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REVIEW ARTICLE

Ghrelin Signalling on Food Reward: A Salient Link Between the Gut and the Mesolimbic System

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'Hunger is the best spice' is an old and wise saying that acknowledges the fact that almost any food tastes better when we are hungry. The neurobiological underpinnings of this lore include activation of the brain's reward system and the stimulation of this system by the hunger-promoting hormone ghrelin. Ghrelin is produced largely from the stomach and levels are higher preprandially. The ghrelin receptor is expressed in many brain areas important for feeding control, including not only the hypothalamic nuclei involved in energy balance regulation, but also reward-linked areas such as the ventral tegmental area. By targeting the mesoaccumbal dopamine neurones of the ventral tegmental area, ghrelin recruits pathways important for food reward-related behaviours that show overlap with but are also distinct from those important for food intake. We review a variety of studies that support the notion that ghrelin signalling at the level of the mesolimbic system is one of the key molecular substrates that provides a physiological signal connecting gut and reward pathways.

Key words: dopamine, ghrelin

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Introduction

The present review is concerned with the actions of a circulating appetite-promoting hormone ghrelin (1), produced mostly by the stomach, on the reward system of the brain. The idea that the gut utilises enteroendocrine hormones to communicate with pathways involved in rewarding aspects of food intake is a new and emerging field (2) suggesting the existence of an endocrine gut–brain reward axis that can modify reward signalling for food, a system important for survival. Ghrelin appears to have a physiological role in hunger and meal initiation (3), orchestrating a variety of behaviours that ensure animals go out into the environment to seek out and consume a variety and plentiful supply of nutrients. We focus especially on the effects of ghrelin on reward-linked behaviour for food, including the mechanisms and pathways involved.

The ghrelin/growth hormone secretagogue receptor type 1A (GHSR-1A) system

Ghrelin is a 28-amino acid octanoylated peptide hormone (1) secreted predominantly from a specific type of endocrine cell of the stomach located within the gastric oxyntic mucosa and named P/D1-type cells in humans (4) or A like-type cells in rodents (5). Ghrelin mediates its actions via its unique specific receptor, the GHSR-1A (6), which is a G protein-coupled receptor highly expressed in the central nervous system (7,8). Ghrelin is the only known naturally-occurring peptide to be post-translationally

modified by a O-octanoylation, a reaction catalysed by the enzyme ghrelin O-acyl transferase (GOAT) (9). This modification is essential for ghrelin-induced activation of GHSR-1A. Ghrelin plays a well-defined variety of physiological roles and is recognised for being the only known circulating peptide hormone that stimulates food intake (10,11). As discussed below, ghrelin stimulates appetite via diverse mechanisms that promote food intake and that stimulate food reward-related behaviours. In plasma, a non-octanoylated form of ghrelin, named desacyl-ghrelin that represents more than 90% of total ghrelin immunoreactivity, has been shown to exist (12). The GHSR-1A-independent effects of this peptide on food intake regulation have been described, although the physiological significance of desacyl-ghrelin remains a matter of debate (13).

Plasma ghrelin levels fluctuate with meal cycle and energy status. One of the most striking features about the 24-h secretory pattern of ghrelin in man is the large rise in plasma levels that occur just before mealtimes (3). The precise mechanism governing preprandial ghrelin release remains an open question (14). One possibility is that ghrelin release is controlled locally in the gastrointestinal tract (15). Some of the metabolic and hormonal factors able to directly regulate ghrelin secretion have been identified. Using ghrelin-producing cell lines, it has been shown that low D-glucose concentrations stimulate ghrelin release, whereas high D-glucose and glucose metabolism block ghrelin release (16). Also, norepinephrine enhances ghrelin release by binding to β_1-adrenergic receptors on ghrelin cells; indeed, this mechanism has been proposed to modulate ghrelin secretion during fasting when the sympathetic tone is increased (17). It has also been shown that natural (i.e. α-linolenic acid) or chemical agonist for the G protein-coupled receptor 120 inhibit the secretion of ghrelin, suggesting that the decrease of ghrelin release after feeding is induced partially by long-chain fatty acids acting directly on gastric cells (18). Importantly, ghrelin release is also coordinated by a descending pathway (e.g. via the vagus nerve) signalling either a food anticipatory signal (19) or a cry for nutrients by the brain. Anticipatory cues associated with the delivery of food have been shown to trigger ghrelin release in mice (20). Food anticipatory behaviour, including anticipation for palatable food, is decreased in models of suppressed ghrelin signalling, suggesting that ghrelin may, in turn, activate food anticipatory pathways (21,22).

One of the key physiological targets of the ghrelin/GHSR-1A system is the brain. The dedicated ghrelin receptor, GHSR-1A, was identified in 1996 and was found to be expressed in many brain areas linked to feeding control, including hypothalamic, brainstem and mesolimbic pathways (6–8). Ghrelin is able to drive a feeding response when microinjected into many of these sites, including the arcuate nucleus of the hypothalamus (ARC), ventromedial nucleus (23), lateral hypothalamic area (LHA) (24), caudal brainstem (25), ventral tegmental area (VTA), nucleus accumbens (NAc) (26,27), lateral amygdala (28) and ventral hippocampus (29). The orexigenic effects of ghrelin appear to be exclusively signalled through GHSR-1A (6) because ghrelin fails to elicit a feeding response in mice lacking this receptor (30), as well as in rats pre-treated with central injection with a GHSR-1A antagonist (31). Food intake appears to be a common goal of the ghrelin-appetitory networks, an endpoint achieved through integration with brain pathways involved in many diverse constructs and behaviours. Indeed, ghrelin signalling pathways are involved in hunger sensation (32), food anticipatory behaviour (21,33), food reward (34), motivated (reward-linked) behaviour for food (35,36), memory (37), novelty-seeking behaviour (38) and anxiety/stress-like behaviour (28,39,40). In the present review, we focus especially on recent advances implicating the central ghrelin signalling system in food reward.

The first indication that central GHSR-1A signalling could be implicated in food intake regulation was noted in 1993, when it was found that the growth hormone-releasing peptide 6 (a growth hormone secretagogue that is now recognised to be a ghrelin mimetic) activates cells in the ARC, as reflected by an increase in the number of cells detected that express Fos protein (41). It emerged that approximately half of the ARC cells activated by this ghrelin mimetic expressed neuropeptide Y (NPY) mRNA, a potent orexigenic signal (42). These ghrelin-sensitive NPY neurones, which co-express another orexigenic peptide, agouti-related protein (AgRP), are considered as a major node in the appetite-regulatory circuitry. In line with this hypothesis, it was later found that NPY/AgRP neurones of the ARC express high levels of GHSR-1A (43,44). Moreover, ghrelin fails to increase food intake in mice lacking NPY and AgRP (45,46), suggesting that these peptides play a pivotal role in the effects of ghrelin on food intake. Selective re-expression of GHSR-1A in AgRP neurones partially restored this phenotype (47).

Ghrelin signalling engages a complex network of neuronal circuitries to modulate feeding-linked behaviour. For example, ghrelin-induced food intake also appears to depend on orexin neurones of the LHA, where GHSR-1A is expressed (24,48) and the action of ghrelin on food reward requires intact orexin signalling (35). GHSR-1A is expressed in vagal afferent neurones of the nodose ganglia and in the dorsal vagal complex (8,49), providing an alternative route through which peripheral ghrelin could signal to appetitive neurocircuitry. Indeed, it has been suggested that vagus nerve integrity is required for ghrelin-induced food intake (50,51), although a subsequent study did not find this to be the case (52). The presence of GHSR-1A in dopaminergic VTA neurones (8,53,54), as well as other cell types in this region, supports the possibility that ghrelin can regulate rewarding aspects of eating. Ghrelin may also regulate mesolimbic circuits indirectly via the cholinergic neurones of the laterodorsal tegmental area (LDTg), which also express GHSR-1A (55,56). The action of ghrelin at the level of the hippocampus, another brain area where GHSR-1A is present in abundance (7,8), also appears to be important for both motivational and learned aspects of feeding behaviour (29). Thus, the neuroanatomical distribution of the ghrelin receptor supports a role for the ghrelin/GHSR-1A system in the regulation of both homeostatic and rewarding aspects of feeding.

In humans, the response of the brain's reward system to food cues, as measured using functional resonance imaging, is enhanced by fasting (57). The relevance of the ghrelin signalling in this observation was suggested by a functional magnetic resonance imaging study showing that ghrelin can mimic the effects of fasting on the reward networks (58) and was further confirmed by another imaging study in humans showing that ghrelin increases the neural

response in brain centres implicated in rewarding aspects of feeding (59). In particular, ghrelin administration to human subjects increases the activation of some reward-related brain centres, including the substance nigra and the VTA, in response to tempting food pictures (59). A more recent imaging study has not only confirmed previous observations, but also shown that subjects with polymorphisms in the fat mass and obesity-associated gene genotype exhibited divergent neural responsiveness to peripheral ghrelin within brain regions that regulate rewarding aspects of appetite (60). Thus, several functional resonance imaging studies in healthy subjects strongly support a role for ghrelin in rewarding aspects of eating in human subjects.

Food reward: motivational and hedonic aspects of eating

All organisms have a capacity to seek out and consume food. In mammals, the neurobiological mechanisms sensing energy need, food availability and coordinating appetitive and food-seeking behaviours are complex. Feeding control involves an integrated regulatory system of homeostatic brain circuits that drive food intake depending on energy store levels: processes for which the hypothalamus and brainstem have a primary role. Food intake is also regulated by reward pathways that process information about the pleasurable (hedonic) and incentive (motivational) aspects of food intake. Homeostatic and reward circuits mediating food intake are inter-related: 'Hunger is the best spice', is a Swedish saying acknowledging that all foods have a rewarding value that is influenced by hunger and food availability. From an evolutionary perspective, food reward has a dual role: it not only promotes food seeking and eating when food is scarce, but also promotes overeating when food becomes available, aiming to establish sufficient energy stores for a future famine. If food is found rewarding, this will motivate animals to go out into the environment to seek it out, ensuring not only an adequate supply of calories, but also that consumed foods are of diverse nutritional composition. In the current obesogenic environment, however, food reward is no longer an evolutionary advantage for modern human beings. Instead, it contributes to the maladaptation to that environment, encouraging people to overeat calorie-dense foods to a level far beyond metabolic need.

Neuronal circuits involved in food reward

A key feature of reward processing is the activation of a major dopaminergic cell group located in the VTA of the midbrain. These dopaminergic neurones project to the NAc and the prefrontal cortex, as well as to several other brain areas, including the hippocampus and the hypothalamus (61). The VTA receives projections from many brain nuclei, including the aforementioned areas that receive projections from the VTA, cholinergic neurones of the LDTg (55), as well as taste information via afferent sensory fibres (62,63). Activation of dopaminergic VTA neurones occurs in response to both natural rewards (such as food or sex) and artificial rewards (such as alcohol or other addictive drugs of abuse). As is the case for addictive drugs, the consumption of a food reward has been found to trigger dopamine overflow/release in the NAc, as measured by microdialysis (64), and to increase phasic dopamine release in the striatum, as measured by fast-scan cyclic voltammetry (65). Accumbal dopamine overflow is coupled to VTA dopamine neurone activity (66) and so it may be inferred that foods, especially palatable foods, have the capacity to activate the VTA-NAc dopamine projection. Indeed, acute consumption of a high-fat diet activates the mesolimbic circuit by neuronal pathways that require orosensory stimulation (67). Dopamine release in the NAc potently augments the drive to obtain food rewards (68). The shell part of the NAc is particularly important for eating behaviours engaging projections to the LHA neurones that control food intake. Orexigenic LHA neurones appear to be under a tonic inhibition that can be relieved by activation of reward pathways (69,70). In addition, LHA orexin neurones send projections to the VTA, where they activate dopaminergic neurones (71,72). Thus, LHA orexin neurones have been proposed as a potential link between homeostatic and reward circuits regulating food intake (73).

One of the primary roles attributed to the VTA-NAc dopamine pathway is 'wanting' or motivational component of reward that is important for craving behaviour and that is linked to but distinct from the 'liking' or hedonic component of reward (74). As reviewed elsewhere (75), the mechanisms linking dopamine to rewarding aspects of eating are rather sophisticated. Novelty of the reward appears to be critical for achieving a maximal dopamine signal (76). With repeated exposure to that same reward (conditioning), the NAc dopamine response lessens (habituates) and transfers instead onto a predictive cue associated with its delivery (77–79). Thus, dopamine signalling is crucially important for the formation of associations between rewards and anticipatory cues (78,80,81). As a consequence of conditioning, the dopamine signal takes on a new role: as a predictor of reward; motivational behaviours are recruited as part of this mechanism ensuring that the expected reward is consumed. One hypothesis for the role of the dopamine signal in reward is that it serves as a 'reward prediction error' (82). It follows that NAc dopamine release could be important for assigning increasing reward value to food cues (83).

Multifaceted actions of ghrelin signalling on food reward

In 2006, it became apparent that ghrelin activates the dopamine system, triggering NAc dopamine release (84), dopamine turnover and VTA dopamine neurone activity (54). Consistent with this, a subpopulation of VTA dopamine neurones was found to express GHSR-1A, although the receptor was also found to be located on other cell types within the VTA (54). The idea that ghrelin may provide a physiological signal connecting gut and reward pathways paved the way to studies exploring the effects of ghrelin on behaviours linked to dopamine signalling, including hedonic and motivational aspects of eating.

In rodents, reward can be assessed in the condition place preference (CPP) test in which the animals learn to associate the experience of reward with a particular environment/chamber. They will return to that chamber, spending more time there, even when the

reward is no longer available. In satiated rats, the peripheral delivery of a GHSR-1A antagonist has been shown to abolish CPP for chocolate (85). Similarly, in mice, the delivery of a GHSR-1A antagonist suppresses/abolishes CPP for a high-fat diet (35), and even for alcohol (86) or psychostimulant drugs such as cocaine or amphetamine (87). These data using GHSR-1A antagonists, together with data from ghrelin receptor knockout mice (35,85,86), indicate that central ghrelin signalling is required for the rodents to experience reward from alcohol (an artificial reward) or food (a natural reward). Consistent with this, ghrelin appears to enhance CPP for a high-fat diet (35) and psychostimulant drugs such as cocaine (88).

Craving-like behaviour (i.e. 'wanting', motivated, goal-direct behaviour) for food or other reward reinforcers can be explored in rodents using the 'operant conditioning' paradigm in which the animals have to progressively work harder (e.g. by pressing a lever) to obtain a reward. The animals are first trained on a fixed ratio schedule to associate the pressing of the lever a fixed number of times with the delivery of a reward. Subsequently, they are tested using a progressive ratio schedule in which the animal has to work increasingly hard for each reward obtained. The number of lever presses or the number of reward earned can be used to estimate motivation or goal-directed behaviour. Motivated behaviour for sugar treats has been shown to be increased by ghrelin (administered peripherally or centrally to satiated rats) and decreased by a GHSR-1A antagonist administered to fasted rats (also delivered both peripherally and centrally) (36). Central ghrelin administration increases operant lever-pressing for sucrose in rats (36,89). Similarly, operant behaviour for a high-fat diet in mice, measured by nose pokes instead of lever presses, was increased by ghrelin (35). Also, GOAT-deficient mice display an attenuated motivation for a high-fat diet in an operant responding model and a decreased reward-linked feeding response examined in a 'dessert effect' protocol, in which the intake of a palatable high-fat diet pellet 'dessert' is assessed in calorically-satiated mice (90).

It is clear that ghrelin enhances preference for pleasurable, sweet and fatty foods. In particular, ghrelin administration shifts food preference towards a high-fat diet (91). Ghrelin administration also increases intake of palatable saccharin solution and preference for saccharin-flavored foods in mice (92). Similarly, rats treated with a GHSR-1A antagonist consume less peanut butter and the liquid nutritional supplement Ensure® (Abbott Laboratories, Chicago, IL, USA) but fail to change intake of regular chow in a free choice protocol (85). On the other hand, ghrelin also increases food anticipatory activity, which is characterised by increased arousal, increased locomotor activity and an elevated body temperature in anticipation of a predicted meal (21,22). Ghrelin secreted in anticipation of a meal correlates with anticipatory locomotor activity and the administration of ghrelin increases locomotor activity and foraging-like activities in rodents (93–95). Moreover, GHSR-1A antagonists decrease anticipatory behaviour for a palatable meal (22). Interestingly, i.c.v. ghrelin fails to alter the avidity of licking, when lick motor patterns were recorded using lickometry, suggesting that ghrelin does not affect the hedonic valuation (i.e. 'liking') in rats (89). Odour also plays a role in conferring information about food availability, and ghrelin may have a beneficial role in to help animals seek out because it has been shown to stimulate sniffing and to increase olfactory sensitivity in mice (96). Overall, there is a great deal of evidence supporting a role for ghrelin in a variety of food reward-related eating behaviours. It is perhaps not surprising therefore that ghrelin-sensitive brain networks overlap considerably with those important for feeding control (97).

Neuronal circuits mediating the effects of ghrelin on food reward

The neurobiological substrates and circuits underpinning the effects of ghrelin on food-motivated behaviour show some overlap with (but also divergence from) those involved in food intake (2,97). Although food intake can be driven by ghrelin delivery to both the VTA (26,54,85) and the NAc (26,85), food-motivated behaviour occurs only after VTA (but not Nac) microinjection of ghrelin (27). VTA-lesioned rats spend less time than control rats exploring tubes containing peanut butter in response to centrally-administered ghrelin (85). Similar effects are observed in food-restricted rats in which chronic intra-VTA administration of ghrelin enhances, whereas chronic intra-VTA delivery of a GHSR-1A antagonist blunts, operant responding for chocolate-flavored pellets (98). Collectively, these findings identify the VTA as a key target brain area for the effects of ghrelin on food-motivated behaviour and suggest that direct action of ghrelin at the level of the VTA is sufficient to drive food-motivated behaviour. Moreover, the uncoupling between food intake and food motivated behaviour elicited by intra-VTA ghrelin resonates with the collective findings that NAc dopamine signalling is not coupled to normal feeding (99,100) but rather in motivated behaviour towards palatable foods, independent of the calories consumed (101,102). Importantly, only the effect of intra-VTA ghrelin on food-motivated behaviour was found to require NAc dopamine receptor 1 and 2 signalling, whereas food intake driven by VTA ghrelin was dopamine-independent in this paradigm (103). Pretreatment of rats with a dopamine receptor 1 antagonist eliminates ghrelin-induced increases in bar pressing, without compromising generalised licking motor control, supporting a role for dopamine receptor 1 signalling mainly in the motivational feeding effects of ghrelin (89). It has been shown that mice expressing GHSR-1A selectively in tyrosine hydroxylase-containing cells, including a subset of VTA dopaminergic neurones, display a significant, albeit reduced, response to the orexigenic effects of ghrelin and a full CPP for a high-fat diet when treated with exogenous ghrelin or exposed to a chronic social defeat stress (CSDS) protocol. In the study, a nontraditional mouse model was used in which GHSR-1A gene expression is disrupted by a transcriptional blocking cassette flanked by loxP sites that enable Cre recombinase-mediated GHSR-1A gene re-expression (53). Thus, a variety of studies in rodents suggest that the action of ghrelin at the level of the dopaminergic neurones of the VTA is essential for the actions of ghrelin on both food intake and food reward.

Apart from the VTA-NAc dopamine system, the rest of the neuronal circuitry engaged by ghrelin to modulate food reward-related behaviours is quite unclear. For example, it has been suggested that ghrelin can regulate mesolimbic circuits indirectly via the

cholinergic neurones of the LDTg, which express GHSR-1A (55,56). Also, the action of ghrelin on food reward appears to require intact orexin signalling, as indicated by the finding that the effects of ghrelin with respect to conditioning food CPP or operant conditioning for a food reward were blocked in orexin-deficient mice and wild-type mice given an orexin 1 receptor antagonist (35). A potential pathway would involve direct binding of ghrelin to GHSR-1A present on orexin neurones of the LHA. This is supported by the finding that GHSR-1A is expressed within the LHA of the rat (8), as well as by studies showing that ghrelin can induce action potentials and depolarisation in isolated orexin neurones (104). The ghrelin-engaged orexin neurones would then project to the VTA, where orexin signalling is critical in the activation of mesolimbic dopaminergic circuit and reward-seeking behaviours (105,106). A recent study using fast-scan cyclic voltammetry in awake rats to record dopamine spikes in the NAc core showed that the central infusion of ghrelin increases, whereas a GHSR-1A antagonist suppresses, the magnitude of dopamine spikes evoked by food, respectively. In addition, potentiation of food-evoked dopamine spikes was increased by intra-LHA ghrelin and intra-VTA blockade of orexin receptors attenuated food intake induced by central ghrelin (107). Thus, orexin signalling also appears as a key mediator of the actions of ghrelin on food reward.

Additionally, ghrelin might indirectly regulate food reward-related behaviours by engaging central NPY/AgRP neurones of the ARC. In this regard, studies using DREADD technology (designer receptors exclusively activated by designer drugs) have revealed that selective activation of AgRP neurones is sufficient to drive food motivated behaviour in mice (108). NPY has been shown to be able to induce CPP when administered to the NAc (109). NPY also induces sucrose-motivated behaviour when administered to the VTA or Nac, although it only increased sucrose consumption when administered to the NAc (110). Cross-talk between these NPY- and ghrelin-sensitive networks at the level of the VTA for food intake has been suggested by studies showing that the feeding effects, but not food-motivated effects, of ghrelin were abolished by VTA pretreatment with a NPY receptor 1 antagonist. Interestingly, the converse was true for the VTA delivery of a mu-preferring opioid receptor antagonist, which suppressed the effects of VTA ghrelin on food motivation but not food intake (111). Kappa opioid receptor pathways at the hypothalamic level also appear to be a component of the ghrelin sensitive circuitry that is important for feeding control (112). Collectively, these studies outline the divergence of behaviours (food motivation and food intake), involving overlapping but also distinct neurochemical pathways.

Physiological role of the ghrelin signalling system on food reward

Fifteen years after its discovery, the physiological relevance of the ghrelin signalling on food intake regulation, in general, and food reward pathways, in particular, is still a matter of discussion. Notably, some evidence strongly supports a role for ghrelin in short-term food intake regulation. Ghrelin administration triggers eating in both human beings and rodents (10,11). As described above, human plasma ghrelin levels decrease rapidly in response to nutrient ingestion, and 24-h plasma profiles display marked preprandial increases and postprandial decreases associated with every meal (3). In rodents, ghrelin levels are suppressed within minutes by re-feeding or enteral infusions of nutrients (113). Interestingly, blockade of ghrelin signalling in adult animals using anti-ghrelin antibodies, GHSR-1A antagonists or anti-sense oligonucleotides report decreases in spontaneous food intake and body weight (114–116). By contrast, genetically modified mouse models, including mice overexpressing ghrelin or mice with genetic deletion of ghrelin, GHSR-1A or GOAT, are almost indistinguishable from wild-type mice in terms of *ad lib.* standard rodent chow diet feeding, food intake and body weights (117). The argument invariably put forward for the lack of a robust phenotype in these mutant mice is that compensatory physiological mechanisms arise during development. However, a recent study has reported that genetic ablation of ghrelin-producing cells in adult mice fails to lead to a loss of appetite or body weight, or resistance to a high-fat diet (118). Even a physiological role for plasma ghrelin concentrations in the regulation of basal food intake was questioned because the study found that only doses of ghrelin resulting in supraphysiological plasma levels of the hormone are able to increase food intake. In line with this possibility, no increase in appetite was observed in normal-weight volunteers when plasma ghrelin is raised four-fold above basal levels (119). By contrast to these data, ghrelin has been shown to increase hunger scores and food intake in a buffet test meal in both normal weight and obese subjects (120,121). Thus, further studies are required to better clarify the role of basal ghrelin signalling on food intake and food reward-linked behaviours.

The significance of ghrelin signalling likely becomes more evident in situations in which ghrelin signalling is physiologically more relevant, such as fasting, caloric restriction or stress (122). In this regard, GHSR-1A deficient mice show important eating behaviour alterations under specific experimental conditions. For example, wild-type mice subjected to prolonged caloric restriction show enhanced CPP for a high-fat diet, whereas GHSR-1A deficient mice lack such response (35,92). Moreover, GHSR-1A deficient mice in response to scheduled meals have both attenuated anticipatory hyper-locomotion and reduced expression of the marker of cellular activation c-Fos in the mesolimbic pathway (93,123). Similarly, GHSR-1A deficient mice do not anticipate food when exposed to an activity-based anorexia model, in which mice are given free access to a running wheel and fed once per day for 2 h (21). Most humans change in their eating habits in response to stress, and an emerging literature has started to support the existence of a strong association between the ghrelin/GHSR-1A system and stress. For example, the CSDS procedure, which subjects mice to daily bouts of social defeat by aggressive male mice, has been used to study the physiological effect of ghrelin on feeding behaviours (39,53,124). Wild-type mice exposed to CSDS increase their plasma ghrelin levels and regular chow intake during and for at least 1 month after the defeat period. By contrast, GHSR-1A deficient mice fail to show CSDS-induced hyperphagia (39,124). In wild-type mice, CSDS also increases CPP for a high-fat diet, whereas such a stress-induced food reward response is not observed in CSDS-exposed GHSR-1A deficient mice (53). Thus, ghrelin signalling appears to be required

for stress-induced change in rewarding aspects of eating in mice, under these particular conditions. By contrast to these findings, wild-type mice exposed to a chronic unpredictable stress procedure, which also elevates plasma ghrelin levels, decrease food intake and body weight gain, whereas similarly-treated GHSR-1A deficient mice lack these changes (124). Notably, elevations of plasma ghrelin are observed in several stress models and ghrelin administration to either humans or rodents has been shown to induce a strong activation of the hypothalamus-pituitary-adrenal neuroendocrine axis (125,126). The physiological implications of ghrelin-induced activation of stress responses or, conversely, the impact of stress on ghrelin responsiveness are currently unknown. Thus, further work is needed to clarify the inter-relationship between of ghrelin, stress and food intake.

The ability of ghrelin to act in the brain to regulate food intake depends on the accessibility of circulating ghrelin to the above mentioned brain areas. Circulating ghrelin cannot freely cross the blood–brain barrier and it is currently unclear how this hormone enters the brain (127). In mice, ghrelin can be transported from the brain to the circulation via a saturable transport system; however, no such system has been identified for blood to brain transport (128). A recent study using ghrelin-fluorescent tracer has shown that peripheral increases of plasma ghrelin mainly access to the ARC (127,129). The ARC is a hypothalamic nucleus located in close apposition to the median eminence, a circumventricular organ with fenestrated capillaries that allows plasma ghrelin to diffuse to the brain parenchyma (130). The area postrema is another circumventricular organ also known to participate in food intake regulation and to express GHSR-1A (8,127). Early studies using GHSR-1A agonists showed that these compounds activate cells in the area postrema and in some closely-adjacent structures (131). In the study using the fluorescent ghrelin tracer, it was also found that high levels of circulating ghrelin can directly act on area postrema neurones, which then innervate several hypothalamic and brainstem feeding centres (127,129). In line with this possibility, there are indications that long-term effects of ghrelin on feeding depend on intact signalling at the area postrema (132,133). The study using the fluorescent ghrelin tracer could not provide direct evidence for increases of plasma ghrelin reaching centres of the mesolimbic pathway, at least in an acute fashion. However, an acute effect of peripheral ghrelin on the mesolimbic pathways is supported by a study showing that intra-VTA delivery of a selective GHSR-1A antagonist blocked the orexigenic effect of peripherally-administrated ghrelin (54). Also, it has been shown that peripheral administration of ghrelin in mice increases the extracellular concentration of dopamine in the NAc measured by *in vivo* microdialysis (134). However, the extent to which implantation of permanent cannulas in the brain affects the blood–brain

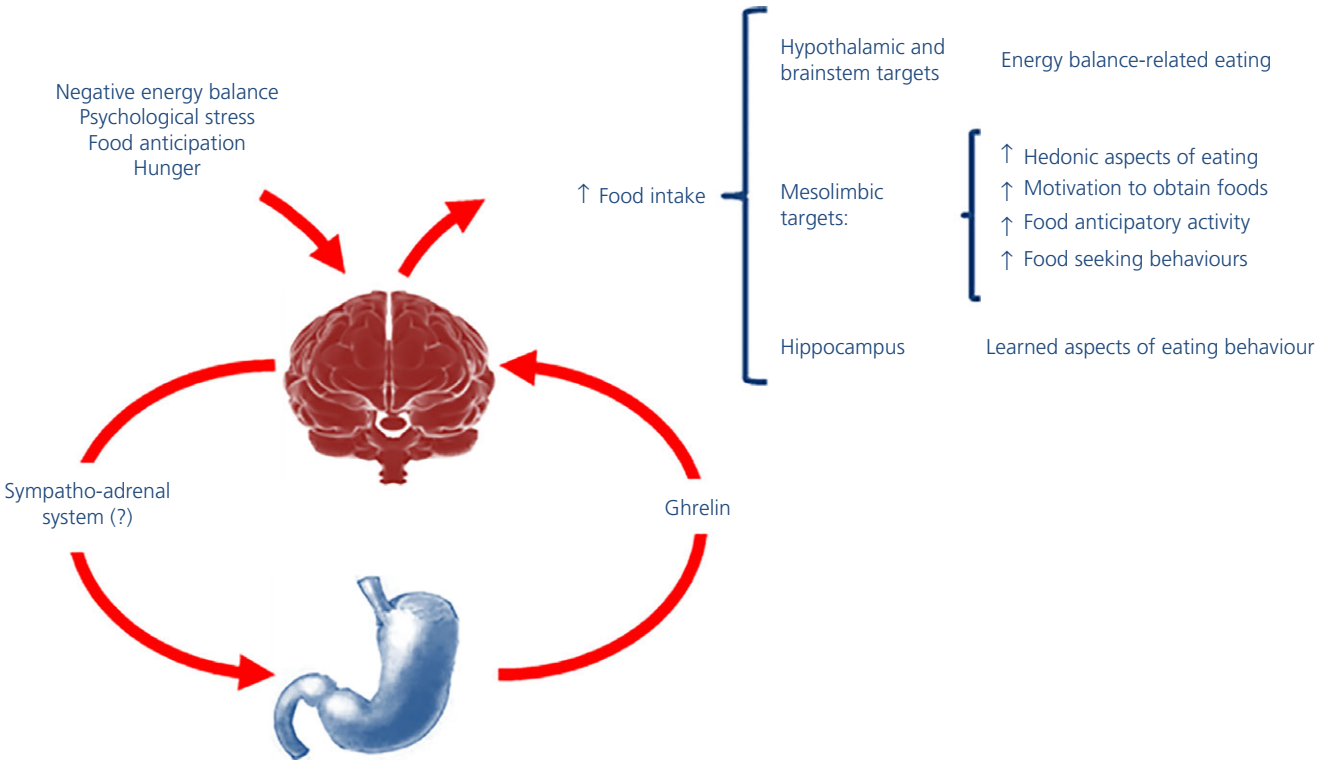

Fig. 1. Endocrine gut–brain reward axis: a model of the effects of ghrelin on eating behaviour. Some specific conditions are known to influence eating regulation by affecting homeostatic brain circuits, which drive food intake depending on energy store levels, and/or reward brain circuits, which drive consumption based on the rewarding properties of foods. Several lines of evidence suggest a key role for the ghrelin/growth hormone secretagogue receptor type 1A system in mediating these eating behaviours. These specific conditions increase ghrelin, which in turn reaches the brain where, upon interaction with its receptor on dopaminergic neurones in the ventral tegmental area and likely in other brain nuclei, mediates an integrated and complex eating behavioural response.

barrier integrity in these studies is unclear. Interestingly, mice expressing GHSR-1A selectively in tyrosine hydroxylase-containing cells partially respond to ghrelin-induced food intake and fully develop CPP for a high-fat diet in response to either peripheral ghrelin administration during the conditioning sessions or after CSDS (53). Thus, future studies are required to clarify the physiological relevance of the action of peripheral ghrelin on the mesolimbic pathway.

The relevance of the expression of GHSR-1A in brain areas without obvious access to circulating ghrelin is, in general, unclear. Although earlier studies suggested that ghrelin could be produced in the brain, more recent studies have clearly shown that ghrelin is not synthesised in the central nervous system (135–137). GHSR-1A mainly signals through Gαq/11, phospholipase C, inositol phosphate and calcium mobilisation from intracellular stores; although it also activates other signalling pathways (138). An interesting feature of GHSR-1A is its strong constitutive activity that makes it capable to signal in a ghrelin-independent manner (139,140). Thus, the increase of GHSR-1A expression would accordingly increase activation of the downstream signalling pathways affecting, as a consequence, food intake and body weight regulation (124). Additionally, it has been proposed that an alternative mechanism by which GHSR-1A regulates food intake involves its dimerisation with other G protein-coupled receptors. GHSR-1A has been shown to heterodimerise with the melanocortin 3 receptor, the serotonin 2C receptor and the dopamine receptors, which are all involved in food intake and food reward regulation. Heterodimerisation could serve to modulate specific functions of GHSR-1A, such as signalling pathways, or to act as an allosteric mechanism to regulate signalling pathways of the other receptors, independently of ghrelin binding (141–144).

Concluding remarks

The evidence reviewed here suggests that ghrelin/GHSR-1A system is strongly linked to food reward-related pathways in addition to and partially separate from those which drive food intake. Notably, the mechanisms by which ghrelin/GHSR-1A system promotes food intake are multifaceted and are summarised in Fig. 1. The mesoaccumbal dopamine pathway appears to be a key target for ghrelin/GHSR-1A system, opening the possibility that a primary role for ghrelin is to regulate rewarding aspects of eating. The ghrelin/GHSR-1A system is not only up-regulated by hunger and in anticipation to food, orchestrating a feeding response, but also by negative energy balance conditions, or psychological stress when the activation of the mesoaccumbal dopamine pathway helps animals cope with these detrimental conditions. Thus, the action of the ghrelin/GHSR-1A system on the mesolimbic pathway is very advantageous for the survival of the animal in times of food scarcity. The constant abundance of palatable foods together to the excessive stress levels that we suffer in modern societies places the ghrelin/GHSR-1A system in a new role in which it likely cause adverse consequences, including overeating beyond metabolic need and body weight gain. Therefore, the action of ghrelin on the mesolimbic system may have been a 'great spice' from an evolutionary perspective, although it no longer represents an advantage for modern human beings. Our knowledge of the neuronal circuits and molecular mechanisms mediating the actions of the ghrelin/GHSR-1A system on the mesolimbic pathways has progressed considerably in recent years, yet still many novel and exciting aspects of this endocrine gut–brain reward axis likely remain to be discovered and will deserve intense research in the near future.

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References

1 Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656–660.
2 Skibicka KP, Dickson SL. Enteroendocrine hormones – central effects on behavior. *Curr Opin Pharmacol* 2013; **13**: 977–982.
3 Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714–1719.
4 Rindi G, Necchi V, Savio A, Torsello A, Zoli M, Locatelli V, Raimondo F, Cocchi D, Solcia E. Characterisation of gastric ghrelin cells in man and other mammals: studies in adult and fetal tissues. *Histochem Cell Biol* 2002; **117**: 511–519.
5 Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. 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* 2000; **141**: 4255–4261.
6 Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, DeMartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; **273**: 974–977.
7 Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, Van der Ploeg LH, Howard AD. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res Mol Brain Res* 1997; **48**: 23–29.
8 Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK. Expression of ghrelin receptor mRNA in the rat and the mouse brain. *J Comp Neurol* 2006; **494**: 528–548.

9 Gutierrez JA, Solenberg PJ, Perkins DR, Willency JA, Knierman MD, Jin Z, Witcher DR, Luo S, Onyia JE, Hale JE. Ghrelin octanoylation mediated by an orphan lipid transferase. *Proc Natl Acad Sci USA* 2008; **105**: 6320–6325.

10 Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992.

11 Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR. Ghrelin causes hyperphagia and obesity in rats. *Diabetes* 2001; **50**: 2540–2547.

12 Patterson M, Murphy KG, le Roux CW, Ghatei MA, Bloom SR. Characterization of ghrelin-like immunoreactivity in human plasma. *J Clin Endocrinol Metab* 2005; **90**: 2205–2211.

13 Delhanty PJ, Neggers SJ, van der Lely AJ. Des-acyl ghrelin: a metabolically active peptide. *Endocr Dev* 2013; **25**: 112–121.

14 Yin X, Li Y, Xu G. Ghrelin fluctuation, what determines its production? *Acta Biochim Biophys Sin (Shanghai)* 2009; **41**: 188–197.

15 Dornonville de la Cour C, *et al.* A-like cells in the rat stomach contain ghrelin and do not operate under gastrin control. *Regul Pept* 2001; **99**: 141–150.

16 Sakata I, Park WM, Walker AK, Piper PK, Chuang JC, Osborne-Lawrence S, Zigman JM. Glucose-mediated control of ghrelin release from primary cultures of gastric mucosal cells. *Am J Physiol Endocrinol Metab* 2012; **302**: E1300–E1310.

17 Zhao TJ, Liang G, Li RL, Xie X, Sleeman MW, Murphy AJ, Valenzuela DM, Yancopoulos GD, Goldstein JL, Brown MS. Ghrelin O-acyltransferase (GOAT) is essential for growth hormone-mediated survival of calorie-restricted mice. *Proc Natl Acad Sci USA* 2010; **107**: 7467–7472.

18 Gong Z, Yoshimura M, Aizawa S, Kurotani R, Zigman JM, Sakai T, Sakata I. G protein-coupled receptor 120 signaling regulates ghrelin secretion in vivo and in vitro. *Am J Physiol Endocrinol Metab* 2014; **306**: E28–E35.

19 Natalucci G, Riedl S, Gleiss A, Zidek T, Frisch H. Spontaneous 24-h ghrelin secretion pattern in fasting subjects: maintenance of a meal-related pattern. *Eur J Endocrinol* 2005; **152**: 845–850.

20 Walker AK, Ibia IE, Zigman JM. Disruption of cue-potentiated feeding in mice with blocked ghrelin signaling. *Physiol Behav* 2012; **108**: 34–43.

21 Verhagen LA, Egecioglu E, Luijendijk MC, Hillebrand JJ, Adan RA, Dickson SL. Acute and chronic suppression of the central ghrelin signaling system reveals a role in food anticipatory activity. *Eur Neuropsychopharmacol* 2011; **21**: 384–392.

22 Merkestein M, Brans MA, Luijendijk MC, de Jong JW, Egecioglu E, Dickson SL, Adan RA. Ghrelin mediates anticipation to a palatable meal in rats. *Obesity (Silver Spring)* 2012; **20**: 963–971.

23 Currie PJ, Khelemsky R, Rigsbee EM, Dono LM, Coiro CD, Chapman CD, Hinchcliff K. Ghrelin is an orexigenic peptide and elicits anxiety-like behaviors following administration into discrete regions of the hypothalamus. *Behav Brain Res* 2012; **226**: 96–105.

24 Olszewski PK, Li D, Grace MK, Billington CJ, Kotz CM, Levine AS. Neural basis of orexigenic effects of ghrelin acting within lateral hypothalamus. *Peptides* 2003; **24**: 597–602.

25 Faulconbridge LF, Cummings DE, Kaplan JM, Grill HJ. Hyperphagic effects of brainstem ghrelin administration. *Diabetes* 2003; **52**: 2260–2265.

26 Naleid AM, Grace MK, Cummings DE, Levine AS. Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* 2005; **26**: 2274–2279.

27 Skibicka KP, Hansson C, Alvarez-Crespo M, Friberg PA, Dickson SL. Ghrelin directly targets the ventral tegmental area to increase food motivation. *Neuroscience* 2011; **180**: 129–137.

28 Alvarez-Crespo M, Skibicka KP, Farkas I, Molnar CS, Egecioglu E, Hrabovszky E, Liposits Z, Dickson SL. The amygdala as a neurobiological target for ghrelin in rats: neuroanatomical, electrophysiological and behavioral evidence. *PLoS One* 2012; **7**: e46321.

29 Kanoski SE, Fortin SM, Ricks KM, Grill HJ. Ghrelin signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. *Biol Psychiatry* 2013; **73**: 915–923.

30 Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci USA* 2004; **101**: 4679–4684.

31 Salome N, Haage D, Perrissoud D, Moulin A, Demange L, Egecioglu E, Fehrentz JA, Martinez J, Dickson SL. Anorexigenic and electrophysiological actions of novel ghrelin receptor (GHS-R1A) antagonists in rats. *Eur J Pharmacol* 2009; **612**: 167–173.

32 Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 2004; **287**: E297–E304.

33 Merkestein M, van Gestel MA, van der Zwaal EM, Brans MA, Luijendijk MC, van Rozen AJ, Hendriks J, Garner KM, Boender AJ, Pandit R, Adan R. GHS-R1a signaling in the DMH and VMH contributes to food anticipatory activity. *Int J Obes (Lond)* 2014; **38**: 610–618.

34 Nutu M, Feng Y, Egecioglu E, Weijdegard B, Stener-Victorin E, Shao R. Stromal cell-specific apoptotic and antiestrogenic mechanisms may explain uterine defects in humans after clomiphene citrate therapy. *Am J Obstet Gynecol*, 2010; **203**: 65 e1–65 e10.

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

36 Skibicka KP, Hansson C, Egecioglu E, Dickson SL. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor gene expression. *Addict Biol* 2012; **17**: 95–107.

37 Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB, Banks WA, Morley JE, Pinto S, Sherwin RS, Xu L, Yamada KA, Sleeman MW, Tschop MH, Horvath TL. Ghrelin controls hippocampal spine synapse density and memory performance. *Nat Neurosci* 2006; **9**: 381–388.

38 Hansson C, Shirazi RH, Naslund J, Vogel H, Neuber C, Holm G, Anckarsater H, Dickson SL, Eriksson E, Skibicka KP. Ghrelin influences novelty seeking behavior in rodents and men. *PLoS One* 2012; **7**: e50409.

39 Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ, Zigman JM. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci* 2008; **11**: 752–753.

40 Hansson C, Haage D, Taube M, Egecioglu E, Salome N, Dickson SL. Central administration of ghrelin alters emotional responses in rats: behavioural, electrophysiological and molecular evidence. *Neuroscience* 2011; **180**: 201–211.

41 Dickson SL, Leng G, Robinson IC. Systemic administration of growth hormone-releasing peptide activates hypothalamic arcuate neurons. *Neuroscience* 1993; **53**: 303–306.

42 Dickson SL, Luckman SM. Induction of c-fos messenger ribonucleic acid in neuropeptide Y and growth hormone (GH)-releasing factor neurons in the rat arcuate nucleus following systemic injection of the GH secretagogue, GH-releasing peptide-6. *Endocrinology* 1997; **138**: 771–777.

43 Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194–198.

44 Willesen MG, Kristensen P, Romer J. Co-localization of growth hormone secretagogue receptor and NPY mRNA in the arcuate nucleus of the rat. *Neuroendocrinology* 1999; **70**: 306–316.

45 Chen HY, Trumbauer ME, Chen AS, Weingarth DT, Adams JR, Frazier EG, Shen Z, Marsh DJ, Feighner SD, Guan XM, Ye Z, Nargund RP, Smith RG, Van der Ploeg LH, Howard AD, MacNeil DJ, Qian S. Orexigenic action of peripheral ghrelin is mediated by neuropeptide Y and agouti-related protein. *Endocrinology* 2004; **145**: 2607–2612.
46 Luquet S, Phillips CT, Palmiter RD. NPY/AgRP neurons are not essential for feeding responses to glucoprivation. *Peptides* 2007; **28**: 214–225.
47 Wang Q, Liu C, Uchida A, Chuang JC, Walker A, Liu T, Osborne-Lawrence S, Mason BL, Mosher C, Berglund ED, Elmquist JK, Zigman JM. Arcuate AgRP neurons mediate orexigenic and glucoregulatory actions of ghrelin. *Mol Metab* 2014; **3**: 64–72.
48 Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 2003; **144**: 1506–1512.
49 Sakata I, Yamazaki M, Inoue K, Hayashi Y, Kangawa K, Sakai T. Growth hormone secretagogue receptor expression in the cells of the stomach-projected afferent nerve in the rat nodose ganglion. *Neurosci Lett* 2003; **342**: 183–186.
50 Date Y, Murakami N, Toshinai K, Matsukura S, Niijima A, Matsuo H, Kangawa K, Nakazato M. The role of the gastric afferent vagal nerve in ghrelin-induced feeding and growth hormone secretion in rats. *Gastroenterology* 2002; **123**: 1120–1128.
51 Date Y. Ghrelin and the vagus nerve. *Methods Enzymol* 2012; **514**: 261–269.
52 Arnold M, Mura A, Langhans W, Geary N. Gut vagal afferents are not necessary for the eating-stimulatory effect of intraperitoneally injected ghrelin in the rat. *J Neurosci* 2006; **26**: 11052–11060.
53 Chuang JC, Hrabovszky E, Hansson C, Jerlhag E, Alvarez-Crespo M, Skibicka KP, Molnar CS, Liposits Z, Engel JA, Egecioglu E. Ghrelin mediates stress-induced food-reward behavior in mice. *J Clin Invest* 2011; **121**: 2684–2692.
54 Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH, Gao XB, Horvath TL. Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *J Clin Invest* 2006; **116**: 3229–3239.
55 Dickson SL, Hrabovszky E, Hansson C, Jerlhag E, Alvarez-Crespo M, Skibicka KP, Molnar CS, Liposits Z, Engel JA, Egecioglu E. Blockade of central nicotine acetylcholine receptor signaling attenuate ghrelin-induced food intake in rodents. *Neuroscience* 2010; **171**: 1180–1186.
56 Jerlhag E, Egecioglu E, Dickson SL, Svensson L, Engel JA. Alpha-conotoxin MII-sensitive nicotinic acetylcholine receptors are involved in mediating the ghrelin-induced locomotor stimulation and dopamine overflow in nucleus accumbens. *Eur Neuropsychopharmacol* 2008; **18**: 508–518.
57 Goldstone AP, Prechtl de Hernandez CG, Beaver JD, Muhammed K, Croese C, Bell G, Durighel G, Hughes E, Waldman AD, Frost G, Bell JD. Fasting biases brain reward systems towards high-calorie foods. *Eur J Neurosci* 2009; **30**: 1625–1635.
58 Goldstone AP, Prechtl CG, Scholtz S, Miras AD, Chhina N, Durighel G, Deliran SS, Beckmann C, Ghatei MA, Ashby DR, Waldman AD, Gaylinn BD, Thorner MO, Frost GS, Bloom SR, Bell JD. Ghrelin mimics fasting to enhance human hedonic, orbitofrontal cortex, and hippocampal responses to food. *Am J Clin Nutr* 2014; **99**: 1319–1330.
59 Malik S, McGlone F, Bedrossian D, Dagher A. Ghrelin modulates brain activity in areas that control appetitive behavior. *Cell Metab* 2008; **7**: 400–409.
60 Karra E, O'Daly OG, Choudhury AI, Yousseif A, Millership S, Neary MT, Scott WR, Chandarana K, Manning S, Hess ME, Iwakura H, Akamizu T, Millet Q, Gelegen C, Drew ME, Rahman S, Emmanuel JJ, Williams SC, Ruther UU, Bruning JC, Withers DJ, Zelaya FO, Batterham RL. A link between FTO, ghrelin, and impaired brain food-cue responsivity. *J Clin Invest* 2013; **123**: 3539–3551.
61 Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. *Trends Neurosci* 2007; **30**: 194–202.
62 Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* 2006; **29**: 565–598.
63 DiLeone RJ, Taylor JR, Picciotto MR. The drive to eat: comparisons and distinctions between mechanisms of food reward and drug addiction. *Nat Neurosci* 2012; **15**: 1330–1335.
64 Hernandez L, Hoebel BG. Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sci* 1988; **42**: 1705–1712.
65 Day JJ, Roitman MF, Wightman RM, Carelli RM. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat Neurosci* 2007; **10**: 1020–1028.
66 Sombers LA, Beyene M, Carelli RM, Wightman RM. Synaptic overflow of dopamine in the nucleus accumbens arises from neuronal activity in the ventral tegmental area. *J Neurosci* 2009; **29**: 1735–1742.
67 Valdivia S, Patrone A, Reynaldo M, Perello M. Acute high fat diet consumption activates the mesolimbic circuit and requires orexin signaling in a mouse model. *PLoS One* 2014; **9**: e87478.
68 Palmiter RD. Is dopamine a physiologically relevant mediator of feeding behavior? *Trends Neurosci* 2007; **30**: 375–381.
69 Zheng H, Patterson LM, Berthoud HR. Orexin signaling in the ventral tegmental area is required for high-fat appetite induced by opioid stimulation of the nucleus accumbens. *J Neurosci* 2007; **27**: 11075–11082.
70 Stratford TR, Kelley AE. Evidence of a functional relationship between the nucleus accumbens shell and lateral hypothalamus subserving the control of feeding behavior. *J Neurosci* 1999; **19**: 11040–11048.
71 Korotkova TM, Sergeeva OA, Eriksson KS, Haas HL, Brown RE. Excitation of ventral tegmental area dopaminergic and nondopaminergic neurons by orexins/hypocretins. *J Neurosci* 2003; **23**: 7–11.
72 Nakamura T, Uramura K, Nambu T, Yada T, Goto K, Yanagisawa M, Sakurai T. Orexin-induced hyperlocomotion and stereotypy are mediated by the dopaminergic system. *Brain Res* 2000; **873**: 181–187.
73 Mahler SV, Smith RJ, Aston-Jones G. Interactions between VTA orexin and glutamate in cue-induced reinstatement of cocaine seeking in rats. *Psychopharmacology* 2013; **226**: 687–698.
74 Berridge KC. Food reward: brain substrates of wanting and liking. *Neurosci Biobehav Rev* 1996; **20**: 1–25.
75 Volkow ND, Wang GJ, Tomasi D, Baler RD. Obesity and addiction: neurobiological overlaps. *Obes Rev* 2013; **14**: 2–18.
76 Norgren R, Hajnal A, Mungarndee SS. Gustatory reward and the nucleus accumbens. *Physiol Behav* 2006; **89**: 531–535.
77 Epstein LH, Temple JL, Roemmich JN, Bouton ME. Habituation as a determinant of human food intake. *Psychol Rev* 2009; **116**: 384–407.
78 Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science* 1997; **275**: 1593–1599.
79 Schultz W. Dopamine signals for reward value and risk: basic and recent data. *Behav Brain Funct* 2010; **6**: 24.
80 Stuber GD, Klanker M, de Ridder B, Bowers MS, Joosten RN, Feenstra MG, Bonci A. Reward-predictive cues enhance excitatory synaptic strength onto midbrain dopamine neurons. *Science* 2008; **321**: 1690–1692.
81 Salamone JD, Correa M. The mysterious motivational functions of mesolimbic dopamine. *Neuron* 2012; **76**: 470–485.
82 Steinberg EE, Keiflin R, Boivin JR, Witten IB, Deisseroth K, Janak PH. A causal link between prediction errors, dopamine neurons and learning. *Nat Neurosci* 2013; **16**: 966–973.
83 Roitman MF, Stuber GD, Phillips PE, Wightman RM, Carelli RM. Dopamine operates as a subsecond modulator of food seeking. *J Neurosci* 2004; **24**: 1265–1271.

84 Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA. Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. *Addict Biol* 2006; **11**: 45–54.
85 Egecioglu E, Jerlhag E, Salome N, Skibicka KP, Haage D, Bohlooly YM, Andersson D, Bjursell M, Perrissoud D, Engel JA, Dickson SL. Ghrelin increases intake of rewarding food in rodents. *Addict Biol* 2010; **15**: 304–311.
86 Jerlhag E, Egecioglu E, Landgren S, Salome N, Heilig M, Moechars D, Datta R, Perrissoud D, Dickson SL, Engel JA Requirement of central ghrelin signaling for alcohol reward. *Proc Natl Acad Sci USA* 2009; **106**: 11318–11323.
87 Jerlhag E, Egecioglu E, Dickson SL, Engel JA. Ghrelin receptor antagonism attenuates cocaine- and amphetamine-induced locomotor stimulation, accumbal dopamine release, and conditioned place preference. *Psychopharmacology* 2010; **211**: 415–422.
88 Wellman PJ, Davis KW, Nation JR. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. *Regul Pept* 2005; **125**: 151–154.
89 Overduin J, Figlewicz DP, Bennett-Jay J, Kittleson S, Cummings DE. Ghrelin increases the motivation to eat, but does not alter food palatability. *Am J Physiol Regul Integr Comp Physiol* 2012; **303**: R259–R269.
90 Davis JF, Perello M, Choi DL, Magrisso IJ, Kirchner H, Pfluger PT, Tschoep M, Zigman JM, Benoit SC. GOAT induced ghrelin acylation regulates hedonic feeding. *Horm Behav* 2012; **62**: 598–604.
91 Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, Date Y, Nakazato M. Central administration of ghrelin preferentially enhances fat ingestion. *Neurosci Lett* 2004; **369**: 75–79.
92 Disse E, Bussier AL, Veyrat-Durebex C, Deblon N, Pfluger PT, Tschop MH, Laville M, Rohner-Jeanrenaud F. Peripheral ghrelin enhances sweet taste food consumption and preference, regardless of its caloric content. *Physiol Behav* 2010; **101**: 277–281.
93 Blum ID, Patterson Z, Khazall R, Lamont EW, Sleeman MW, Horvath TL, Abizaid A. Reduced anticipatory locomotor responses to scheduled meals in ghrelin receptor deficient mice. *Neuroscience* 2009; **164**: 351–359.
94 Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA. Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. *Addict Biol* 2007; **12**: 6–16.
95 Keen-Rhinehart E, Bartness TJ. Peripheral ghrelin injections stimulate food intake, foraging, and food hoarding in Siberian hamsters. *Am J Physiol Regul Integr Comp Physiol* 2005; **288**: R716–R722.
96 Tong J, Mannea E, Aime P, Pfluger PT, Yi CX, Castaneda TR, Davis HW, Ren X, Pixley S, Benoit S, Julliard K, Woods SC, Horvath TL, Sleeman MM, D'Alessio D, Obici S, Frank R, Tschop MH. Ghrelin enhances olfactory sensitivity and exploratory sniffing in rodents and humans. *J Neurosci* 2011; **31**: 5841–5846.
97 Skibicka KP, Dickson SL. Ghrelin and food reward: the story of potential underlying substrates. *Peptides* 2011; **32**: 2265–2273.
98 King SJ, Isaacs AM, O'Farrell E, Abizaid A. Motivation to obtain preferred foods is enhanced by ghrelin in the ventral tegmental area. *Horm Behav* 2011; **60**: 572–580.
99 Koob GF, Riley SJ, Smith SC, Robbins TW. Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *J Comp Physiol Psychol* 1978; **92**: 917–927.
100 Baldo BA, Sadeghian K, Basso AM, Kelley AE. Effects of selective dopamine D1 or D2 receptor blockade within nucleus accumbens subregions on ingestive behavior and associated motor activity. *Behav Brain Res* 2002; **137**: 165–177.
101 Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D, Mahan K. Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. *Psychopharmacology* 1991; **104**: 515–521.
102 Koch M, Schmid A, Schnitzler HU. Role of muscles accumbens dopamine D1 and D2 receptors in instrumental and Pavlovian paradigms of conditioned reward. *Psychopharmacology* 2000; **152**: 67–73.
103 Skibicka KP, Shirazi RH, Rabasa-Papio C, Alvarez-Crespo M, Neuber C, Vogel H, Dickson SL. 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* 2013; **73**: 274–283.
104 Yamanaka A, Beuckmann CT, Willie JT, Hara J, Tsujino N, Mieda M, Tominaga M, Yagami K, Sugiyama F, Goto K, Yanagisawa M, Sakurai T. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 2003; **38**: 701–713.
105 Narita M, Nagumo Y, Hashimoto S, Narita M, Khotib J, Miyatake M, Sakurai T, Yanagisawa M, Nakamachi T, Shioda S, Suzuki T. Direct involvement of orexinergic systems in the activation of the mesolimbic dopamine pathway and related behaviors induced by morphine. *J Neurosci* 2006; **26**: 398–405.
106 Harris GC, Wimmer M, Aston-Jones G. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature* 2005; **437**: 556–559.
107 Cone JJ, McCutcheon JE, Roitman MF. Ghrelin acts as an interface between physiological state and phasic dopamine signaling. *J Neurosci* 2014; **34**: 4905–4913.
108 Krashes MJ, Koda S, Ye C, Rogan SC, Adams AC, Cusher DS, Maratos-Flier E, Roth BL, Lowell BB. Rapid, reversible activation of AgRP neurons drives feeding behavior in mice. *J Clin Invest* 2011; **121**: 1424–1428.
109 Josselyn SA, Beninger RJ. Neuropeptide Y: intraaccumbens injections produce a place preference that is blocked by cis-flupenthixol. *Pharmacol Biochem Behav* 1993; **46**: 543–552.
110 Pandit R, Luijendijk MC, Vanderschuren LJ, la Fleur SE, Adan RA. Limbic substrates of the effects of neuropeptide Y on intake of and motivation for palatable food. *Obesity (Silver Spring)* 2014; **22**: 1216–1219.
111 Skibicka KP, Shirazi RH, Hansson C, Dickson SL. Ghrelin interacts with neuropeptide Y Y1 and opioid receptors to increase food reward. *Endocrinology* 2012; **153**: 1194–1205.
112 Romero-Pico A, Vazquez MJ, Gonzalez-Touceda D, Folgueira C, Skibicka KP, Alvarez-Crespo M, Van Gestel MA, Velasquez DA, Schwarzer C, Herzog H, Lopez M, Adan RA, Dickson SL, Dieguez C, Nogueiras R. Hypothalamic kappa-opioid receptor modulates the orexigenic effect of ghrelin. *Neuropsychopharmacology* 2013; **38**: 1296–1307.
113 Williams DL, Cummings DE, Grill HJ, Kaplan JM. Meal-related ghrelin suppression requires postgastric feedback. *Endocrinology* 2003; **144**: 2765–2767.
114 Murakami N, Hayashida T, Kuroiwa T, Nakahara K, Ida T, Mondal MS, Nakazato M, Kojima M, Kangawa K. Role for central ghrelin in food intake and secretion profile of stomach ghrelin in rats. *J Endocrinol* 2002; **174**: 283–288.
115 Bagnasco M, Tulipano G, Melis MR, Argiolas A, Cocchi D, Muller EE. Endogenous ghrelin is an orexigenic peptide acting in the arcuate nucleus in response to fasting. *Regul Pept* 2003; **111**: 161–167.
116 Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, Kasuga M. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003; **52**: 947–952.
117 Uchida A, Zigman JM, Perello M. Ghrelin and eating behavior: evidence and insights from genetically-modified mouse models. *Front Neurosci* 2013; **7**: 121.
118 McFarlane MR, Brown MS, Goldstein JL, Zhao TJ. Induced ablation of ghrelin cells in adult mice does not decrease food intake, body weight, or response to high-fat diet. *Cell Metab* 2014; **20**: 54–60.

119 Lippl F, Erdmann J, Steiger A, Lichter N, Czogalla-Peter C, Bidlingmaier M, Tholl S, Schusdziarra V. Low-dose ghrelin infusion–evidence against a hormonal role in food intake. *Regul Pept* 2012; **174**: 26–31.

120 Druce MR, Neary NM, Small CJ, Milton J, Monteiro M, Patterson M, Ghatei MA, Bloom SR. Subcutaneous administration of ghrelin stimulates energy intake in healthy lean human volunteers. *Int J Obes (Lond)* 2006; **30**: 293–296.

121 Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes (Lond)* 2005; **29**: 1130–1136.

122 Perello M, Zigman JM. The role of ghrelin in reward-based eating. *Biol Psychiatry* 2012; **72**: 347–353.

123 Lamont EW, Patterson Z, Rodrigues T, Vallejos O, Blum ID, Abizaid A. Ghrelin-deficient mice have fewer orexin cells and reduced cFOS expression in the mesolimbic dopamine pathway under a restricted feeding paradigm. *Neuroscience* 2012; **218**: 12–19.

124 Patterson ZR, Khazall R, Mackay H, Anisman H, Abizaid A. Central ghrelin signaling mediates the metabolic response of C57BL/6 male mice to chronic social defeat stress. *Endocrinology* 2013; **154**: 1080–1091.

125 Cabral A, Suescun O, Zigman JM, Perello M. Ghrelin indirectly activates hypophysiotropic CRF neurons in rodents. *PLoS One* 2012; **7**: e31462.

126 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. Ghrelin strongly stimulates growth hormone release in humans. *J Clin Endocrinol Metab* 2000; **85**: 4908–4911.

127 Fry M, Ferguson AV. Ghrelin: central nervous system sites of action in regulation of energy balance. *Int J Pept* 2010; **2010**: 616757.

128 Banks WA. The blood–brain barrier: connecting the gut and the brain. *Regul Pept* 2008; **149**: 11–14.

129 Cabral A, Valdivia S, Fernandez G, Reynaldo M, Perello M. Divergent neuronal circuitries underlying acute orexigenic effects of peripheral or central ghrelin: critical role of brain accessibility. *J Neuroendocrinol* 2014; **26**: 542–554.

130 Schaeffer M, Langlet F, Lafont C, Molino F, Hodson DJ, Roux T, Lamarque L, Verdie P, Bourrier E, Dehouck B, Baneres JL, Martinez J, Mery PF, Marie J, Trinquet E, Fehrentz JA, Prevot V, Mollard P. Rapid sensing of circulating ghrelin by hypothalamic appetite-modifying neurons. *Proc Natl Acad Sci USA* 2013; **110**: 1512–1517.

131 Bailey AR, von Engelhardt N, Leng G, Smith RG, Dickson SL. Growth hormone secretagogue activation of the arcuate nucleus and brainstem occurs via a non-noradrenergic pathway. *J Neuroendocrinol* 2000; **12**: 191–197.

132 Gilg S, Lutz TA. The orexigenic effect of peripheral ghrelin differs between rats of different age and with different baseline food intake, and it may in part be mediated by the area postrema. *Physiol Behav* 2006; **87**: 353–359.

133 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. Peripheral ghrelin transmits orexigenic signals through the noradrenergic pathway from the hindbrain to the hypothalamus. *Cell Metab* 2006; **4**: 323–331.

134 Jerlhag E. Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. *Addict Biol* 2008; **13**: 358–363.

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

136 Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 2003; **37**: 649–661.

137 Sakata I, Nakano Y, Osborne-Lawrence S, Rovinsky SA, Lee CE, Perello M, Anderson JG, Coppari R, Xiao G, Lowell BB, Elmquist JK, Zigman JM. Characterization of a novel ghrelin cell reporter mouse. *Regul Pept* 2009; **155**: 91–98.

138 Cong WN, Golden E, Pantaleo N, White CM, Maudsley S, Martin B. Ghrelin receptor signaling: a promising therapeutic target for metabolic syndrome and cognitive dysfunction. *CNS Neurol Disord Drug Targets* 2010; **9**: 557–563.

139 Mokrosinski J, Holst B. Modulation of the constitutive activity of the ghrelin receptor by use of pharmacological tools and mutagenesis. *Methods Enzymol* 2010; **484**: 53–73.

140 Damian M, Marie J, Leyris JP, Fehrentz JA, Verdie P, Martinez J, Baneres JL, Mary S. High constitutive activity is an intrinsic feature of ghrelin receptor protein: a study with a functional monomeric GHS-R1a receptor reconstituted in lipid discs. *J Biol Chem* 2012; **287**: 3630–3641.

141 Schellekens H, van Oeffelen WE, Dinan TG, Cryan JF. Promiscuous dimerization of the growth hormone secretagogue receptor (GHS-R1a) attenuates ghrelin-mediated signaling. *J Biol Chem* 2013; **288**: 181–191.

142 Rediger A, Piechowski CL, Yi CX, Tarnow P, Strotmann R, Gruters A, Krude H, Schoneberg T, Tschop MH, Kleinau G, Biebermann H. Mutually opposite signal modulation by hypothalamic heterodimerization of ghrelin and melanocortin-3 receptors. *J Biol Chem* 2011; **286**: 39623–39631.

143 Kern A, Albarran-Zeckler R, Walsh HE, Smith RG. Apo-ghrelin receptor forms heteromers with DRD2 in hypothalamic neurons and is essential for anorexigenic effects of DRD2 agonism. *Neuron* 2012; **73**: 317–332.

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