Published in final edited form as:

Behav Brain Res. 2012 January 1; 226(1): 96–105. doi:10.1016/j.bbr.2011.08.037.

Ghrelin is an Orexigenic Peptide and Elicits Anxiety-Like Behaviors Following Administration into Discrete Regions of the Hypothalamus

Paul J. Currie*, Renata Khelemsky, Elizabeth M. Rigsbee, Lindsey M. Dono, Christina D. Coiro, Colin D. Chapman, and Kate Hinchcliff

Department of Psychology, Reed College, 3203 SE Woodstock Blvd., Portland, OR 97202

Abstract

Previous evidence indicates that peripherally administered ghrelin significantly increases corticotropin releasing hormone (CRH) mRNA and serum corticosterone. In addition, intraventricular administration of ghrelin has been reported to elicit anxiety-like behaviors suggesting that the peptide plays a role in mediating neuroendocrine and behavioral responses to stress. In the present study, we characterized the orexigenic, metabolic, and anxiogenic action of ghrelin following microinjection into the arcuate nucleus (ARN), paraventricular nucleus (PVN), perifornical hypothalamus (PFH), and the ventromedial nucleus (VMN). To assess ghrelin's role in anxiogenic behavior, rats were injected with vehicle or 50–800 pmol of ghrelin and then placed in an elevated plus maze (EPM) for 10 min. Each test was performed as a single trial per animal. In separate behavioral testing we measured the induction of stereotypic behaviors. Doses of 200 pmol or higher administered into the ARN and PVN elicited anxiety-like behaviors, including an increased avoidance of the open arms of the EPM. However, in the PFH and VMN, higher doses of ghrelin (400–800 pmol) were required to induce anxiety. Ghrelin doses as low as 50 pmol stimulated eating and altered energy substrate oxidation (respiratory quotient; RQ) when injected into the ARN and PVN. Injections into the PFH and VMN elicited more modest effects on eating and RQ at doses of 400 pmol or greater. Our findings indicate that regions of the hypothalamus appear to be differentially sensitive and responsive to the feeding-stimulant, metabolic and anxiogenic actions of ghrelin and that the ARN and PVN, in particular, exert a primary role in mediating these effects.

Keywords

Anxiogenic effects; Appetite; Carbohydrate oxidation; Energy substrate utilization; Ghrelin; Hypothalamic nuclei; Respiratory quotient

© 2011 Elsevier B.V. All rights reserved.

*Corresponding Author, Department of Psychology, Reed College, Portland, OR, 97202. Tel: 503-777-7267; Fax: 503-777-7769; pcurrie@reed.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1. Introduction

The current understanding of hypothalamic circuits in metabolic regulation implicates a complex network of neuropeptide and transmitter systems [1–5]. It is also clear that extra-hypothalamic mechanisms participate in this process [2,4]. Within the hypothalamus, distinct effects on food intake and energy metabolism have been reported in discrete regions or nuclei [6–9]. In addition to ghrelin, other appetite-stimulating peptides include neuropeptide Y (NPY), melanin concentrating hormone, hypocretins/orexins, and agouti-related protein (AGRP) [1,10–15]. AGRP is co-produced with NPY in the same hypothalamic neurons [15–18]. Appetite suppressive neuropeptides include the pro-opiomelanocortin (POMC) derivative, α -melanocyte-stimulating hormone (α MSH), and cocaine and amphetamine related transcript (CART), which are co-produced in a separate population of hypothalamic neurons [19–22]. With respect to specific hypothalamic peptidergic circuits, particular focus is currently directed at arcuate neurons producing α -MSH, an anorectic peptide that enhances energy expenditure [18] and the interrelationship with a separate group of arcuate neurons producing both NPY and AGRP [17]. AGRP is an endogenous antagonist of α -MSH [17].

The interaction of these two distinct populations of neurons is considered significant in the regulation of energy homeostasis and these circuits have been proposed to mediate the effects of ghrelin on energy balance [1,12,15,23–26]. Ghrelin itself increases AGRP and NPY mRNA levels in the hypothalamus [27]. Conversely, the orexigenic action of ghrelin is abolished or reduced by NPY Y1 receptor antagonism, melanocortin agonists, and antisera to both NPY and AGRP [24–28]. Further, studies examining the effects of ghrelin on appetite after central injections indicate that the peptide stimulates eating when administered into various hypothalamic [6–8,29,30] or extra-hypothalamic brain sites [31,32,34]. Consistent with a direct action on hypothalamic neurons, ventricular ghrelin administration activates c-Fos expression in multiple areas within the medial and lateral hypothalamus [35]. Hypothalamic paraventricular nucleus (PVN) injections of ghrelin induce c-Fos immunoreactivity in the PVN and ARN when microinjected at feeding-stimulant doses [36]. We have previously reported that ghrelin's metabolic effects within the PVN and ARN include an increase in carbohydrate substrate oxidation as measured using indirect calorimetry [7,8]. Other recent evidence indicates that reducing PVN ghrelin (GHSR1a) receptor gene expression significantly alters energy balance by reducing body weight [37].

In addition, emerging evidence suggests that ghrelin signaling contributes to the neural control of anxiety and emotional states. Ghrelin expression in the stomach is reported to increase following tail pinch stress or starvation stress [38]. Chronic administration of ghrelin into the lateral ventricle is reported to alter emotional reactivity in rats by inducing pro-anxiety and pro-depressive effects [39]. Also, acute third ventricular ghrelin administration elicits a number of anxiogenic behaviors in mice and rats such as increased avoidance of the open arms of the elevated plus maze (EPM), a widely used test paradigm in anxiety behavior [38,40]. The proximity of the hypothalamus to the third ventricle suggests that ghrelin receptor mechanisms within this region are involved in mediating the peptide's anxiogenic activity. Ghrelin receptor expression and binding are localized within the

hypothalamus, including nuclei synthesizing neuroactive peptides and transmitters controlling food intake [41–44].

Overall, the above findings suggest that ghrelin may play an important role in the neuroendocrine and behavioral responses to stress and anxiety in addition to its known role in energy metabolism and homeostasis. In the present study, we attempted to characterize the anxiogenic, orexigenic, and metabolic effects of hypothalamic ghrelin. We hypothesized that if ghrelin plays a critical role in the regulation of both emotional and feeding states, this could be expressed selectively within discrete regions of the hypothalamus. The specific hypothalamic areas under investigation included the arcuate (ARN), paraventricular (PVN) and ventromedial (VMN) nuclei as well as the perifornical region of the lateral hypothalamus (PFH).

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats (N=408; Harlan, Kent, WA) weighed 275–315 g at the time of surgery. Rats were housed individually in polypropylene cages with free access to water and rodent chow pellets (LabDiet). The animal colony room was maintained on a 12 h light/dark cycle (lights on at 0300 h) and at a temperature of $22 \pm 2^\circ\text{C}$. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Reed College.

2.2. Surgery

Rats were anesthetized with pentobarbital sodium (50 mg/kg IP) and placed in a Kopf stereotaxic frame with the incisor bar set 3.5 mm below the interaural line. Coordinates for the guide cannulae relative to bregma were as follows: ARN, posterior 2.3 mm, lateral ± 0.3 mm, and ventral 5.8 mm; PVN, posterior 1.8 mm, lateral ± 0.3 mm, and ventral 3.9 mm; PFH, posterior 2.1 mm, lateral ± 1.4 mm, and ventral 4.4 mm; VMN, posterior 2.5 mm, lateral ± 0.5 mm, and ventral 5.4 mm [45]. Unilateral guide cannulae (22-gauge; Plastics One, Roanoke, VA) were implanted 4 mm dorsal target sites and secured with acrylic cement. A 28-gauge stainless-steel inner stylet was inserted into the guide cannula to maintain patency. All rats were administered 0.4 mg/kg SC of the analgesic buprenorphine at the time of surgery and once again 12 h later. Behavioral and metabolic testing began after a postoperative recovery period of two weeks.

2.3. Apparatus

Oxygen consumption (O_2) and carbon dioxide (CO_2) production were measured using an AccuScan open-circuit indirect calorimeter (AccuScan Instruments, Columbus, OH). Detectors measured O_2 and CO_2 sequentially across each acrylic test chamber. The flow rate was set at 1500 ml/min. Concentrations of the oxygen and carbon dioxide gases were recorded in ml/kg body weight/min. Respiratory quotient (RQ) was calculated as the volume of CO_2 produced (VCO_2) divided by the volume of O_2 consumed (VO_2) and used as a measure of energy substrate oxidation. The analyzers were calibrated prior each test using primary gas standards of high purity (Polar Cryogenics, Portland, OR).

2.4. Experimental Design and Procedures

To examine the induction of anxiety-like behavior, rats were injected with vehicle or rat acylated ghrelin (Biopeptides, San Diego, CA) at doses of 50–800 pmol and after 10 min, placed in the elevated plus maze (EPM) for the 10-min test. Injections were administered into the ARN, PVN, PFH, and VMN (n=12–14/treatment dose). The EPM apparatus (Med Associates) consisted of two open arms (10 × 50 cm) and two closed arms (10 × 50 cm with walls 40 cm in height) that intersected at a central neutral zone (10×10 cm). The floor of the maze consisted of a near infrared backlight runway. Measurement of the percentage of time spent in the open arms was carried out using a digital camera and video tracking software (Version 1.14; Med Associates). The apparatus was elevated 50 cm from the floor. In separate groups of rats, ghrelin was administered into the ARN, PVN, PFH and VMN (n=9–11/anatomical site) and behaviors were digitally recorded under very low lighting conditions for 30 minutes. The frequencies of the following behaviors were subsequently scored: digging, freezing, grooming, oral stereotypies (chewing/gnawing/licking), rearing and body (wet dog) shakes. Behaviors were scored without prior knowledge of each rat's treatment condition.

To evaluate the dose-response effects of the peptide on eating behavior, additional groups of rats (n=8–10/site) were injected with vehicle or ghrelin (50–800 pmol). After injections, rats were returned to their home cages, where pre-weighed amounts of food had been placed. Food intake, corrected for spillage, was measured 2 h postinjection. Similar injection protocols were followed in a series of metabolic tests. Specifically, the RQ effects of varying doses ghrelin (50–800 pmol) were examined upon administration into the ARN, PVN, PFH, or VMN (n=7/group). Animals were placed in the metabolic apparatus for 2 h. Food and water were not available during metabolic testing. Feeding and metabolic testing utilized a repeated measures design with separate groups of rats used for each experiment. Treatment conditions were separated by at least 4 days.

In all experiments ghrelin was dissolved in sterile 0.15 M NaCl and injections were administered in a volume of 0.2 µl using a microinjector extending 4 mm beyond the permanent guide cannula. Ghrelin was injected over a 1 min period with the injector left in place for an additional 2 min to permit peptide diffusion. After injections, the injector was slowly extracted from the guide cannula. Doses were selected based on previously published reports [7–9] and preliminary work in our lab [46]. In repeated measures testing, ghrelin doses were administered in a randomized order. All testing was conducted during the early dark period of the rat's active cycle.

2.5. Histological and statistical analyses

Cannulae placements were confirmed via histological analysis. Immediately prior to brain extractions, black ink was injected in a volume equal to that administered in test microinjections. The black ink assisted in localizing the site of injection as well as its spread. Coronal sections (40 µm) were cut through the hypothalamus using a cryostat and then stained with Cresyl violet. Sections were examined using light microscopy and viewed relative to the stereotaxic atlas of Paxinos and Watson [45]. All rats reported here were found to have injector tracks extending into appropriate target sites. A schematic of coronal

sections of the rat brain showing representative placement of cannulae in hypothalamic areas is shown in Figure 1. For statistical evaluations, data were analyzed using one-way or two-way analyses of variance (ANOVA). Specific comparisons between means were evaluated using post hoc Tukey tests. The criterion for statistical significance was $p < 0.05$.

3. Results

The anxiogenic effects of ghrelin as measured using the EMP and following microinjections into discrete regions of the hypothalamus are shown in Figures 2–5. Separate one-way between groups ANOVA indicated that ghrelin, at doses of 200 pmol or higher, elicited an increased avoidance of percent time spent in the open arms and in the open arm entries when administered into the ARN ($F(5,69)=5.01$, $p < 0.0006$; $F(5,69)=8.12$, $p < 0.00002$) and PVN ($F(5,70)=5.79$, $p < 0.0001$; $F(5,70)=7.62$, $p < 0.00001$). In the VMN, doses of 400 pmol or higher elicited similar effects ($F(5,69)=3.16$, $p < 0.01$; $F(5,69)=4.49$, $p < 0.001$) while only the highest dose appeared to be anxiogenic after injection into the PFH ($F(5,70)=3.20$, $p < 0.01$; $F(5,70)=3.41$, $p < 0.008$). We did not observe any alterations in total number of entries, under any of the above conditions, suggesting that the peptide did not negatively impact locomotor function (ARN, $F(5,69)=1.47$, $p > 0.05$; PVN, $F(5,70)=1.09$, $p > 0.05$; PFH, $F(5,70)=1.13$, $p > 0.05$; VMN, $F(5,69)=0.73$, $p > 0.05$).

Figures 6–9 illustrate the effects on ghrelin on the behavioral profile of rats. These data were evaluated using one-way ANOVA for repeated measures. In the ARN, ghrelin increased digging ($F(5,48)=2.52$, $p < 0.04$), freezing ($F(5,48)=2.83$, $p < 0.02$) grooming ($F(5,48)=6.11$, $p < 0.00002$), oral stereotypies ($F(5,48)=5.11$, $p < 0.0008$), and wet dog/body shakes ($F(5,48)=4.66$, $p < 0.002$) at doses of 200–800 pmol. Similar effects were observed after PVN injections at doses of 400–800 pmol with significant F statistics reported for digging ($F(5,54)=2.86$, $p < 0.02$), freezing ($F(5,54)=3.60$, $p < 0.007$) grooming ($F(5,54)=4.04$, $p < 0.003$), oral stereotypies ($F(5,54)=4.37$, $p < 0.002$), and wet dog/body shakes ($F(5,54)=6.20$, $p < 0.0001$). Ghrelin also increased some behaviors when injected into the PFH (digging ($F(5,60)=3.24$, $p < 0.02$); grooming ($F(5,60)=4.33$, $p < 0.002$), oral stereotypies ($F(5,60)=2.82$, $p < 0.02$) and body shakes ($F(5,60)=2.61$, $p < 0.04$) and VMN (freezing ($F(5,60)=3.10$, $p < 0.02$), oral stereotypies ($F(5,60)=3.49$, $p < 0.008$) and body shakes ($F(5,60)=3.03$, $p < 0.02$), and at only higher doses.

The orexigenic effects of ghrelin, measured 2 h postinjection, are shown in Figure 10. Data were analyzed using a two-way (site $\times$ dose) ANOVA with repeated measures on the second factor. Doses as low as 50 pmol stimulated eating after administration into the ARN and PVN. VMN injections of 400–800 pmol were orexigenic while only the highest dose of ghrelin increased food intake when injected into the PFH ($F(15,165)=2.07$, $p < 0.01$). The dose-response effects of ghrelin on energy metabolism are shown in Figures 11–14. Two-way ANOVA confirmed that ghrelin increased RQ when injected into the ARN ($F(30,216)=2.56$, $p < 0.00005$), PVN ($F(30,216)=2.49$, $p < 0.0001$), PFH ($F(30,216)=1.58$, $p < 0.03$) and VMN ($F(30,216)=1.82$, $p < 0.008$). All doses increased RQ after injection into the ARN and PVN, with RQ increases observed within the first 20 min of testing. RQs remained elevated for the duration of the 2-h test. In the VMN and PFH, ghrelin induced

more modest and transient effects on RQ and only at higher doses of 400–800 pmol. Increases in RQ were no longer observed by the end of the test session.

4.0. Discussion

The present study characterized the orexigenic, metabolic, and anxiogenic effects of hypothalamic ghrelin. While the peptide stimulated eating and evoked alterations in energy substrate oxidation following injections into multiple hypothalamic sites, the ARN and PVN were particularly responsive to these effects. These findings are consistent with our previous work showing that ghrelin administration into the PVN and ARN stimulate food intake and alter energy metabolism via an increase in carbohydrate oxidation [7–9]. VMN and PFH injections elicited similar increases in eating and energy metabolism, although the effects were generally more modest and transient, and occurred only at higher doses. While lower doses of ghrelin effectively stimulated eating, higher doses also induced anxiogenesis. That is, hypothalamic injections of ghrelin, particularly into the PVN and ARN, remained orexigenic, but also induced anxiety-like behaviors such as an increased avoidance of the open arms of the EPM and an increase in grooming, oral stereotypies, freezing, wet dog shakes and prolonged, frequent rearing. Overall locomotor behavior was not altered as indicated by a lack of effect of ghrelin on total arm entries in the EPM.

As a 28-amino acid acylated peptide initially identified in the rat stomach, ghrelin is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) [12,47,48]. The peptide is primarily synthesized by endocrine cells of the gastric oxyntic mucosa. When secreted by the stomach, ghrelin has a variety of regulatory actions in the central nervous system (CNS) and in the periphery [41,47,48]. Further, extensive evidence now indicates that ghrelin is involved in the regulation of food intake and energy balance. The peptide's effects on body weight are due in part to altered metabolism and energy expenditure [1,12,37]. Fasting increases ghrelin mRNA expression and increases peptide levels in plasma [49]. Systemic or central administration of ghrelin in rodents rapidly increases food intake and body weight gain [26,30,50,51] while ingestion of food decreases plasma ghrelin concentrations [26]. Ghrelin O-acyl transferase (GOAT) expression, the enzyme mediating ghrelin octanoylation, is expressed in hypothalamic tissue and is regulated by metabolic status [52]. Indeed ghrelin's effects on energy homeostasis appear to be elicited through activation of hypothalamic neurons [7–9, 53,54]. At the same time, it is known that the orexigenic effects of ghrelin are mediated by other forebrain and hindbrain structures. Recent work indicates, for example, that direct injections of ghrelin into the VTA stimulate food intake (32,34) and that systemic administration of ghrelin fails to induce food intake when the ghrelin receptor antagonist JMV2959 is infused into the VTA [33]. Our findings are consistent with other evidence demonstrating that the peptide elicits eating following injection into multiple hypothalamic areas [7,8,29,30]. This is in turn consistent with the actions of other orexigenic and anorexigenic peptides [2,3,4,55,56,57] and clearly implicates ghrelin in feeding motivation and energy homeostasis.

Hypothalamic ghrelinergic receptor mechanisms have also been investigated. Ghrelin binds to a G-protein coupled receptor, the GHS-R, which has two subtypes, 1a and 1b. The GHS-R sequences do not show significant homology with other known receptors [58]. The

GHSR1a is believed to be highly conserved across species and the human 1a subtype shares 96% identity with the rat 1a receptor [58]. While ghrelin and synthetic GHS bind with high affinity to the GHSR1a receptor, this is not the case with the GHSR1b, and in fact the functional role of the 1b receptor remains poorly defined [59,23]. Expression for the GHS1a receptor is localized within the hypothalamus and anterior pituitary [45,59,60]. A transgenic rat model over expressing antisense oligonucleotides against the GHSR1a has been reported to exhibit decreased food intake and lower body fat [61], consistent with ghrelin's hypothesized positive action on energy balance. However, the identification of GHS receptors with binding profiles different from 1a and 1b [23] suggests the additional subtypes of the GHS receptor are likely to be cloned in the future.

Ghrelin is reported to elicit a stimulatory effect on the activity of the hypothalamo-pituitary-adrenal (HPA) axis [62,23], implicating the peptide in the central control of stress and anxiety. For example, Wren et al. (63) reported that the HPA axis was stimulated after acute intracerebroventricular administration of ghrelin but not after intraperitoneal ghrelin treatment. Stress has been described as a state of threatened homeostasis and includes a complex array of physiological, behavioral and adaptive responses [64]. Adaptive responses may be inadequate to reinstate homeostasis or may be excessive or prolonged, eliciting pathogenesis. Homeostatic stress includes disturbances resulting from pain, hunger or similar stimuli [64,65]. Emotional stress, in turn, relates to perception processes and includes behavioral responses such as anxiety and fear [64,66]. It is argued that the hypothalamus plays a critical role in the organization of the behavioral response to stress. This includes emotional stress, such as anxiogenic behaviors, where the hypothalamus may act in close association with other limbic structures [64].

In rats, ventricular ghrelin administration increases anxiety-like behavior as measured using open field and elevated plus maze paradigms [40]. Direct injections into the amygdala, dorsal raphe nucleus and hippocampus [67] have been reported to be anxiogenic, typically at doses higher (1.5–3 nmol) than those administered in the present study. Ghrelin was also found to be orexigenic when injected into these same structures at lower dosing (300 pmol). Further, ventricular administration of ghrelin antisense oligonucleotides elicits anxiolytic-like effects [68] and chronic ventricular administration has recently been reported to elicit similar anxiety and depressive-like behavioral responses [39]. In this latter study, chronic ghrelin administration was associated with alterations in cannabinoid, glutamatergic, GABA-ergic and dopaminergic function in the amygdala, an important structure mediating the behavioral responses to stress and anxiety in association with the hypothalamus [69–71]. Such effects appear to be consistent with depression and anxiety disorders in humans [72–74]. However, in contrast to ghrelin's previously reported anxiogenic effects, recent work indicates that the peptide may exert anxiolytic action under certain conditions [75]. Peripheral injections of ghrelin and caloric restriction have been reported to produce antidepressant and anti-anxiety behavioral responses as measured using the elevated plus maze and forced swim test paradigms [76]. Although the neural mechanisms underlying the anxiogenic and anxiolytic effects of ghrelin continue to be elucidated, the differential effects of the peptide on anxiety might be related to dose, route of administration, receptor distribution, and anatomical localization.

It is well documented that corticotropin-releasing hormone (CRH) plays a significant role in the mediation of behavioral responses to stress [64,77,78]. However, in contrast with ghrelin, CRH potently suppresses appetite [64,77]. In fact, it now appears that CRH is a significant mediator of endocrine, autonomic, and immune responses to stressful stimuli and it is argued that CRH may coordinate autonomic and behavioral responses to stress, including anxiety-like behaviors, food intake, and arousal [64,78]. Moreover, initial evidence indicates that ghrelin may exhibit anxiogenic activity through an action on CRH mechanisms. In mice, intraperitoneal injection of ghrelin increases hypothalamic CRH gene expression and produces a reliable dose-dependent increase in serum corticosterone levels [38]. Ventricular injections of the CRH antagonist α -helical CRH9-41 attenuate the anxiogenic action of ICV ghrelin in mice by reversing ghrelin's suppression of time spent in the open arm of the plus maze [38]. We have recently reported that the CRH-related peptide, urocortin I, attenuates the feeding-stimulant and metabolic effects of ghrelin [9]. Together with the above findings, therefore, it seems reasonable to propose that ghrelin interacts with CRH and CRH-related peptides in the hypothalamic control of eating, energy homeostasis, and anxiety/stress-related neural circuits.

5. Conclusions

In summary, the present study investigated the effects of ghrelin on eating, energy metabolism, and the induction of anxiety-like behaviors. Our findings indicate that specific nuclei or areas of the hypothalamus appear to be differentially sensitive and responsive to the orexigenic, metabolic, and anxiogenic actions of the peptide. Whereas lower doses of ghrelin injected into the PVN and ARN, in particular, were effective in increasing food intake and inducing alterations in substrate metabolism, higher doses were anxiogenic. Indeed our findings are consistent with the argument that enhanced ghrelin signaling within these hypothalamic structures plays a critical role in mediating anxiety-like behaviors. The fact that higher doses are required to induce anxiogenesis, while still being orexigenic, suggests that these nuclei mediate both motivational and emotional states, consistent with limbic system function.

Research Highlights

Hypothalamic ghrelin elicits anxiety-like behaviors

Ghrelin is orexigenic in discrete hypothalamic nuclei

Hypothalamic ghrelin alters energy substrate oxidation

Acknowledgments

Supported by NIH 070496-01A1 to PJC and the Undergraduate Biological Sciences Education Program of the Howard Hughes Medical Institute.

References

1. Briggs DI, Andrews ZB. Metabolic Status Regulates Ghrelin Function on Energy Homeostasis. *Neuroendocrinology*. 2011; 93:48–57. [PubMed: 21124019]

2. Seeley RJ, Schwartz MW. The regulation of energy balance: peripheral hormonal signals and hypothalamic neuropeptides. *Current Directions Psych Sci.* 1997; 6:39–44.
3. Schwartz MW, Seeley RJ. Seminars in medicine of the Beth Israel Deaconess Medical Center. Neuroendocrine responses to starvation and weight loss. *N Engl J Med.* 1997; 336:1802–1811. [PubMed: 9187072]
4. Schwartz MW, Woods S, Porte D, Seeley RJ, Baskin DG. Central nervous system controls food intake. *Nature.* 2000; 404:661–671. [PubMed: 10766253]
5. Stanley, BG. Neuropeptide Y in multiple hypothalamic sites controls eating behavior, endocrine and autonomic systems for body energy balance. In: Colmers, WF.; Wahlestedt, C., editors. *The biology of neuropeptide Y and related peptides.* Totowa, NJ: Humana Press; 1993. p. 457–509.
6. Currie PJ. Integration of hypothalamic feeding and metabolic signals: focus on neuropeptide Y. *Appetite.* 2003; 41:335–337. [PubMed: 14637334]
7. Currie PJ, John CS, Nicholson ML, Chapman CD, Loera KE. Hypothalamic paraventricular 5-hydroxytryptamine inhibits the effects of ghrelin on eating and energy substrate utilization. *Pharmacol Biochem Behav.* 2010; 97:152–155. [PubMed: 20573591]
8. Currie PJ, Mirza A, Fuld R, Park D, Vasselli JR. Ghrelin is an orexigenic and metabolic signaling peptide in the arcuate and paraventricular nuclei. *Am J Physiol Regul Integr Comp Physiol.* 2005; 289:R353–R358. [PubMed: 15817841]
9. Currie PJ, Coiro CD, Duenas R, Guss JL, Mirza A, Tal N. Urocortin I inhibits the effects of ghrelin and neuropeptide Y on feeding and energy substrate utilization. *Brain Research.* 2011; 1385:127–134. [PubMed: 21303672]
10. Bittencourt JC, Presse F, Arias C, Peto C, Vaughan J, Nahon JL, et al. The melanin-concentrating hormone system of the rat brain: an immuno- and hybridization histochemical characterization. *J Comp Neurol.* 1992; 319:218–245. [PubMed: 1522246]
11. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, et al. The hypocretins: two hypothalamic peptides with neuroexcitatory activity. *Proc Natl Acad Sci USA.* 1998; 95:322–327. [PubMed: 9419374]
12. Lim TC, Kola B, Korbonits M, Grossman AB. Ghrelin's role as a major regulator of appetite and its other functions in neuroendocrinology. *Prog Brain Res.* 2010; 182:189–205. [PubMed: 20541666]
13. Qu D, Ludwig DS, Gammeltoft S, Piper M, Pelleymounter MA, Cullen MJ, et al. A role for melanin-concentrating hormone in the central regulation of feeding behavior. *Nature.* 1996; 380:243–247. [PubMed: 8637571]
14. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka H, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell.* 1998; 92:573–585. [PubMed: 9491897]
15. Semjonous NM, Smith KL, Parkinson JRC, Gunner DJL, Liu Y-L, Murphy KG, et al. Coordinated changes in energy intake and expenditure following hypothalamic administration of neuropeptides involved in energy balance. *Int J Obes (Lond).* 2009; 33:775–785. [PubMed: 19488048]
16. Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature.* 1997; 385:165–168. [PubMed: 8990120]
17. Lu D, Willard D, Patel IR, Kadwell S, Overton L, Kost T, et al. Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature.* 1994; 371:799–802. [PubMed: 7935841]
18. Ollmann MM, Wilson BD, Yang YK, Kerns JA, Chen Y, Gantz I, et al. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science.* 1997; 278:135–138. [PubMed: 9311920]
19. Ahima RS, Osei SY. Molecular regulation of eating behavior: new insights and prospects for therapeutic strategies. *Trends Mol Med.* 2001; 7:205–213. [PubMed: 11325632]
20. Hahn TM, Breininger JF, Baskin DG, Schwartz MW. Coexpression of *Agrp* and *NPY* in fasting-activated hypothalamic neurons. *Nature Neurosci.* 1998; 1:271–272. [PubMed: 10195157]
21. Mezey E, Kiss JZ, Mueller GP, Eskay R, Odonohue TL, Palkovits M. Distribution of pro-opiomelanocortin derived peptides, adrenocorticotrope hormone, α -melanocyte-stimulating hormone and β -endorphin (ACTH, α -MSH, β -END) in the rat hypothalamus. *Brain Res.* 1985; 328:341–347. [PubMed: 2985184]

22. Spiegelman BM, Flier JS. Obesity and the regulation of the energy balance. *Cell*. 2001; 104:656–660.
23. Muccioli G, Tschöp M, Papotti M, Deghenghi R, Heiman M, Ghigo E. Neuroendocrine and peripheral activities of ghrelin: implications in metabolism and obesity. *European Journal of Endocrinology*. 2002; 440:235–254.
24. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature*. 2001; 409:194–198. [PubMed: 11196643]
25. Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, et al. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuro peptide Y/Y1 receptor pathway. *Diabetes*. 2001; 50:227–232. [PubMed: 11272130]
26. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature*. 2000; 407:908–913. [PubMed: 11057670]
27. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuro peptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes*. 2001; 50:2438–2443. [PubMed: 11679419]
28. Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, et al. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut*. 2003; 52:947–952. [PubMed: 12801949]
29. Melis MR, Mascia MS, Succu S, Torsello A, Muller EE, Deghenghi R, et al. Ghrelin injected into the paraventricular nucleus of the hypothalamus of male rats induces feeding but not penile erection. *Neurosci Lett*. 2002; 329:339–343. [PubMed: 12183044]
30. Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, et al. Ghrelin causes hyperphagia and obesity in rats. *Diabetes*. 2001; 50:2540–2547. [PubMed: 11679432]
31. Faulconbridge LF, Cummings DE, Kaplan JM, Grill HJ. Hyperphagic effects of brainstem ghrelin administration. *Diabetes*. 2003; 52:2260–2265. [PubMed: 12941764]
32. 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. [PubMed: 16137788]
33. Skibicka KP, Hansson C, Egicioglu E, Dickson SL. Role of ghrelin in food reward: impact of ghrelin on sucrose self-administration and mesolimbic dopamine and acetylcholine receptor expression. *Addict Biol*. 2011 in press.
34. Weinberg ZY, Nicholson ML, Currie PJ. 6-Hydroxydopamine lesions of the ventral tegmental area suppress ghrelin's ability to elicit food-reinforced behavior. *Neurosci Lett*. 2011; 499:70–73. [PubMed: 21640160]
35. Lawrence CB, Snape AC, Baudoin FMH, Luckman SM. Acute central ghrelin and GH secretagogues induce feeding and activate brain appetite centers. *Endocrinology*. 2002; 143:155–162. [PubMed: 11751604]
36. Olzsewski PK, Grace MK, Billington CJ, Levine AS. Hypothalamic paraventricular injections of ghrelin: effect on feeding and c-Fos immunoreactivity. *Peptides*. 2003; 24:919–923. [PubMed: 12948845]
37. Shrestha YB, Wickwire K, Giraudo S. The effect of reducing hypothalamic ghrelin receptor gene expression on energy balance. *Peptides*. 2009; 30:1336–1341. [PubMed: 19540432]
38. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, et al. A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. *Neuroendocrinology*. 2001; 74:143–147. [PubMed: 11528215]
39. Hansson C, Haage D, Taube M, Egicioglu E, Salome N, Dickson SL. Central administration of ghrelin alters emotional responses in rats: behavioral, electrophysiological, and molecular evidence. *Neuroscience*. 2011; 180:201–211. [PubMed: 21303683]
40. Carlini VP, Monzon ME, Varas MM, Cragolini AB, Schioth HB, Scimonelli TN, et al. Ghrelin increases anxiety-like behavior and memory retention in rats. *Biochem Biophys Res Commun*. 2002; 299:739–743.

41. Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, et al. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Res. Mol. Brain Res.* 1997; 48:23–29. [PubMed: 9379845]
42. Tannenbaum GS, Lapointe M, Beaudet A, Howard AD. Expression of growth hormonesecretagogue-receptors by growth hormone-releasing hormone neurons in the mediobasal hypothalamus. *Endocrinology.* 1998; 139:4420–4423. [PubMed: 9751527]
43. 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. [PubMed: 10567856]
44. 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. [PubMed: 16320257]
45. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates.* New York: Academic Press; 2007.
46. Currie PJ, Grueneisen AM, Wall DG, Sarkodie KA. Anxiogenic, orexigenic and metabolic effects of hypothalamic ghrelin. *Appetite.* 2008; 51:360.
47. Casanueva FF, Dieguez C. Growth hormone secretagogues: Physiological role and clinical utility. *Trends Endocrinol. Metab.* 1999; 10:30–38. [PubMed: 10322392]
48. 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. [PubMed: 10604470]
49. Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, et al. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology.* 2001; 120:337–345. [PubMed: 11159873]
50. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature.* 2001; 409:194–198. [PubMed: 11196643]
51. Wren AM, Small CJ, Ward HL, Murphy KG, Dakin CL, Taher S, et al. The novel hypothalamic peptide ghrelin stimulates food intake and GH secretion. *Endocrinology.* 2000; 141:4325–4328. [PubMed: 11089570]
52. Gahete MD, Córdoba-Chacón J, Salvatori R, Castaño JP, Kineman RD, Luque RM. Metabolic regulation of ghrelin O-acyl transferase (GOAT) expression in the mouse hypothalamus, pituitary, and stomach. *Mol Cell Endocrinol.* 2010; 317:154–160. [PubMed: 20035826]
53. Seoane LM, Tovar S, Baldelli R, Arvat E, Ghigo E, Casanueva FF, et al. Ghrelin elicits a marked stimulatory effect on GH secretion in freely-moving rats. *Eur J Endocrinol.* 2000; 143:R7–R9. [PubMed: 11078999]
54. Wang L, Saint-Pierre DH, Tache Y. Peripheral ghrelin selectively increases Fos expression in neuropeptide Y- synthesizing neurons in mouse hypothalamic arcuate nucleus. *Neurosci Lett.* 2002; 325:47–51. [PubMed: 12023064]
55. Heinrichs SC, Menzaghi F, Koob GF. Neuropeptide Y-induced feeding and its control. *Vitam Horm.* 1998; 54:51–66. [PubMed: 9529973]
56. Saper CB, Chou TC, Elmquist JK. The need to feed: Homeostatic and hedonic control of eating. *Neuron.* 2002; 36:199–211. [PubMed: 12383777]
57. Wilding JPH. Neuropeptides and appetite control. *Diabetic Medicine.* 2002; 19:619–627. [PubMed: 12147141]
58. Smith RG, Leonard R, Bailey AR, Palyha O, Feighner S, Tan C, et al. Growth hormone secretagogue receptor family members and ligands. *Endocrine.* 2001; 14:9–14. [PubMed: 11322507]
59. Howard HD, Feighner SD, Cully DF, Arena JP, Liberators PA, Rosenblum CI, et al. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science.* 1996; 273:974–976. [PubMed: 8688086]
60. Shuto Y, Shibasaki T, Wada K, Parhar I, Kamegai J, Sugihara H, et al. Generation of polyclonal antiserum against the growth hormone secretagogue receptor (GHS-R). Evidence the GHS-R exists in the hypothalamus, pituitary and stomach of rats. *Life Sci.* 2001; 68:991–996. [PubMed: 11212874]

61. Shuto Y, Shibasaki T, Otagiri A, Kuriyama H, Ohata H, Tamara H, et al. Hypothalamic growth hormone secretagogue receptor regulates growth hormone secretion, feeding and adiposity. *J Clin Invest.* 2002; 109:1429–1436. [PubMed: 12045256]
62. Broglio F, Gottero C, Arvat E, Ghigo E. Endocrine and Non-endocrine actions of ghrelin. *Horm Res.* 2003; 59:109–117. [PubMed: 12637790]
63. Wren AM, Small CJ, Fribbens CV, Neary NM, Ward HL, Seal LJ, et al. The hypothalamic mechanisms of the hypophysiologic action of ghrelin. *Neuroendocrinology.* 2002; 76:316–324. [PubMed: 12457042]
64. Nandi J, Meguid MM, Inui A, Xu A, Makarenko IG, Tada T, et al. Central mechanisms involved with catabolism. *Current Opinion Clin. Nutr. & Metabol. Care.* 2002; 5:407–418.
65. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, et al. 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. [PubMed: 11089560]
66. Aguilera G, Lightman SL, Kiss A. Regulation of the hypothalamo-pituitary-adrenal axis during water deprivation. *Endocrinology.* 1993; 132:241–248. [PubMed: 8380375]
67. Carlini VP, Varas MM, Cragolini AB, Schiöth HB, Scimonelli TN, de Barioglio SR. Biochem Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biophys Res Commun.* 2004; 313:635–641.
68. Kanehisa M, Akiyoshi J, Kitaichi T, Matsushita H, Tanaka E, Kodama K, et al. Administration of antisense DNA for ghrelin causes an antidepressant and anxiolytic response in rats. *Prog Neuropsychopharmacol Biol Psychiatry.* 2006; 30:1403–1407. [PubMed: 16793188]
69. Davis, M. Neural circuitry of anxiety and stress disorders. In: Davis, KL.; Charney, D.; Coyle, JT.; Nemeroff, C., editors. *Neuropsychopharmacology: The Fifth Generation of Progress.* Philadelphia: American College of Neuropsychopharmacology; 2002. p. 931–951.
70. Sah P, Faber ES, Lopez De Armentia M, Power J. The amygdaloid complex: anatomy and physiology. *Physiol Rev.* 2003; 83:803–834. [PubMed: 12843409]
71. Sajdyk TJ, Fitz SD, Shekhar A. The role of neuropeptide Y in the amygdala on corticotrophin-releasing factor receptor-mediated behavioral stress responses in the rat. *Stress.* 2006; 9:21–28. [PubMed: 16753930]
72. Klimek V, Schenck JE, Han H, Stockmeier CA, Ordway GA. Dopaminergic abnormalities in amygdaloid nuclei in major depression: a postmortem study. *Biol Psychiatry.* 2006; 52:740–748. [PubMed: 12372665]
73. Paez-Pereda M, Hausch F, Holsboer F. Corticotropin releasing factor receptor antagonists for major depressive disorder. *Expert Opin Investig Drugs.* 2011; 20:519–535.
74. Holsboer F, Ising M. Central CRH system in depression and anxiety—evidence from clinical studies with CRH1 receptor antagonists. *Eur J Pharmacol.* 2008; 583:350–357. [PubMed: 18272149]
75. Chuang JC, Zigman JM. Ghrelin's Roles in Stress, Mood, and Anxiety Regulation. *Int J Pept.* 2010; 2010:1–5b.
76. Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, et al. The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nat Neurosci.* 2008; 11:752–753. [PubMed: 18552842]
77. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB. The role of corticotropin-releasing factor in depression and anxiety disorders. *Endocrinology.* 1999; 160:1–12.
78. Smagin GN, Heinrichs SC, Dunn AJ. The role of CRH in behavioral responses to stress. *Peptides.* 2001; 22:713–724. [PubMed: 11337084]

Fig. 1.
Schematic of coronal sections of the rat brain showing representative placements of cannulae in hypothalamic areas.

Fig. 2.

Effect of ghrelin injections into the ARN on the percentage of time spent in the open arms, open arm entries, and combined total open and closed arm entries of the elevated plus maze. Values represent mean $\pm$ S.E.M. *P<0.05 compared to vehicle (Veh).

Fig. 3.

Effect of PVN administration of ghrelin on performance in the elevated plus maze as measured by percent time spent in the open arms, open arm entries, and total entries. Values are mean $\pm$ S.E.M. *P<0.05 compared to vehicle (Veh).

Fig. 4.

Effect of ghrelin administration into the PFH on performance in the elevated plus maze as measured by percentage of time spent in the open arms, open arm entries, and total entries. Values are mean $\pm$ S.E.M. *P<0.05 compared to vehicle (Veh).

Fig. 5.

Effect of ghrelin injections into the VMN on the percent time spent in the open arms of the elevated plus maze as well as open arm entries and total open and closed arm entries combined. Values represent mean $\pm$ S.E.M. * $P < 0.05$ compared to vehicle (Veh).

Fig. 6.

Ghrelin-induced alterations in behavior after administration of into the ARN. Values represent mean $\pm$ S.E.M. * $P < 0.05$ compared to vehicle (Veh).

Fig. 7.

Alterations in the behavioral profile of rats following ghrelin injections into the PVN.

Values represent mean $\pm$ S.E.M. * $P < 0.05$ compared to vehicle (Veh).

Fig. 8.

Ghrelin-induced alterations in behavior after direct injections into the PFH. Values represent mean $\pm$ S.E.M. * $P < 0.05$ compared to vehicle (Veh).

Fig. 9.

Alterations in behavior elicited by VMN microinjections of ghrelin. Values represent mean $\pm$ S.E.M. * $P < 0.05$ compared to vehicle (Veh).

Fig. 10.

Effect of ghrelin injections into different regions of the hypothalamus on food intake. Values represent mean intakes $\pm$ S.E.M measured 2 h postinjection. * $P < 0.05$ compared to vehicle (Veh).

Fig. 11. Mean ($\pm$ S.E.M.) RQ values following ARN administration of ghrelin. Energy substrate utilization was measured over the 2 h period following treatment. * $P < 0.05$ compared to vehicle (Veh).

Fig. 12.

Mean ($\pm$ S.E.M.) RQ values in rats administered ghrelin into the PVN over a 2-h postinjection period. * $P < 0.05$ compared to vehicle (Veh).

Perifornical Hypothalamus (PFH)

Fig. 13.

Mean ($\pm$ S.E.M.) RQ values after ghrelin injections into the PFH. Energy substrate utilization was measured over the 2 h period following treatment. * $P < 0.05$ compared to vehicle (Veh).

Ventromedial Nucleus (VMN)

Fig. 14.

Mean ($\pm$ S.E.M.) RQ values in rats administered ghrelin into the VMN over a 2-h postinjection period. * $P < 0.05$ compared to vehicle (Veh).