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REVIEW ARTICLE

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Stress and obesity: The ghrelin connection

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Ghrelin is a hormone associated with feeding and energy balance. Not surprisingly, this hormone is secreted in response to acute stressors and it is chronically elevated after exposure to chronic stress in tandem with a number of metabolic changes aimed at attaining homeostatic balance. In the present review, we propose that ghrelin plays a key role in these stress-induced homeostatic processes. Ghrelin targets the hypothalamus and brain stem nuclei that are part of the sympathetic nervous system to increase appetite and energy expenditure and promote the use of carbohydrates as a source of fuel at the same time as sparing fat. Ghrelin also targets mesolimbic brain regions such as the ventral segmental area and the hippocampus to modulate reward processes, to protect against damage associated with chronic stress, as well as to potentially increase resilience to stress. In all, these data support the notion that ghrelin, similar to corticosterone, is a critical metabolic hormone that is essential for the stress response.

KEYWORDS
adiposity, anxiety, chronic social stress, depression, feeding, ghrelin, ghrelin receptors, metabolism, motivation, obesity

1 | INTRODUCTION

In general, the stress response can be considered as a homeostatic response that is generated to meet the energetic demands of an environmental threat. As such, it is not surprising that continuous exposure to stressful situations is often associated with metabolic imbalance. For example, acute or unpredictable stressors result in significant decreases in body weight and food intake, whereas chronic social or predictable stressors result in increased caloric intake and weight gain.[1-5] Acute psychogenic or systemic stressors can result in rapid decline in food intake and the utilisation of carbohydrate stores.[2] Although unpredictable chronic stressors also lead to decreased body weight and food intake, this weight loss is not accompanied by a depletion of fat stores but, instead, results from the use of carbohydrate stores leading to altered glucose regulation, an early indicator of insulin resistance.[2,6-8] By contrast, experiments in a wide array of species including non-human and human primates show that exposure to social stressors generates increases in caloric intake, body weight and adiposity.[9-13] The mechanisms underlying metabolic changes in response to social stressors have been commonly associated with negative consequences of repeated stimulation of the hypothalamic-pituitary-adreenal (HPA) axis and chronically elevated glucocorticoid concentrations.[3,14-20] Nevertheless, glucocorticoids are not the only hormones affected by chronic social stressors, and other hormones play an important role in regulating the metabolic responses required to meet the energy demands posed by continued exposure to social stressors. Recent evidence implicates ghrelin as a hormone that is required for appropriate homeostatic responses to different types of stressors.

2 | GHRELIN AS A REGULATOR OF ENERGY STATE

Ghrelin is a 28 amino-acid peptide secreted by the stomach that was first found to be an endogenous growth hormone secretagogue.[1-3] Soon after, it was determined that, in addition to growth hormone secretion, ghrelin was also a potent stimulator of food intake and adiposity.[4-7] The timing of ghrelin secretion was closely associated with the circadian onset of meals in laboratory animals, and with

the timing of scheduled meals in animals and humans.[6,8] Negative energy balance states such as fasting or chronic food restriction resulted in increased plasma concentrations of ghrelin in most species examined whereas eating results in a rapid decline in plasma ghrelin concentrations, suggesting that ghrelin is an important hormone driving appetite.[6,9-11] Furthermore, the biological activity of ghrelin depends on the presence of *n*-octanoic acid, a free fatty acid that is attached through enzymatic acylation to the ghrelin peptide on the third amino acid (serine 3) of the molecule chain.[1] The rate of active ghrelin synthesis is limited by the activity of at least one known enzyme, the ghrelin O-acyltransferase (GOAT), which is synthesised primarily in the stomach.[12,13]

Most biological effects of ghrelin are mediated by the binding of this hormone to the growth hormone secretagogue receptor (GHSR) 1a subtype, which is the only known biologically active ghrelin receptor to date.[2] This receptor is found throughout the body, although it is abundantly expressed in the brain, pituitary and adrenal glands.[2] In the brain, GHSR is particularly abundant throughout the hypothalamus, although it is also expressed in a number of extrahypothalamic brain regions that are implicated in complex processes that include the stress response.[14,15] For example, some of the first studies looking at the expression of mRNA encoding for the GHSR in the brains of rodents and primates showed a relatively strong signal in mesolimbic structures that include the hippocampus, the mid brain ventral tegmental area (VTA) and the substantia nigra.[14-16] More detailed studies using more sensitive mRNA probes or reporter genes confirmed the results from early findings and adding data showing the expression of GHSR in brain stem regions important for integrating nutritional information from the periphery, including the brain stem area postrema, the nucleus of the solitary tract (NTS) and the parabrachial nucleus.[15,17] Moreover, these studies also show GHSR expression in brain regions important for the expression of emotion in response to positive or negative stimuli, including the medial and basolateral amygdala as well as the paraventricular thalamus and lateral septum.[15,17] The widespread distribution of GHSR therefore points to ghrelin as a hormone implicated in complex processes that not only regulate metabolism, but also regulate behavioural and cognitive processes associated with attaining homeostatic balance in response to stress.

3 | GHRELIN AND THE STRESS RESPONSE

Given its role in food intake and metabolism, it is not surprising that ghrelin also participates in the neuroendocrine responses to stressors. It has been recognised that ghrelin is secreted in response to a variety of acute stressors, including fasting, restraint, foot shock and social stressors.[18-22] Interestingly, exposure to chronic social defeat results in elevated plasma concentrations of ghrelin that remain elevated for days beyond the duration of the stress paradigm. Acutely, ghrelin appears to amplify the activity of the HPA axis given that exogenous ghrelin treatment evokes increased adrenocorticotrophic hormone (ACTH) and prolactin release from the pituitary.[19,23] The role of ghrelin during the stress response is not yet fully understood although it is suggested that, similar to cortisol or corticosterone, ghrelin plays a protective role. For example, ghrelin acts locally in the stomach as a buffer against ulceration following chronic stress.[24] However, the role of ghrelin in the stress response serves a number of functions other than gastro-protection. Among them, ghrelin attenuates the display of anxiety and depressive-like behaviours in socially stressed mice.[19,21] By contrast, mice with targeted deletion of the GHSR gene (GHSR KO) display more of these behaviours following chronic social defeat than their wild-type (WT) littermates,[21,25] and some of these effects are reversed by genetic rescue of the GHSR in specific brain regions. For example, restoration of GHSR gene expression in DA cells reverses social stress-induced anhedonia.[25] These latter data are particularly relevant to humans because we have shown that social stress as evoked by the trier stress (or in anticipation of this task), enhances ghrelin secretion particularly in a subset of human subjects that report higher consumption of food in response to stress.[26,27]

Using rodent models of chronic stress, our laboratory has generated considerable evidence supporting the idea that ghrelin mediates stress-induced metabolic alterations. In particular, we have successfully used a variation of chronic social defeat in mice as a model of social stress that reliably increases caloric intake and weight gain in correlation with increased plasma acylated ghrelin concentrations.[28] In this model, experimental mice with free access to water and regular mouse chow, as well as restricted access to a high calorie palatable diet pellets (60% of calories from fat), are monitored daily for food intake and body weight during a 2-week baseline period. At the end of this period, mice assigned to the social stressor condition are transferred to a different room where they are housed in the cage of a CD-1 retired breeder mouse. Physical interaction between the two mice is allowed until the experimental mouse displays natural submissive posture, an effect that usually occurs within 10 minutes of the introduction of the experimental mouse into the cage of the resident "bully" CD-1 mouse. Mice are quickly separated to prevent physical injury but are left in the cage to live with the bully for the next 3 weeks separated only by a screen that allows exposure to visual and olfactory cues to be shared between mice. Once daily, physical interaction is allowed for a maximum of 10 minutes or until submissive posture is observed, and event that can happen as quickly as 10 seconds. After 3 weeks, mice are either killed to determine outcomes, or they are allowed to recover to determine the long-lasting consequences of this type of stressor. As noted above, this paradigm increases caloric intake, body weight and plasma levels of active ghrelin in comparison to non-stressed mice.[28] Similar increases in caloric intake, body weight and plasma ghrelin concentrations are observed with a 10-day social defeat paradigm. Interestingly, increased caloric intake comes not from an increase in the high calorie palatable diet but, instead, from an increase in the intake of regular chow, a diet that is carbohydrate rich (70% calories from carbohydrate sources), suggesting that ghrelin promotes the intake of diets that are rich in carbohydrates over those that are rich in fat.[29] We then conducted similar studies this time using mice

with mutations to the GHSR and replicated our data on WT mice of the same strain, although the effects of chronic social defeat were attenuated in GHSR KO mice.[28] Furthermore, using indirect calorimetry, we found that socially defeated WT mice favoured the utilisation of carbohydrates as a source of fuel, whereas GHSR KO mice continued to use fat to meet the energetic demands generated by chronic social defeat. This resulted in the accumulation of fat in WT mice and the depletion of fat stores in GHSR KO mice.[28] Because the GHSR is found in both the periphery and the central nervous system, we then conducted a study infusing vehicle of the GHSR antagonist D-Lys-GHRP chronically into the lateral ventricle of mice undergoing social defeat or controls. Similar to GHSR KO mice, mice treated with the GHSR antagonist showed attenuated caloric intake and weight gain in response to social defeat. These data showed that GHSR in the central nervous system mediates the metabolic effects of social stress.[28]

4 | GHRELIN AND THE HYPOTHALAMUS IN THE FACE OF STRESS

Ghrelin is a potent stimulator of food intake and these effects are mediated in part through the binding of ghrelin onto the hypothalamic arcuate nucleus (ARC),[4,5,30,31] where ghrelin stimulates neuropeptide Y/agouti-related peptide (NPY/AGRP) neurones almost selectively. Indeed, the appetite-stimulating effects of ghrelin appear to be highly dependent on the presence of NPY and AGRP.[10] Peripheral ghrelin injections increase immediate early gene expression in the ARC, and the activity of inputs to AGRP neurones.[32] It is therefore not surprising that chronic social defeat also results in increased hypothalamic expression of NPY and AGRP.[28] By contrast, GHSR KO mice that are socially defeated do not show these increases, suggesting that stress-induced ghrelin concentrations target GHSR in NPY/AGRP neurones in the ARC to increase food intake and alter metabolism.[28]

There are, however, other hypothalamic brain regions that may play a role in the regulation of stress-induced changes in food intake and energy expenditure. For example, the hypothalamic paraventricular nucleus (PVN) expresses GHSR mRNA, and cells within this region show increased c-fos expression following peripheral, i.c.v. and intra-PVN infusions of ghrelin.[5,14,15,33,34] Furthermore, the effects of ghrelin on c-fos expression in the PVN are not dependent on an intact ARC.[34] Interestingly, ghrelin delivery into the PVN results not only in increased food intake, but also in increased corticotrophin-releasing hormone (CRH) mRNA expression, as well as increased corticosterone secretion.[34] Again, this particular effect that is not associated with the actions of ghrelin on AGRP or NPY neurones in the ARC, which are known to project to the PVN.[34] These effects are also seen despite CRH cells not expressing GHSR. In rats, central delivery of ghrelin increases the secretion of ACTH and corticosterone, whereas ghrelin deficient mice have lower ACTH and corticosterone responses to an acute stressor, suggesting that ghrelin is required for normal HPA function.[19] Paradoxically, mice with targeted deletions of the ghrelin gene (GHRL KO mice) show increased CRH mRNA expression under basal conditions and enhanced c-fos and CRH mRNA expression following an acute stressor.[19] This suggests that ghrelin not only acts to enhance the stress response acutely, but also could play an important role in the negative-feedback loop mechanisms that stop the activity of the HPA axis, although this needs further investigation.

The effects of ghrelin on the PVN under chronic stress conditions are also complex. Blocking GHSR in the PVN during chronic social defeat enhances the intake of a high calorie diet in stressed mice.[35] One possible explaination for these data is that ghrelin acts on the PVN to enhance inhibitory tone onto CRH neurones as part of a negative-feedback loop that tones down the HPA axis, and not producing ghrelin leaves CRH neurones at a lower threshold of activation. The complexity of these effects lies in the heterogeneous nature of the PVN. Indeed, the PVN contains cells that produce a number of different peptides that have either stimulatory feeding effects (ie, galanin), or inhibitory feeding effects (CRH, oxytocin, thyrotrophin-releasing hormone). Moreover, similar to glucocorticoids, ghrelin recruits a number of complex mechanisms to produce its effects in the PVN and these include the release of endocannabinoids and nitric oxide to influence appetite.[36,37] Whether these mechanisms are also recruited by ghrelin during chronic social defeat stress remains undetermined.

Chronic social defeat alters a number of metabolic and feeding processes that are regulated by regions of the brain that coordinate feeding and metabolism with changes light/dark cycles, the availability of food, or the presence of stressors and other relevant stimuli that occur at specific times of the day. Regions such as the suprachiasmatic nucleus, lateral hypothalamus and dorsomedial nucleus of the hypothalamus express ghrelin receptors and may be important in the regulation of energy balance.[14,15] In the suprachiasmatic nucleus, GHSR appears to be important for the normal functioning of the circadian system under conditions where light/dark cycles are absent.[38] Ghrelin also targets GHSR in the dorsomedial hypothalamic nucleus to facilitate the entrainment of circadian rhythms to scheduled meals,[39] and potentially may also alter metabolic processes through effects on energy expenditure.[40] Finally, in the lateral hypothalamus, ghrelin may act to increase food intake, food reward and arousal via the stimulation of orexin neurones.[41] The effects of chronic social defeat on these systems have been sparsely examined, although there is evidence to suggest that the orexin system protects against some of the metabolic consequences produced by this form of stress[42] and also has anxiolytic effects that mimic those seen by caloric restriction.[43] The role of ghrelin in these stress-induced alterations has not been investigated but the link is evident.

5 | STRESS, GHRELIN AND SYMPATHETIC ACTIVITY

The sympathetic response is one that plays a critical role in the homeostatic changes associated with stress. The sympathetic nervous

system is regulated by neurones in the hypothalamus that project caudally to brains stem regions such as the dorsal motor nucleus of the vagus (DMV), comprising a region that contains noradrenergic cells critical for the sympathetic response to stressors and the integration of sensory inputs with ascending and descending information from the enteric nervous system.[15,44,45] Another important region in the brain stem is the NTS, a region also that integrates ascending visceral and hormonal information from.[46] Cells within these structures also express the GHSR.[15] Ghrelin infusions into the fourth ventricle or directly into the DMV or NTS induce feeding.[47,48] Importantly, targeted destruction of noradrenergic cells in the hind brain abolishes peripheral ghrelin-induced feeding.[49] Furthermore, fourth ventricular ghrelin infusions, which presumably bypass the hypothalamus, produce feeding responses in rats akin to those seen after infusions delivered into the lateral ventricles.[50]

The effects of ghrelin on the brain stem, in particular the NTS, are also associated with the role of this hormone in maintaining glucose levels in the face of low glucose levels. Indeed, GHSR, GHRL and GOAT KO mice have difficulties maintaining plasma glucose concentrations following an acute fast, and this can sometimes lead to mortality.[51-53] Stress, such as fasting, requires the availability and increased utilisation of glucose and it appears that ghrelin receptors in the NTS may be important for the glucose level maintenance, given that selective rescue of GHSR in noradrenergic cells in the NTS can restore the ability of GHSR KO mice to maintain glucose concentrations in spite of a fast.[54] During chronic social defeat, ghrelin levels rise and so do glucose and insulin concentrations, an effect that is not seen in GHSR KO mice.[28] It is therefore likely that GHSR in the NTS may be critical for these metabolic adaptations to meet the glucose requirements produced by the chronic stressor.

6 | GHRELIN AND REWARD CIRCUITRY

Feeding behaviour is driven not only by homeostatic processes but also by the incentive value of high calorie foods. This reward-based eating is considered to be elicited by increases in the activity of the mesolimbic dopaminergic system. Key to this system are dopamine cells located in the VTA and their projections to forebrain limbic structures that include the nucleus accumbens, hippocampus, amygdala and prefrontal cortex.[55] Interestingly, cells within the VTA express the message for the GHSR.[14,15] Many of these cells are dopaminergic (although not all GHSR positive cells are dopaminergic), with estimates suggesting that approximately 50%-60% of dopamine cells co-express GHSR.[56] Patch clamp electrophysiological studies show that these GHSR positive dopamine neurones show robust increases in firing rate and remarkably rapid plastic changes in response to ghrelin.[56] These changes lower the threshold of activation of these neurones sensitising these cells to further stimulation. Not surprisingly, systemic or intra-VTA ghrelin treatment increase dopamine release in the nucleus accumbens, resulting in increased feeding responses and increased motivation to eat as measured by the amount of work rodents are willing to perform to obtain food.[56-61] Ghrelin delivered peripherally or into the VTA also enhances the incentive value of cues associated with rewards, as demonstrated by studies where rats will bar press more in response to cues that predict the availability of food even after extinction and when animals are not food restricted.[62,63] By contrast, GHSR KO mice show attenuated cue-induced feeding.[64]

There have been a number of studies investigating at the role of VTA ghrelin receptors on feeding in response to stress. For example, GHSR KO mice show a decrease in the intake and conditioned place preference for high-fat palatable diets, and these effects are reversed in GHSR KO mice where the GHSR is rescued selectively in tyrosine hydroxylase, an enzyme found in dopamine and norepinephrine-producing neurones.[25] Although the model used did not selectively target dopamine neurones in the VTA, it did rescue GHSR to show its highest relative concentrations in this region, suggesting that GHSR in the VTA does play an important role in stress-induced consumption of palatable foods. A more selective approach is required to understand the role of ghrelin sensitive dopamine cells in the VTA, however, and this will be achieved by newly available viruses targeting tyrosine hydroxylase-specific cells. Also important is the potential for ghrelin to target non-dopaminergic cells in the VTA, including cells that produce GABA, as suggested by a recent study and given the potential role of VTA GABA neurones in reward prediction.[65,66] Moreover, given the role of the VTA in stress-induced psychopathology, understanding the relative contribution of dopamine and non-dopamine VTA neurones will be critical to characterise how the VTA mediates the feeding, as well as the anxiogenic and depressive-like behaviours seen following chronic stress.

7 | GHRELIN, STRESS AND THE HIPPOCAMPUS

Outside of the hypothalamus and olfactory bulbs, the greatest concentration of ghrelin receptors is detected in the hippocampus, and particularly in the dentate gyrus, CA2 and CA3 regions of rats, mice and primates.[14-16] Interestingly, all of these regions are affected by chronic stressors including chronic social defeat. Indeed, many of the mechanisms linked to the pathological effects of chronic stress include deficits in hippocampal dendritic spine density, plasticity and neurogenesis.[67-69] All of these deficits are linked to stress induce cognitive deficits and, ultimately, with depression and anxiety. The effects of stress on the hippocampus are often attributed to altered glucocorticoid signalling, although a number of recent studies suggest that, in addition to glucocorticoid signalling, ghrelin signalling has a protective role in the hippocampus in the face of chronic stress. For example, ghrelin signalling in the hippocampus not only increases spine density, hippocampal plasticity and performance in cognitive tasks, but also increases neurogenesis and prevents damage following brain insults such as ischaemia in rats and mice, resulting in a better prognosis.[70-73] By contrast, deficits in ghrelin signalling are associated with decreased neurogenesis, hippocampal spine density and

plasticity, and decreased performance in cognitive tasks, in addition to increased susceptibility of brain damage following brain insults.[74] Indeed, short-term calorie restriction not only enhances memory processes, but also increases neurogenesis in WT mice but not in GHSR KO mice.[70]

Given that ghrelin signalling is important for maintaining resilience to chronic social defeat, it would appear obvious that ghrelin or ghrelin analogues could be used as potential treatments to prevent or treat stress-induced anxiety and depressive-like behaviours. Nevertheless, there is sparse data relating to this question. Lutter et al[43] showed that caloric restriction or ghrelin injections resulted in anxiolytic and anti-depressant-like effects in non-stressed mice as measured in performance in the elevated plus maze and in the forced swim test. A recent study confirmed these results and compared them with the results obtained following ketamine administration showing that ghrelin can reduce depressive-like behaviours by mice to the same extent as ketamine.[75] Finally, it appears that the display of depressive-like behaviours seen following a 2-week chronic unpredictable stress paradigm are attenuated if mice are treated with ghrelin or the ghrelin analogue GHRP-6 when they are being stressed.[76] It is not known, however, if the effects of chronic social defeat on behaviour can be attenuated by ghrelin, nor whether ghrelin restores synaptic plasticity and integrity within the hippocampus following a stressor. We do know, however, that drugs that increase neurogenesis in the hippocampus can enhance resilience in GHSR KO mice.[77] Future research using ghrelin agonists such as anamorelin, a drug that can cross the blood-brain barrier and one that is also used for the treatment of cachexia,[78] are needed to determine their potential neuroprotective and neurogenic action.

Within the context of feeding, the questions that remain to be clarified relate to the role of ghrelin and ghrelin signalling within the hippocampus. The hippocampus is often not considered to be important for feeding regulation, although emerging data suggest that the

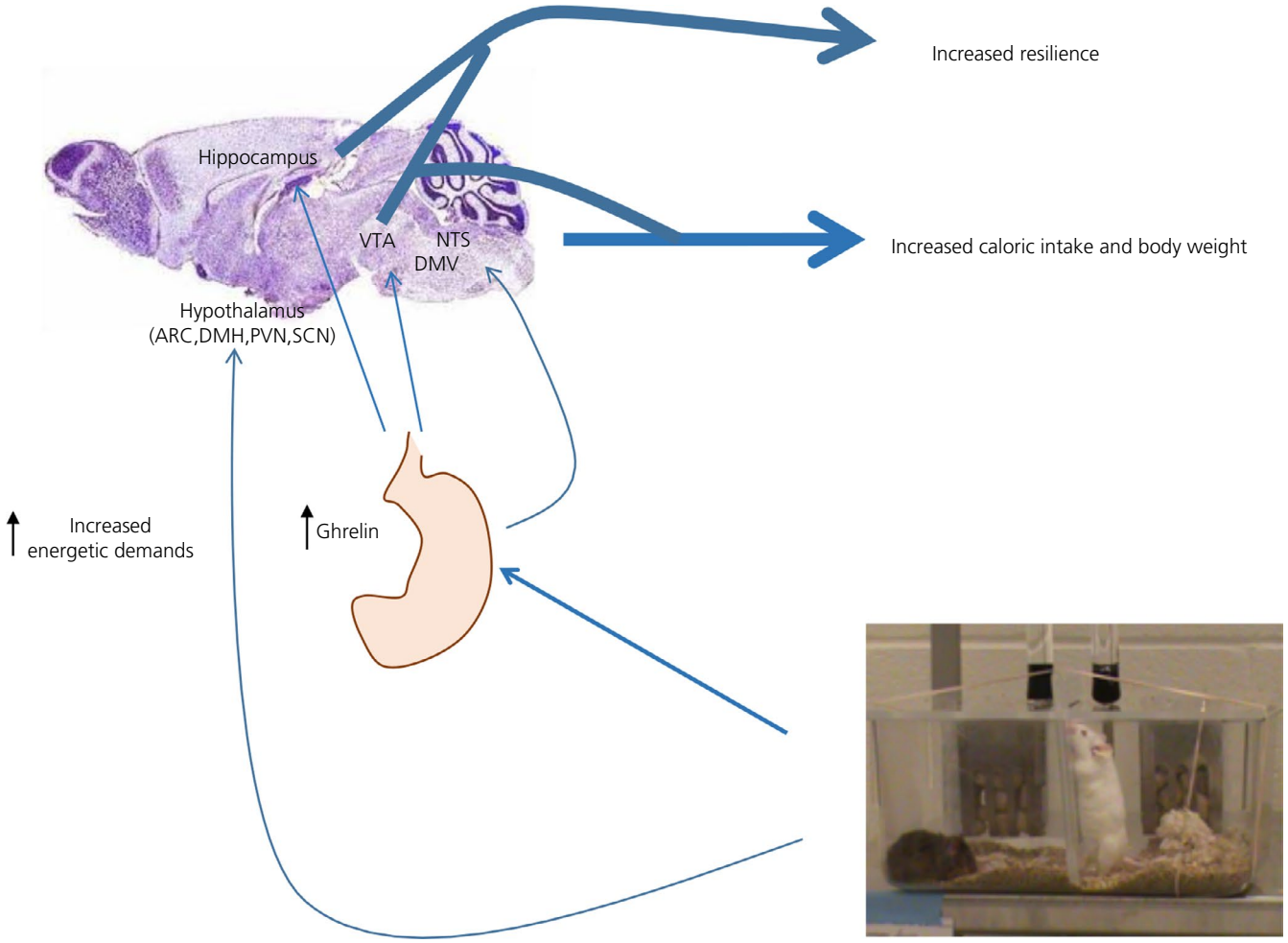

FIGURE 1 Model showing the parallel effect of ghrelin on different brain regions to modulate appetite and metabolism during stress. We propose that, as seen in this figure, ghrelin is secreted during the stress response to meet the energetic demands elicited by chronic social defeat. In the brain, ghrelin targets different hypothalamic and brain stem regions to increase appetite, and alter energy expenditure to facilitate the use of carbohydrate as a metabolic substrate. In addition ghrelin targets hypothalamic and mesolimbic systems to modulate the stress response and ultimately attenuate the deleterious effects of stress. ARC, arcuate nucleus; DMH, dorsomedial nucleus of the hypothalamus; DMV, dorsal motor nucleus of the vagus; NTS, nucleus of the solitary tract; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; VTA, ventral tegmental area

hippocampus may play an important role in the processes underlying time feeding in rodents, and ghrelin may also be implicated in this.[79] Similarly, the effects of ghrelin on motivated behaviours including cue induced feeding and progressive ratio responding have also been linked to ghrelin signalling in the ventral hippocampus of rats.[80] The ventral hippocampus has been linked to the regulation of the HPA axis and the regulation of emotional responses,[81] suggesting that ghrelin signalling in this region may be critical for the regulation of stress induced feeding and anxiety and depressive like behaviours.

8 | CONCLUSIONS AND FUTURE DIRECTIONS

The data that we have reviewed clearly indicate that ghrelin is an important component of the stress response and also that central ghrelin signalling plays an important role in the metabolic and behavioural processes required to meet the energetic demands posed by acute stressors (Figure 1). Furthermore, ghrelin may also be critical in the long-term metabolic adaptations that are produced by chronic stress exposure including carbohydrate depletion. The evidence suggests that ghrelin may protect against the psychological burden produced by prolonged periods of continued exposure to stressors, with the drawback that it may promote an obesogenic phenotype. The sites of ghrelin action include the hypothalamic ARC, PVN and other medial and lateral hypothalamic regions, as well as mesolimbic brain structures implicated in complex emotional and cognitive functions, such as functions associated with feeding, including reward seeking, emotional eating, cue-induced feeding and feeding anticipation. Thus, in addition to the hypothalamus, ghrelin may target the VTA, hippocampus, amygdala and perhaps other structures to enhance feeding in a non-homeostatic manner during a stress response, in a mechanism akin to coping. One critical issue that remains to be explained relates to how or whether ghrelin enters the brain to targets outside circumventricular brain regions such as the ARC or the area postrema.[82] Recent work by Uriarte et al[83] shows that ghrelin can infiltrate the brain through the median eminence and can be transported into the ventricular circulation to access the rest of the brain. Perhaps the transport of ghrelin into the brain also changes with repeated stress given that this form of stress can increase blood-brain barrier permeability.[84] This, however, needs to be confirmed experimentally. In summary, it could therefore be concluded that ghrelin is not only a feeding hormone, but also a hormone that is critical for maintaining an adaptive stress response.

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