colmers, W. F., Cone, R. D., Horvath, T. L., 2003. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 37, 649-661.

Cummings, D. E., Purnell, J. Q., Frayo, R. S., Schmidova, K., Wisse, B. E., Weigle, D. S., 2001. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 50, 1714-1719.

Chen, H. Y., Trumbauer, M. E., Chen, A. S., Weingarth, D. T., Adams, J. R., Frazier, E. G., Shen, Z., Marsh, D. J., Feighner, S. D., Guan, X. M., Ye, Z., Nargund, R. P., Smith, R. G., Van der Ploeg, L. H., Howard, A. D., MacNeil, D. J., Qian, S., 2004. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* 145, 2607-2612.

Chuang, J. C., Perello, M., Sakata, I., Osborne-Lawrence, S., Savitt, J. M., Lutter, M., Zigman, J. M., 2011. Ghrelin mediates stress-induced food-reward behavior in mice. *J Clin Invest* 121, 2684-2692.

Date, Y., Kojima, M., Hosoda, H., Sawaguchi, A., Mondal, M. S., Suganuma, T., Matsukura, S., Kangawa, K., Nakazato, M., 2000. 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, 4255-4261.

Date, Y., Shimbara, T., Koda, S., Toshinai, K., Ida, T., Murakami, N., Miyazato, M., Kokame, K., Ishizuka, Y., Ishida, Y., Kageyama, H., Shioda, S., Kangawa, K., Nakazato, M., 2006. Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell Metab* 4, 323-331.

Davis, K. W., Wellman, P. J., Clifford, P. S., 2007. Augmented cocaine conditioned place preference in rats pretreated with systemic ghrelin. *Regul Pept* 140, 148-152.

de Macedo, I. C., de Freitas, J. S., da Silva Torres, I. L., 2016. The Influence of Palatable Diets in Reward System Activation: A Mini Review. *Adv Pharmacol Sci* 2016, 7238679.

Denis, R. G., Joly-Amado, A., Webber, E., Langlet, F., Schaeffer, M., Padilla, S. L., Cansell, C., Dehouck, B., Castel, J., Delbes, A. S., Martinez, S., Lacombe, A., Rouch, C., Kassis, N., Fehrentz, J. A., Martinez, J., Verdie, P., Hnasko, T. S., Palmiter, R. D., Krashes, M. J., Guler, A. D., Magnan, C., Luquet, S., 2015. Palatability Can Drive Feeding Independent of AgRP Neurons. *Cell Metab* 22, 646-657.

Diano, S., Farr, S. A., Benoit, S. C., McNay, E. C., da Silva, I., Horvath, B., Gaskin, F. S., Nonaka, N., Jaeger, L. B., Banks, W. A., Morley, J. E., Pinto, S., Sherwin, R. S., Xu, L., Yamada, K. A., Sleeman, M. W., Tschop, M. H., Horvath, T. L., 2006. Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* 9, 381-388.

Dieguez, C., Vazquez, M. J., Romero, A., Lopez, M., Nogueiras, R., 2011. Hypothalamic control of lipid metabolism: focus on leptin, ghrelin and melanocortins. *Neuroendocrinology* 94, 1-11.



Drazen, D. L., Vahl, T. P., D'Alessio, D. A., Seeley, R. J., Woods, S. C., 2006. Effects of a fixed meal pattern on ghrelin secretion: evidence for a learned response independent of nutrient status. *Endocrinology* 147, 23-30.

Egecioglu, E., Jerlhag, E., Salome, N., Skibicka, K. P., Haage, D., Bohlooly, Y. M., Andersson, D., Bjursell, M., Perrissoud, D., Engel, J. A., Dickson, S. L., 2010. Ghrelin increases intake of rewarding food in rodents. *Addict Biol* 15, 304-311.

Engel, J. A., Nylander, I., Jerlhag, E., 2015. A ghrelin receptor (GHS-R1A) antagonist attenuates the rewarding properties of morphine and increases opioid peptide levels in reward areas in mice. *Eur Neuropsychopharmacol* 25, 2364-2371.

Favaro-Moreira, N. C., Krausch-Hofmann, S., Matthys, C., Vereecken, C., Vanhauwaert, E., Declercq, A., Bekkering, G. E., Duyck, J., 2016. Risk Factors for Malnutrition in Older Adults: A Systematic Review of the Literature Based on Longitudinal Data. *Adv Nutr* 7, 507-522.

Fazeli, P. K., Lawson, E. A., Faje, A. T., Eddy, K. T., Lee, H., Fiedorek, F. T., Breggia, A., Gaal, I. M., DeSanti, R., Klibanski, A., 2018. Treatment With a Ghrelin Agonist in Outpatient Women With Anorexia Nervosa: A Randomized Clinical Trial. *J Clin Psychiatry* 79.

Feuerbach, L. A., 1863/4. "Der Mensch ist, was er ißt.". Concerning Spiritualism and Materialism.

Finger, B. C., Dinan, T. G., Cryan, J. F., 2012. Diet-induced obesity blunts the behavioural effects of ghrelin: studies in a mouse-progressive ratio task. *Psychopharmacology (Berl)* 220, 173-181.

Fiorillo, C. D., Tobler, P. N., Schultz, W., 2003. Discrete coding of reward probability and uncertainty by dopamine neurons. *Science* 299, 1898-1902.

French, E. D., 1997. delta9-Tetrahydrocannabinol excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci Lett* 226, 159-162.

Furness, J. B., Hunne, B., Matsuda, N., Yin, L., Russo, D., Kato, I., Fujimiya, M., Patterson, M., McLeod, J., Andrews, Z. B., Bron, R., 2011. Investigation of the presence of ghrelin in the central nervous system of the rat and mouse. *Neuroscience* 193, 1-9.

Gahete, M. D., Cordoba-Chacon, J., Salvatori, R., Castano, J. P., Kineman, R. D., Luque, R. M., 2010. Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. *Mol Cell Endocrinol* 317, 154-160.

Garcia, J. M., Boccia, R. V., Graham, C. D., Yan, Y., Duus, E. M., Allen, S., Friend, J., 2015. Anamorelin for patients with cancer cachexia: an integrated analysis of two phase 2, randomised, placebo-controlled, double-blind trials. *Lancet Oncol* 16, 108-116.

Ge, X., Yang, H., Bednarek, M. A., Galon-Tilleman, H., Chen, P., Chen, M., Lichtman, J. S., Wang, Y., Dalmas, O., Yin, Y., Tian, H., Jermutus, L., Grimsby, J., Rondinone, C. M., Konkar, A., Kaplan, D. D., 2018. LEAP2 Is an Endogenous Antagonist of the Ghrelin Receptor. *Cell Metab* 27, 461-469 e466.

Goldstone, A. P., Prechtel, C. G., Scholtz, S., Miras, A. D., Chhina, N., Durighel, G., Deliran, S. S., Beckmann, C., Ghatei, M. A., Ashby, D. R., Waldman, A. D., Gaylinn, B. D., Thorner, M. O., Frost, G. S., Bloom, S. R., Bell, J. D., 2014. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *Am J Clin Nutr* 99, 1319-1330.

Guan, X. M., Yu, H., Palyha, O. C., McKee, K. K., Feighner, S. D., Sirinathsinghji, D. J., Smith, R. G., Van der Ploeg, L. H., Howard, A. D., 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* 48, 23-29.

Herkenham, M., Lynn, A. B., de Costa, B. R., Richfield, E. K., 1991. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. *Brain Res* 547, 267-274.

Hill, J. O., Peters, J. C., 1998. Environmental contributions to the obesity epidemic. *Science* 280, 1371-1374.

Hirose, N., Murakawa, K., Takada, K., Oi, Y., Suzuki, T., Nagase, H., Cools, A. R., Koshikawa, N., 2005. Interactions among mu- and delta-opioid receptors, especially putative delta1- and delta2-opioid receptors, promote dopamine release in the nucleus accumbens. *Neuroscience* 135, 213-225.

Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberatore, P. A., Rosenblum, C. I., Hamelin, M., Hreniuk, D. L., Palyha, O. C., Anderson, J., Paresse, P. S., Diaz, C., Chou, M., Liu, K. K., McKee, K. K., Pong, S. S., Chung, L. Y., Elbrecht, A., Dashkevich, M., Heavens, R., Rigby, M., Sirinathsinghji, D. J., Dean, D. C., Melillo, D. G., Patchett, A. A., Nargund, R., Griffin, P. R., DeMartino, J. A., Gupta, S. K., Schaeffer, J. M., Smith, R. G., Van der Ploeg, L. H., 1996. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 273, 974-977.

Hsu, T. M., Hahn, J. D., Konanur, V. R., Noble, E. E., Suarez, A. N., Thai, J., Nakamoto, E. M., Kanoski, S. E., 2015. Hippocampus ghrelin signaling mediates appetite through lateral hypothalamic orexin pathways. *Elife* 4.

Ikeda, H., Ardianto, C., Yonemochi, N., Yang, L., Ohashi, T., Ikegami, M., Nagase, H., Kamei, J., 2015. Inhibition of opioid systems in the hypothalamus as well as the mesolimbic area suppresses feeding behavior of mice. *Neuroscience* 311, 9-21.

Jang, J. K., Kim, W. Y., Cho, B. R., Lee, J. W., Kim, J. H., 2013. Microinjection of ghrelin in the nucleus accumbens core enhances locomotor activity induced by cocaine. *Behav Brain Res* 248, 7-11.

Jerlhag, E., 2008. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict Biol* 13, 358-363.

Jerlhag, E., Egecioglu, E., Dickson, S. L., Andersson, M., Svensson, L., Engel, J. A., 2006. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict Biol* 11, 45-54.

Jerlhag, E., Egecioglu, E., Dickson, S. L., Douhan, A., Svensson, L., Engel, J. A., 2007. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol* 12, 6-16.

Jerlhag, E., Egecioglu, E., Dickson, S. L., Engel, J. A., 2010. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology (Berl)* 211, 415-422.

Jerlhag, E., Engel, J. A., 2011. Ghrelin receptor antagonism attenuates nicotine-induced locomotor stimulation, accumbal dopamine release and conditioned place preference in mice. *Drug Alcohol Depend* 117, 126-131.

Jerlhag, E., Janson, A. C., Waters, S., Engel, J. A., 2012. Concomitant release of ventral tegmental acetylcholine and accumbal dopamine by ghrelin in rats. *PLoS One* 7, e49557.

Jiang, H., Betancourt, L., Smith, R. G., 2006. Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. *Mol Endocrinol* 20, 1772-1785.

Kalafateli, A. L., Vallof, D., Jornulf, J. W., Heilig, M., Jerlhag, E., 2018. A cannabinoid receptor antagonist attenuates ghrelin-induced activation of the mesolimbic dopamine system in mice. *Physiol Behav* 184, 211-219.

Kanoski, S. E., Fortin, S. M., Ricks, K. M., Grill, H. J., 2013. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol Psychiatry* 73, 915-923.

Karra, E., O'Daly, O. G., Choudhury, A. I., Youssef, A., Millership, S., Neary, M. T., Scott, W. R., Chandarana, K., Manning, S., Hess, M. E., Iwakura, H., Akamizu, T., Millet, Q., Gelegen, C., Drew, M. E., Rahman, S., Emmanuel, J. J., Williams, S. C., Ruther, U. U., Bruning, J. C., Withers, D. J., Zelaya, F. O., Batterham, R. L., 2013. A link between FTO, ghrelin, and impaired brain food-cue responsivity. *J Clin Invest* 123, 3539-3551.

Kern, A., Albarran-Zeckler, R., Walsh, H. E., Smith, R. G., 2012. Apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* 73, 317-332.

Kirkham, T. C., 2009. Cannabinoids and appetite: food craving and food pleasure. *Int Rev Psychiatry* 21, 163-171.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 402, 656-660.

Kola, B., Farkas, I., Christ-Crain, M., Wittmann, G., Lolli, F., Amin, F., Harvey-White, J., Liposits, Z., Kunos, G., Grossman, A. B., Fekete, C., Korbonits, M., 2008. The orexigenic effect of ghrelin is mediated through central activation of the endogenous cannabinoid system. *PLoS One* 3, e1797.

Korotkova, T. M., Sergeeva, O. A., Eriksson, K. S., Haas, H. L., Brown, R. E., 2003. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J Neurosci* 23, 7-11.

Kroemer, N. B., Krebs, L., Kobiella, A., Grimm, O., Pilhatsch, M., Bidlingmaier, M., Zimmermann, U. S., Smolka, M. N., 2013. Fasting levels of ghrelin covary with the brain response to food pictures. *Addict Biol* 18, 855-862.

Kroemer, N. B., Wuttig, F., Bidlingmaier, M., Zimmermann, U. S., Smolka, M. N., 2015. Nicotine enhances modulation of food-cue reactivity by leptin and ghrelin in the ventromedial prefrontal cortex. *Addict Biol* 20, 832-844.

Lawrence, C. B., Snape, A. C., Baudoin, F. M., Luckman, S. M., 2002. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology* 143, 155-162.

Lockie, S. H., Andrews, Z. B., 2013. The hormonal signature of energy deficit: Increasing the value of food reward. *Mol Metab* 2, 329-336.

Lockie, S. H., Dinan, T., Lawrence, A. J., Spencer, S. J., Andrews, Z. B., 2015. Diet-induced obesity causes ghrelin resistance in reward processing tasks. *Psychoneuroendocrinology* 62, 114-120.

Lopez-Ferreras, L., Richard, J. E., Anderberg, R. H., Nilsson, F. H., Olandersson, K., Kanoski, S. E., Skibicka, K. P., 2017. Ghrelin's control of food reward and body weight in the lateral hypothalamic area is sexually dimorphic. *Physiol Behav* 176, 40-49.

Luquet, S., Perez, F. A., Hnasko, T. S., Palmiter, R. D., 2005. NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates. *Science* 310, 683-685.

Malik, S., McGlone, F., Bedrossian, D., Dagher, A., 2008. Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metab* 7, 400-409.

Melis, T., Succu, S., Sanna, F., Boi, A., Argiolas, A., Melis, M. R., 2007. The cannabinoid antagonist SR 141716A (Rimonabant) reduces the increase of extra-cellular dopamine release in the rat nucleus accumbens induced by a novel high palatable food. *Neurosci Lett* 419, 231-235.

Mequinion, M., Langlet, F., Zgheib, S., Dickson, S., Dehouck, B., Chauveau, C., Viltart, O., 2013. Ghrelin: central and peripheral implications in anorexia nervosa. *Front Endocrinol (Lausanne)* 4, 15.

Merkestein, M., Brans, M. A., Luijendijk, M. C., de Jong, J. W., Egecioglu, E., Dickson, S. L., Adan, R. A., 2012. Ghrelin mediates anticipation to a palatable meal in rats. *Obesity (Silver Spring)* 20, 963-971.

Muller, T. D., Nogueiras, R., Andermann, M. L., Andrews, Z. B., Anker, S. D., Argente, J., Batterham, R. L., Benoit, S. C., Bowers, C. Y., Broglio, F., Casanueva, F. F., D'Alessio, D., Depoortere, I., Geliebter, A., Ghigo, E., Cole, P. A., Cowley, M., Cummings, D. E., Dagher, A., Diano, S., Dickson, S. L., Dieguez, C., Granata, R., Grill, H. J., Grove, K., Habegger, K. M., Heppner, K., Heiman, M. L., Holsen, L., Holst, B., Inui, A., Jansson, J. O., Kirchner, H., Korbonits, M., Laferrere, B., LeRoux, C. W., Lopez, M., Morin, S., Nakazato, M., Nass, R., Perez-Tilve, D., Pfluger, P. T., Schwartz, T. W., Seeley, R. J., Sleeman, M., Sun, Y., Sussel, L., Tong, J., Thorner, M. O., van der Lely, A. J., van der Ploeg, L. H., Zigman, J. M., Kojima, M., Kangawa, K., Smith, R. G., Horvath, T., Tschop, M. H., 2015. Ghrelin. *Mol Metab* 4, 437-460.

Murtuza, M. I., Isokawa, M., 2018. Endogenous ghrelin-O-acyltransferase (GOAT) acylates local ghrelin in the hippocampus. *J Neurochem* 144, 58-67.

Naleid, A. M., Grace, M. K., Cummings, D. E., Levine, A. S., 2005. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 26, 2274-2279.

NCD-RisC, N. R. F. C., 2017. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* 390, 2627-2642.

Nieh, E. H., Matthews, G. A., Allsop, S. A., Presbrey, K. N., Leppla, C. A., Wichmann, R., Neve, R., Wildes, C. P., Tye, K. M., 2015. Decoding neural circuits that control compulsive sucrose seeking. *Cell* 160, 528-541.

Nogueiras, R., Romero-Pico, A., Vazquez, M. J., Novelle, M. G., Lopez, M., Dieguez, C., 2012. The opioid system and food intake: homeostatic and hedonic mechanisms. *Obes Facts* 5, 196-207.

Olds, J., Milner, P., 1954. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47, 419-427.

Oleson, E. B., Beckert, M. V., Morra, J. T., Lansink, C. S., Cachope, R., Abdullah, R. A., Loriaux, A. L., Schettters, D., Pattij, T., Roitman, M. F., Lichtman, A. H., Cheer, J. F., 2012. Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. *Neuron* 73, 360-373.

Olszewski, P. K., Li, D., Grace, M. K., Billington, C. J., Kotz, C. M., Levine, A. S., 2003. Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* 24, 597-602.

Otto, B., Cuntz, U., Fruehauf, E., Wawarta, R., Folwaczny, C., Riepl, R. L., Heiman, M. L., Lehnert, P., Fichter, M., Tschop, M., 2001. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* 145, 669-673.

Palmiter, R. D., 2007. Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci* 30, 375-381.

Palotai, M., Bagosi, Z., Jaszberenyi, M., Csabafi, K., Dochnal, R., Manczinger, M., Telegdy, G., Szabo, G., 2013. Ghrelin and nicotine stimulate equally the dopamine release in the rat amygdala. *Neurochem Res* 38, 1989-1995.

Parent, M. A., Amarante, L. M., Swanson, K., Laubach, M., 2015. Cholinergic and ghrelinergic receptors and KCNQ channels in the medial PFC regulate the expression of palatability. *Front Behav Neurosci* 9, 284.

Perello, M., Dickson, S. L., 2015. Ghrelin signalling on food reward: a salient link between the gut and the mesolimbic system. *J Neuroendocrinol* 27, 424-434.

Perello, M., Sakata, I., Birnbaum, S., Chuang, J. C., Osborne-Lawrence, S., Rovinsky, S. A., Woloszyn, J., Yanagisawa, M., Lutter, M., Zigman, J. M., 2010. Ghrelin increases the rewarding value of high-fat diet in an orexin-dependent manner. *Biol Psychiatry* 67, 880-886.

Perello, M., Zigman, J. M., 2012. The role of ghrelin in reward-based eating. *Biol Psychiatry* 72, 347-353.

Pfaus, J. G., Damsma, G., Wenkstern, D., Fibiger, H. C., 1995. Sexual activity increases dopamine transmission in the nucleus accumbens and striatum of female rats. *Brain Res* 693, 21-30.

Poulin, J. F., Caronia, G., Hofer, C., Cui, Q., Helm, B., Ramakrishnan, C., Chan, C. S., Dombeck, D. A., Deisseroth, K., Awatramani, R., 2018. Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. *Nat Neurosci*.

Romero-Pico, A., Vazquez, M. J., Gonzalez-Touceda, D., Folgueira, C., Skibicka, K. P., Alvarez-Crespo, M., Van Gestel, M. A., Velasquez, D. A., Schwarzer, C., Herzog, H., Lopez, M., Adan, R. A., Dickson, S. L., Dieguez, C., Nogueiras, R., 2013. Hypothalamic kappa-opioid receptor modulates the orexigenic effect of ghrelin. *Neuropsychopharmacology* 38, 1296-1307.

Sakata, I., Nakano, Y., Osborne-Lawrence, S., Rovinsky, S. A., Lee, C. E., Perello, M., Anderson, J. G., Coppari, R., Xiao, G., Lowell, B. B., Elmquist, J. K., Zigman, J. M., 2009a. Characterization of a novel ghrelin cell reporter mouse. *Regul Pept* 155, 91-98.

Sakata, I., Yang, J., Lee, C. E., Osborne-Lawrence, S., Rovinsky, S. A., Elmquist, J. K., Zigman, J. M., 2009b. Colocalization of ghrelin O-acyltransferase and ghrelin in gastric mucosal cells. *Am J Physiol Endocrinol Metab* 297, E134-141.

Sarvari, M., Kocsis, P., Deli, L., Gajari, D., David, S., Pozsgay, Z., Hegedus, N., Tihanyi, K., Liposits, Z., 2014. Ghrelin modulates the fMRI BOLD response of homeostatic and hedonic brain centers regulating energy balance in the rat. *PLoS One* 9, e97651.

Schaeffer, M., Langlet, F., Lafont, C., Molino, F., Hodson, D. J., Roux, T., Lamarque, L., Verdie, P., Bourrier, E., Dehouck, B., Baneres, J. L., Martinez, J., Mery, P. F., Marie, J., Trinquet, E., Fehrentz, J. A., Prevot, V., Mollard, P., 2013. Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc Natl Acad Sci U S A* 110, 1512-1517.

Schele, E., Bake, T., Rabasa, C., Dickson, S. L., 2016. Centrally Administered Ghrelin Acutely Influences Food Choice in Rodents. *PLoS One* 11, e0149456.

Schele, E., Cook, C., Le May, M., Bake, T., Luckman, S. M., Dickson, S. L., 2017. Central administration of ghrelin induces conditioned avoidance in rodents. *Eur Neuropsychopharmacol* 27, 809-815.

Schellekens, H., van Oeffelen, W. E., Dinan, T. G., Cryan, J. F., 2013. Promiscuous dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates ghrelin-mediated signaling. *J Biol Chem* 288, 181-191.

Schultz, W., Apicella, P., Scarnati, E., Ljungberg, T., 1992. Neuronal activity in monkey ventral striatum related to the expectation of reward. *J Neurosci* 12, 4595-4610.

Senin, L. L., Al-Massadi, O., Folgueira, C., Castelao, C., Pardo, M., Barja-Fernandez, S., Roca-Rivada, A., Amil, M., Crujeiras, A. B., Garcia-Caballero, T., Gabellieri, E., Leis, R., Dieguez, C., Pagotto, U., Casanueva, F. F., Seoane, L. M., 2013. The gastric CB1 receptor modulates ghrelin production through the mTOR pathway to regulate food intake. *PLoS One* 8, e80339.

Seoane, L. M., Al-Massadi, O., Caminos, J. E., Tovar, S. A., Dieguez, C., Casanueva, F. F., 2007. Sensory stimuli directly acting at the central nervous system regulate gastric ghrelin secretion. an ex vivo organ culture study. *Endocrinology* 148, 3998-4006.

Seoane, L. M., Tovar, S., Baldelli, R., Arvat, E., Ghigo, E., Casanueva, F. F., Dieguez, C., 2000. Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Eur J Endocrinol* 143, R7-9.

Skibicka, K. P., Hansson, C., Alvarez-Crespo, M., Friberg, P. A., Dickson, S. L., 2011. Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 180, 129-137.

Skibicka, K. P., Hansson, C., Egecioglu, E., Dickson, S. L., 2012a. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict Biol* 17, 95-107.

Skibicka, K. P., Shirazi, R. H., Hansson, C., Dickson, S. L., 2012b. Ghrelin interacts with neuropeptide Y Y1 and opioid receptors to increase food reward. *Endocrinology* 153, 1194-1205.

Skibicka, K. P., Shirazi, R. H., Rabasa-Papio, C., Alvarez-Crespo, M., Neuber, C., Vogel, H., Dickson, S. L., 2013. 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, 274-283.

Small, D. M., Jones-Gotman, M., Dagher, A., 2003. Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *Neuroimage* 19, 1709-1715.

Solinas, M., Justinova, Z., Goldberg, S. R., Tanda, G., 2006. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J Neurochem* 98, 408-419.

Spanagel, R., Herz, A., Shippenberg, T. S., 1992. Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proc Natl Acad Sci U S A* 89, 2046-2050.

St-Onge, V., Watts, A., Abizaid, A., 2016. Ghrelin enhances cue-induced bar pressing for high fat food. *Horm Behav* 78, 141-149.

Stuber, G. D., Wise, R. A., 2016. Lateral hypothalamic circuits for feeding and reward. *Nat Neurosci* 19, 198-205.

Su, J., Geng, J., Bao, J., Tang, Y., Liu, M., Yu, H., Han, Y., Huang, W., Zhou, S., 2016. Two ghrelin receptor agonists for adults with malnutrition: a systematic review and meta-analysis. *Nutr J* 15, 97.

Sugino, T., Hasegawa, Y., Kikkawa, Y., Yamaura, J., Yamagishi, M., Kurose, Y., Kojima, M., Kangawa, K., Terashima, Y., 2002. A transient ghrelin surge occurs just before feeding in a scheduled meal-fed sheep. *Biochem Biophys Res Commun* 295, 255-260.

Sun, Y., Wang, P., Zheng, H., Smith, R. G., 2004. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci U S A* 101, 4679-4684.

Sustkova-Fiserova, M., Charalambous, C., Havlickova, T., Lapka, M., Jerabek, P., Puskina, N., Syslova, K., 2017. Alterations in Rat Accumbens Endocannabinoid and GABA Content during Fentanyl Treatment: The Role of Ghrelin. *Int J Mol Sci* 18.

Sustkova-Fiserova, M., Jerabek, P., Havlickova, T., Kacer, P., Krsiak, M., 2014. Ghrelin receptor antagonism of morphine-induced accumbens dopamine release and behavioral stimulation in rats. *Psychopharmacology (Berl)* 231, 2899-2908.

Takaya, K., Ariyasu, H., Kanamoto, N., Iwakura, H., Yoshimoto, A., Harada, M., Mori, K., Komatsu, Y., Usui, T., Shimatsu, A., Ogawa, Y., Hosoda, K., Akamizu, T., Kojima, M., Kangawa, K., Nakao, K., 2000. Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 85, 4908-4911.

Tong, J., Mannea, E., Aime, P., Pfluger, P. T., Yi, C. X., Castaneda, T. R., Davis, H. W., Ren, X., Pixley, S., Benoit, S., Julliard, K., Woods, S. C., Horvath, T. L., Sleeman, M. M., D'Alessio, D., Obici, S., Frank, R., Tschop, M. H., 2011. Ghrelin enhances olfactory sensitivity and exploratory sniffing in rodents and humans. *J Neurosci* 31, 5841-5846.

Tschop, M., Weyer, C., Tataranni, P. A., Devanarayan, V., Ravussin, E., Heiman, M. L., 2001. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 50, 707-709.

Uriarte, M., De Francesco, P. N., Fernandez, G., Cabral, A., Castrogiovanni, D., Lalonde, T., Luyt, L. G., Trejo, S., Perello, M., 2018. Evidence Supporting a Role for the Blood-Cerebrospinal Fluid Barrier Transporting Circulating Ghrelin into the Brain. *Mol Neurobiol*.

Valdivia, S., Cornejo, M. P., Reynaldo, M., De Francesco, P. N., Perello, M., 2015. 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, 206-216.

van der Plasse, G., van Zessen, R., Luijendijk, M. C., Erkan, H., Stuber, G. D., Ramakers, G. M., Adan, R. A., 2015. Modulation of cue-induced firing of ventral tegmental area dopamine neurons by leptin and ghrelin. *Int J Obes (Lond)* 39, 1742-1749.

Walker, A. K., Ibia, I. E., Zigman, J. M., 2012. Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. *Physiol Behav* 108, 34-43.

Wang, Q., Liu, C., Uchida, A., Chuang, J. C., Walker, A., Liu, T., Osborne-Lawrence, S., Mason, B. L., Mosher, C., Berglund, E. D., Elmquist, J. K., Zigman, J. M., 2014. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Mol Metab* 3, 64-72.

Wellman, M., Abizaid, A., 2015. Knockdown of central ghrelin O-acyltransferase by vivo-morpholino reduces body mass of rats fed a high-fat diet. *Peptides* 70, 17-22.

Wellman, P. J., Clifford, P. S., Rodriguez, J., Hughes, S., Eitan, S., Brunel, L., Fehrentz, J. A., Martinez, J., 2011. Pharmacologic antagonism of ghrelin receptors attenuates development of nicotine induced locomotor sensitization in rats. *Regul Pept* 172, 77-80.

Wellman, P. J., Clifford, P. S., Rodriguez, J. A., Hughes, S., Di Francesco, C., Melotto, S., Tessari, M., Corsi, M., Bifone, A., Gozzi, A., 2012. Brain reinforcement system function is ghrelin dependent: studies in the rat using pharmacological fMRI and intracranial self-stimulation. *Addict Biol* 17, 908-919.

Wellman, P. J., Davis, K. W., Nation, J. R., 2005. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. *Regul Pept* 125, 151-154.

Yamanaka, A., Beuckmann, C. T., Willie, J. T., Hara, J., Tsujino, N., Mieda, M., Tominaga, M., Yagami, K., Sugiyama, F., Goto, K., Yanagisawa, M., Sakurai, T., 2003. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38, 701-713.

Yoshida, Y., Koide, S., Hirose, N., Takada, K., Tomiyama, K., Koshikawa, N., Cools, A. R., 1999. Fentanyl increases dopamine release in rat nucleus accumbens: involvement of mesolimbic mu- and delta-2-opioid receptors. *Neuroscience* 92, 1357-1365.

Zheng, D., Liu, S., Cabeza de Vaca, S., Carr, K. D., 2013. Effects of time of feeding on psychostimulant reward, conditioned place preference, metabolic hormone levels, and nucleus accumbens biochemical measures in food-restricted rats. *Psychopharmacology (Berl)* 227, 307-320.

Zigman, J. M., Bouret, S. G., Andrews, Z. B., 2016. Obesity Impairs the Action of the Neuroendocrine Ghrelin System. *Trends Endocrinol Metab* 27, 54-63.

Zigman, J. M., Jones, J. E., Lee, C. E., Saper, C. B., Elmquist, J. K., 2006. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* 494, 528-548.

## Figure legends

**Figure 1: Gastric ghrelin regulation and action on the hypothalamus.** Ghrelin is mainly secreted by the stomach during fasting and its release is inhibited by food-associated stimuli locally and through innervation. Circulating ghrelin can cross the blood-brain barrier (BBB) through the fenestrated endothelium of the median eminence and activate GHSR1a in the arcuate nucleus (ARC) of the hypothalamus. Ghrelin has also been proposed to activate GHSR1a in the terminals of the vagus nerve and to trigger neuronal signaling to the nucleus of the solitary tract (NTS) in the brain stem and indirectly the hypothalamus. ARC: hypothalamic arcuate nucleus; GHSR1a: growth hormone secretagogue receptor 1a or ghrelin receptor; NTS: nucleus of the solitary tract.

**Figure 2: Ghrelin in food reward.** Interaction between the mesolimbic pathway and the hypothalamus in ghrelin-induced food reward and crosstalk of ghrelin with other neuronal systems regulating feeding such as dopamine, opioid peptides, and endocannabinoids. Peripheral ghrelin can reach the hypothalamus and act on AgRP neurons in the arcuate nucleus (ARC) and orexin neurons in the lateral hypothalamus area (LHA). AgRP neurons are connected to neurons that express pro-opiomelanocortin (POMC), the precursor of  $\alpha$ -MSH. All these hypothalamic neurons could act directly or indirectly on VTA through their neuropeptides or GABA or glutamate. One important intermediate are the cholinergic neurons which activate DA neurons. Although ghrelin can act on several of these neurons in which its receptor GHSR1a has been demonstrated, it is still unclear how it reaches these deep brain regions.

ACh: acetylcholine; AgRP: agouti related peptide; ARC: hypothalamic arcuate nucleus; CB1R: cannabinoid receptor 1; DA: dopamine;  $\delta$ -OR: delta opioid receptor; GABA: gamma aminobutyric acid; GHSR1a: growth hormone secretagogue receptor 1a; JMV-2959: GHSR1a antagonist;  $\kappa$ -OR: kappa opioid receptor; LHA: lateral hypothalamic area; NAc: nucleus accumbens; MC4R: melanocortin 4 receptor;  $\mu$ -OR; mu opioid receptor; OX-R: orexin receptor; POMC: pro-opiomelanocortin; VTA: ventral tegmental area.



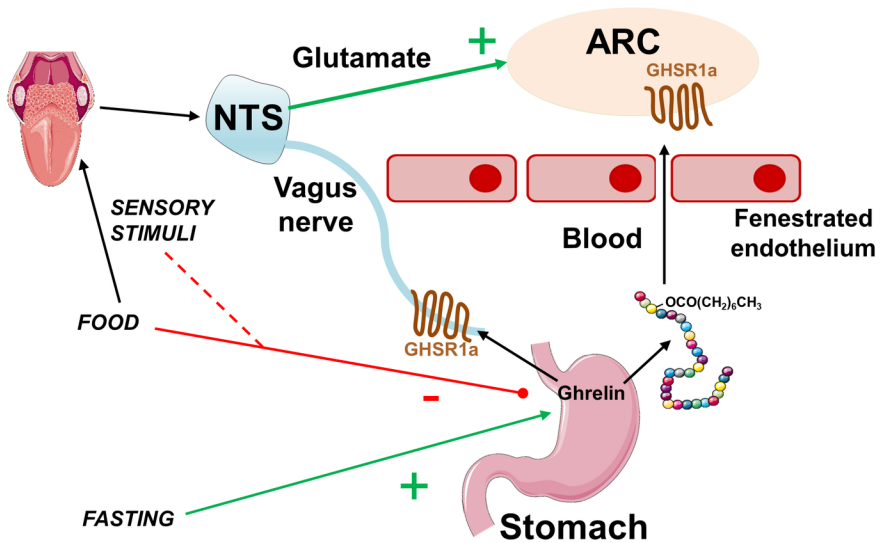


Figure 1

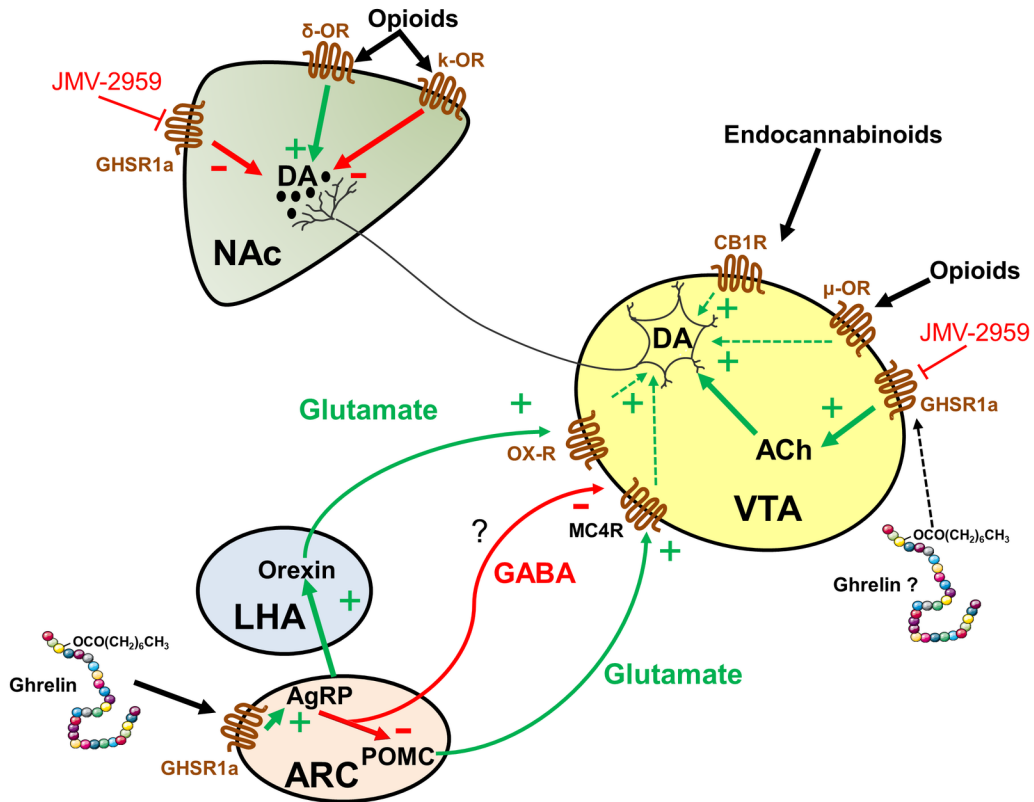


Figure 2