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Divergent associations between ghrelin and neural responsivity to palatable food in hyperphagic and hypophagic depression

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Abstract

Background: The neurobiological mechanisms involved in divergent appetitive phenotypes in major depressive disorder (MDD) are not well understood, although recent data suggest disruption in mesolimbic reward circuitry. Ghrelin, an orexigenic hormone, has been shown to modulate the reward circuitry. We aimed to investigate the relationship between acylated ghrelin levels and the neural response to food stimuli in individuals with hyperphagic and hypophagic MDD in remission.

Methods: Women with hyperphagic MDD ($n=10$), hypophagic MDD ($n=18$), and healthy controls (HC; $n=18$) underwent fMRI scanning during which they viewed images of food. The fMRI session was followed by a standardized meal, appetite ratings, and serial blood draws.

Results: In individuals with hyperphagic MDD, greater change in acylated ghrelin in response to a meal was associated with increased BOLD response to high-calorie food in the bilateral ventral

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Contributions

HC was responsible for conducting the analyses, preparing the first draft of the manuscript, and preparing the manuscript for submission. LMH was responsible for obtaining funding for the study, developing the study design, collecting data, supervised the conduction of the analyses and was responsible for editing drafts of the manuscript. KC and PM were responsible for data collection and initial data preprocessing. AK contributed towards study design and validation of ghrelin data and analyses. JMG contributed towards study design, clinical diagnostic validation, and analytic approach. All authors contributed to and approved the final manuscript.

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tegmental area and left hypothalamus. In contrast, negative associations were observed between acylated ghrelin AUC and BOLD activity in the right hypothalamus in the hypophagic MDD group.

Limitations: Unbalanced group sizes with a relatively small sample in the hyperphagic MDD group.

Conclusions: In the absence of differences in absolute ghrelin levels between the hyperphagic MDD and HC groups, results in hyperphagic MDD might suggest a ghrelinergic signaling mechanism for increased appetite during an MDD episode in this group. Our findings shed light on interactions between appetite hormones and mesolimbic circuitry which could contribute to development of therapeutic targets for opposing appetite phenotypes in depression.

Keywords

Major depressive disorder; hyperphagia; ghrelin; mesolimbic reward circuitry; dopamine

Introduction

Major Depressive Disorder (MDD) is one of the major causes of disability worldwide due to the negative impacts on functioning and re-occurring nature (WHO, 2017). One of the key challenges for the successful treatment of MDD is the highly heterogeneous nature of the disorder. Efforts to distinguish endophenotypes have revealed several pathophysiological mechanisms underlying depression, including HPA axis dysregulation, inflammation, circadian rhythm disturbances and altered reward sensitivity (Dean and Keshavan, 2017; Goldstein et al., in press.; Schmidt et al., 2011). Appetitive phenotypes, in particular, are of significant clinical importance and yet understudied. These consist of hyperphagic MDD, which is characterized by increased appetite, and hypophagic MDD, which is characterized by decreased appetite. Hyperphagic MDD overlaps with the more commonly studied endophenotype of atypical depression, which, compared to typical depression, is associated with greater suicidality and heightened risk to develop obesity and related comorbid conditions (Blanco et al., 2012; Goldstein et al., 2016; Horwath et al., 1992; Levitan et al., 1997; Matza et al., 2003). Thus, understanding the pathophysiology underlying hyperphagic MDD may contribute to the development of novel treatment approaches, and therefore, has the potential to lessen the disease burden of MDD.

The neurobiological mechanisms of overeating in MDD may be due to a disruption in the mesolimbic reward circuitry, which is defined as the dopaminergic pathway connecting the ventral tegmental area (VTA), nucleus accumbens (NAcc), and the connected striatal regions. A recent fMRI study showed that a mixed-sex group of individuals with current hyperphagic MDD exhibited elevated neural activity in response to visual food stimuli in mesolimbic reward regions, including the ventral striatum, putamen, ventral pallidum, and orbitofrontal cortex (OFC), compared to age- and BMI-matched individuals with hypophagic MDD and healthy controls (Simmons et al., 2016). Individuals with hypophagic MDD, on the other hand, exhibited hypoactivation to food cues in the mid-insula, a region in which individuals with hyperphagic MDD and healthy controls showed no significant differences. These findings suggest distinct neural pathways implicating mesolimbic reward

circuitry in hyperphagic MDD (Simmons et al., 2016). Similarly, abnormal dopamine (DA) signalling has been reported in obesity and depression (Brunswick et al., 2003; Simmons et al., 2016; Volkow et al., 2011); however, the question of how appetite-regulating hormones interact with reward circuitry in hyperphagic MDD is still not well understood.

An important candidate involved in this interplay might be ghrelin, an orexigenic hormone secreted by the stomach which acts on targets in the central nervous system to regulate food intake by acting on neurons in the arcuate nucleus of the hypothalamus (Nakazato et al., 2001). Preclinical studies have shown that ghrelin modulates dopaminergic mesolimbic signalling, extending the role of ghrelin beyond the regulation of homeostatic control of food intake to the hedonic control of food intake (Abizaid et al., 2006; Jerlhag et al., 2007a; Naleid et al., 2005). Among regions associated with reward and motivation, distribution of ghrelin receptors (GHS-R1a) beyond the hypothalamus shows highest localization in the VTA (projecting to nucleus accumbens) and amygdala (Guan et al., 1997; Zigman et al., 2006). Within the VTA, ghrelin receptors are expressed on dopaminergic neurons (Jiang et al., 2006; Kern et al., 2012; Schellekens et al., 2013). Binding of ghrelin to these receptors increases dopamine neuronal activity in the VTA and dopamine turnover in the nucleus accumbens, a region that receives dopaminergic projections from the VTA (Abizaid et al., 2006). Furthermore, direct administration of ghrelin into the VTA not only increases extracellular concentrations of dopamine in the nucleus accumbens (Jerlhag et al., 2007a), but also increases food intake (Naleid et al., 2005).

The modulatory role of ghrelin in the dopaminergic mesolimbic reward circuitry has also been shown in humans (Karra et al., 2013; Kroemer et al., 2013; Malik et al., 2008; Sun et al., 2014). In healthy volunteers, plasma levels of ghrelin were positively correlated with neural responses to images of food in brain regions implicated in reward processing specifically the caudate, pallidum, VTA, and limbic structures (Kroemer et al., 2013). Similarly, ghrelin administration to healthy volunteers increased the neural response to food pictures in regions such as the striatum, anterior insula, amygdala, OFC (Malik et al., 2008).

Although these studies in healthy individuals demonstrated the association between ghrelin and neural activity in regions where ghrelin receptors are abundant, studies on the ghrelinergic regulation of food-related reward in clinical conditions is still sparse. Animal studies suggest a critical role of ghrelin in mediating neuroendocrine responses in disorders associated with chronic stress and mood fluctuation (Asakawa et al., 2001; Lutter et al., 2008; Ochi et al., 2008; Zheng et al., 2009); however, the role of ghrelin in the modulation of the mesolimbic reward in depression and interactions with appetitive functioning in humans requires further investigation.

In the current study, we aimed to investigate the relationship between peripheral ghrelin levels and blood-oxygen-level-dependant (BOLD) responses to high- and low-calorie food stimuli in individuals with hyperphagic MDD in remission, hypophagic MDD in remission, and healthy controls. We chose to examine individuals with MDD currently in remission to investigate traits (as distinct from clinical state) of opposing appetite phenotypes in MDD. Given preclinical evidence of ghrelinergic action on dopamine neurons in mesolimbic regions, our primary hypothesis was that hyperphagic MDD would be associated with a

positive relationship between acylated ghrelin and BOLD response in homeostatic (hypothalamus) and hedonic (i.e., ventral tegmental area, nucleus accumbens, amygdala) reward circuitry in response to high-calorie (vs. low-calorie) food stimuli. Further, as a secondary hypothesis based on data from animal models (Boersma et al., 2016; Denis et al., 2015; Liu et al., 2007) and clinical samples (Dardennes et al., 2007; Vink et al., 2001) indicating abnormalities in the agouti-related protein (AgRP) signalling system (specifically, an association between stress-induced hypophagia/anorexia and downregulation of AgRP responses which results in dysfunction in ghrelinergic tone and action in the arcuate nucleus), we expected that acylated ghrelin would be negatively correlated with BOLD response in these regions, particularly the hypothalamus, in hypophagic MDD. Finally, in exploratory analyses, we examined whether previously reported differences between acutely depressed individuals with hypophagic vs. hyperphagic MDD in BOLD response to food stimuli (independent of acylated ghrelin levels) extended to the remitted state.

Methods

Subjects

A community sample of women with hyperphagic MDD, in remission (hyperphagic MDD; $n=10$), those with hypophagic MDD, in remission (hypophagic MDD; $n=18$), and healthy controls ($n=18$) between 22 and 42 years of age, with a BMI between 18.5–45, were recruited from the Boston, Massachusetts area through advertisements. Exclusion criteria included current pregnancy or breastfeeding; neurological disease; diabetes; endocrine disorders; comorbid current major psychiatric disorders; significant change in weight within the last three months; or current use of glucocorticoids, steroids, neuroleptics or stimulants. All MDD participants met diagnostic criteria for MDD (in remission) as assessed by the Structured Clinical Interview for Diagnoses (SCID; DSM-IV-TR; (Spitzer et al., 2002) administered by a trained clinician interviewer with over 20 years of experience. Allocation to hyperphagic/hypophagic MDD group was based on reported appetite changes during the most severe prior episode, as assessed during the SCID interview. Specifically, Module A of the SCID (i.e., Mood Episodes - Past Major Depressive Episode) was used to assess appetite changes including the direction of the appetite change. Participants in the HC group did not meet criteria for any psychiatric disorders (assessed by SCID; DSM-IV-TR; (Spitzer et al., 2002). All participants provided written informed consent. All study procedures were approved by the Partners Healthcare Human Research Committee.

Procedures

Following a screening visit at the Brigham and Women's Hospital Center for Clinical Investigation to ascertain height, weight, and other clinical variables, eligible participants underwent the primary study visit during the follicular phase (days 1–10) of their menstrual cycle. Participants (hyperphagic MDD $n=2$; hypophagic MDD $n=2$; HC $n=5$) on oral contraceptives (prescribed for contraceptive purposes) completed study procedures during the inactive (placebo) week of their regimen.

On the morning of the visit at 7:30 am, following an overnight (12 hr) fast, the Beck Depression Inventory-II (BDI-II) (Beck et al., 1996) was administered to assess current

depressive symptoms. After a pregnancy test, a saline-lock IV line was placed in the participant's non-dominant arm to enable serial blood draws. Participants then completed the State-Trait Anxiety Inventory (STAI) (Spielberger et al., 1970), and underwent an fMRI scanning session (see below). After the fMRI scan, participants were asked to consume a standardized meal and asked to complete it in entirety, preceded and followed by blood draws. The meal contained 30% of their recommended daily caloric intake (varying according to each participant's basal metabolic rate/activity level, measured by the Harris-Benedict equation; with 18% calories from protein, 23% calories from fat, and 59% calories from carbohydrates). After consumption of the standardized meal by the participants, unconsumed portions were weighed and for each participant the a) actual kcal consumed during the standardized meal, and b) percentage of the meal consumed (i.e., proportion of kcals consumed vs. provided, the latter of which varied across subjects according to basal metabolic rate/activity), were calculated.

Appetite ratings on desire to eat sweet foods, desire to eat fatty foods, and hunger were collected using 10-cm visual analogue scales (VAS) before and after the meal.

Blood samples were drawn immediately prior to the scan (i.e., baseline draw), after the scan/prior to the meal (Time 0; T0), and 30 (T30), 60 (T60), and 120 (T120) minutes following meal commencement (i.e., post-meal draws included the blood draws at time points: T30, T60 and T120). Blood samples were drawn on ice and immediately processed by trained nursing staff. Plasma samples were stored at -80°C in plastic tubes containing a 10-mg/ml solution of PMSF (phenylmethanesulfonylfluoride) in methanol. Acylated ghrelin levels were determined using a radioimmunoassay (LINCO Research, St. Charles, MO, USA; intra-assay coefficient of variation (CV) 6.5–9.5%; inter-assay CV 9.6–16.2%). After the meal, participants completed the Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith et al., 1995) and Three-Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985), and rated a selection of the food and non-food stimuli using a 10-cm VAS (food images: pleasantness, likeability, desire to eat, and caloric content; nonfood images: pleasantness). At the end of the visit participants were debriefed and compensated for their participation.

Behavioral and hormone data analysis

Behavioral and hormone data were analyzed using SPSS software (version 19; SPSS, Inc., Chicago, IL, USA). Inspection of the data showed that ghrelin AUC was normally distributed and parametric testing approach was applied to the data. Between-group differences were assessed using independent samples t-test and χ^2 tests or overall analysis of variance (ANOVA) with significantly different values compared by the Modified Fisher's Least Significant Difference Test. A p value of <0.05 was designated for statistical significance. Area under the curve (AUC) for ghrelin was calculated using the trapezoidal method.

fMRI paradigm

A visual food stimuli task during which participants viewed high- and low-calorie food stimuli, non-food stimuli (household objects), and fixation cross in a block design was presented while participants underwent standard gradient echo planar functional imaging on

a Siemens 3T scanner. This task was validated in previous publications (Holsen et al., 2012; Holsen et al., 2014). During fMRI scanning, participants viewed 120 high-calorie sweet (e.g., cake, doughnuts) and savory (e.g., pizza, chips) food stimuli and 120 low-calorie (e.g., fruit, vegetables, grilled fish) food stimuli, 120 nonfood stimuli (household objects) and 120 blurred baseline images in a block design with the block order pseudorandomized and counterbalanced. To create the blurred slides, food and nonfood stimuli were Fourier-transformed to create images with the same physical properties but without recognizable content. Six 4-minute runs (5 images/block; 16 blocks/run) were presented. The full color stimuli were matched for luminance and were presented for 3 seconds; each image was presented 1 time only to each participant. To ensure participants kept their attention to the stimuli, they were instructed to look at each image closely and to tap their right pointer finger when pictures changed (adherence to the latter instruction was closely monitored and verified by study staff). A Power Desktop P5000 computer (Milwaukee PC, Milwaukee, WI, USA) running Presentation software (Neurobehavioral Systems, Berkeley, CA, USA) projected visual stimuli onto a screen at the end of the magnet bore; participants viewed the stimuli through a head coil-mounted mirror.

fMRI data acquisition

Whole-brain functional imaging was performed on a Siemens 3T Magnetom Verio MR scanner (Siemens, Munich, German) equipped with a 12-channel head coil using a gradient-echo, echo planar imaging (EPI) pulse sequence (36 oblique-axial slices, 3mm thick, repetition time [TR]=2000ms, echo time [TE]=30ms, flip angle=90°, field of view [FOV]=200×200mm, 122 images per run). Immediately before EPI data acquisition, a magnetic (B0) fieldmap (magnitude and phase images with the same slice prescription, number and thickness as the EPI scans) was collected for later use in distortion correction of the EPI scans. A T1-weighted 3-dimensional spoiled gradient recalled scan was also acquired (176 sagittal slices, 1.0mm isotropic, TR=2530ms, TE=3.36ms, flip angle=7°, FOV 256×256mm).

fMRI data analysis

The fMRI data were preprocessed using Statistical Parametric Mapping (SPM8) (Wellcome Trust Centre for Neuroimaging, London, UK). The following preprocessing steps were applied to the echo planar imaging (EPI) data set: realignment and geometric unwarping using magnetic fieldmaps, motion correction, nonlinear volume-based spatial normalization [standard Montreal Neurological Institute (MNI), spatial smoothing using a Gaussian kernel of 6 mm full width at half maximum (FWHM)]. Outliers in the global mean image time series (threshold: 3.5 SD) and movement (threshold: 0.8 mm, measured as scan-to-scan movement) were detected using Artifact Detection Tools (ART) (<http://web.mit.edu/swg/software.htm>). A default high-pass filter cut-off of 128 seconds was applied. Outliers were censored by entering them as nuisance regressors in the first-level, single-subject analysis. A generic binary mask was applied to ensure that voxels in regions with signal dropout were not arbitrarily excluded. For single-subject analyses, a generalized linear model (GLM) was applied to the fMRI data of each participant, with anticipated hemodynamic responses to visual stimuli (high-calorie foods, low-calorie foods, objects) convolved with a canonical hemodynamic response function and subsequently used as regressors. Specific comparisons

of interest (high-calorie foods vs. low-calorie foods) were tested using linear contrasts, and SPM maps were created based on these contrasts. Results from the single-subject level for the contrasts of interest (i.e., high-calorie foods vs. low-calorie foods) were fed into to a second level random effects regression analysis to examine the relationship between ghrelin AUC and neural activity in response to foods of varying reward value. For each group (hyperphagic MDD, hypophagic MDD, healthy controls), ghrelin AUC values were entered as covariates of interest. Hypothesis-driven, specific relationships of interest (i.e., positive association in the hyperphagic group, negative association in the hypophagic group) were tested using linear contrasts, and SPM maps were created based on these contrasts. Effects of interest were examined in each group using the small-volume correction approach in SPM8, which restricts voxel-wise analyses to voxels within a hypothesized *a priori* regions of interest. Predefined ROIs for *a priori* hypotheses were: hypothalamus, ventral tegmental area, nucleus accumbens, amygdala. An exploratory set of *post hoc* ROIs included the caudate, pallidum, putamen, hippocampus, and anterior insula. The *a priori* ROI approach was based on known GHS-R1a receptor distribution in homeostatic and reward-related regions (Guan et al., 1997; Zigman et al., 2006), while *post hoc* ROIs were based on connected striatal regions and previous reports in the literature on ghrelin–neural activity relationships (Holsen et al., 2014; Kroemer et al., 2013; Malik et al., 2008; Skibicka et al., 2012). Anatomical borders of hypothesized regions were defined using a manually segmented Montreal Neurological Institute-152 brain and implemented as overlays on the SPM8 canonical brain using the Wake Forest University PickAtlas (Maldjian et al., 2003) toolbox for SPM. False positives were controlled using family-wise error (FWE) correction. Within an anatomical ROI (left and right, separately), significant results were identified using small volume correction (initial voxel-wise height threshold: $p < 0.05$ uncorrected) and were reported as significant if they additionally met the peak level threshold of $p < 0.05$, FWE-corrected.

Following direct testing of our *a priori* hypotheses (focused on within-group positive or negative relationships between ghrelin AUC and BOLD activity) carried out in SPM as detailed above, percent signal change (psc) values were obtained and used *post hoc* to quantify brain-ghrelin relationships. First, areas significant within each anatomical ROI for the within-group contrasts for positive and negative associations between ghrelin and brain activity were identified and 2mm spheres were created around the peak voxel using the MarsBaR toolbox (Brett et al., 2002). Next, we used the REX toolbox (Whitfield-Gabrieli, 2009) to extract psc values within these 2mm spheres for each subject from their individual (first level) statistical parameter maps. Psc values were extracted from the same anatomical spheres for all three groups. Psc values for individual subjects were exported to SPSS for analyses carried out for illustrative purposes only: (1) calculation of Pearson correlation coefficients within each group; (2) tests of statistical differences between correlations quantified using r to Z Fisher transformation; (3) differences in the slopes between groups for each ROIs using a univariate general linear model.

Exploratory analyses.—To explore whether trends from *a priori* hypotheses extended to other contrasts, a similar approach (as above) was used to examine: 1) differences between hyperphagic MDD, hypophagic MDD, and HC groups in BOLD response to high-calorie vs.

low-calorie foods, independent of acylated ghrelin AUC; 2) differential relationships between acylated ghrelin AUC and BOLD activity in response to high-calorie foods vs. objects in the hyperphagic MDD and hypophagic MDD groups; and 3) differences between hyperphagic MDD, hypophagic MDD, and HC groups in BOLD response to high-calorie vs. objects, independent of acylated ghrelin AUC. ROIs for these analyses included both *a priori* and *post hoc* regions listed above for the primary analyses, and results of these exploratory analyses are reported for ROIs meeting $p < 0.10$, FWE-corrected.

Results

Subject characteristics

Demographics and clinical data of the participants are presented in Table 1. Subject groups (hyperphagic MDD, hypophagic MDD, and healthy control) were comparable in handedness ($p > 0.5$) and age ($p > 0.2$). The groups had similar body mass index (BMI) ($p > 0.5$) and percent ideal body weight (%IBW) ($p > 0.5$). There were no significant differences between the hyperphagic MDD and hypophagic MDD groups in age of symptom onset, duration of illness, remission rate, or current use of medication (all p values > 0.4). As expected, compared to the HC group, hyper- and hypophagic MDD groups had higher depressive symptoms (BDI) and trait and state anxiety (STAI) scores (see Table 1), although hyper- and hypophagic MDD groups were comparable on BDI and STAI scores (all $p > 0.4$). There were no significant group differences between healthy control, hyperphagic MDD, and hypophagic MDD groups on SHAPS or TFEQ scores (all p values > 0.13). Healthy control subjects consumed a higher percentage of provided kcal during the standardized meal compared to hypophagic MDD group ($p = 0.003$). However, total kcal consumed during the standardized meal did not significantly differ between HC, hypophagic MDD, and hyperphagic MDD groups ($p > 0.06$).

Ghrelin AUC and appetite ratings

Acylated ghrelin AUC was significantly higher in the hypophagic MDD group compared to the hyperphagic MDD and healthy control groups (see Table 2). Repeated-measures ANOVAs were conducted for each scale (desire to eat sweet foods, desire to eat fatty foods, and hunger) with time (pre-meal, post-meal) as within-subject factors and group (healthy control, hyperphagic MDD, hypophagic MDD) as between-subjects factor. This analysis revealed a main effect of time for each appetite rating ($p < 0.001$), but there was no time $\times$ group interaction (all $p > 0.05$) (see Table 2). The desire to eat sweet and fatty food was higher pre-meal compared to post-meal. Also, hunger scores were higher pre-meal compared to post-meal (all $p < 0.001$). Stimuli ratings of high- and low-calorie foods did not differ between groups (all $p > 0.3$).

Brain-hormone associations

For *a priori* regions, acylated ghrelin AUC was positively associated with BOLD activity to high-calorie food vs. low-calorie food stimuli in the left and right VTA and left hypothalamus in the hyperphagic MDD group (see Table 3, Figure 1A and 1B). In contrast, negative association was observed between acylated ghrelin AUC and BOLD activity in the right hypothalamus in the hypophagic MDD group (see Table 3; Figure 1C). Results of the

relationships between BOLD activity to high-calorie vs. low-calorie food stimuli and acylated ghrelin AUC in *post hoc* ROIs are displayed in Table S1 (Supplementary Material). At the whole brain level, no additional regions were identified as showing positive or negative relationships between acylated ghrelin AUC and BOLD response to high-calorie food (vs. low-calorie food) stimuli at the $p < 0.001$, FWE-corrected level for each group independently.

Percent signal change (psc) values were extracted for each subject from within the significant ROI clusters, and Pearson correlation coefficients between these psc values and ghrelin AUC were computed for each group to quantify differences between groups in ghrelin-psc associations. For illustrative purposes, results of the r to Z Fisher transformations for each ROI demonstrate the strength of the differences between hyperphagic MDD and two other groups (i.e., healthy control and hypophagic MDD) in the correlations between acylated ghrelin AUC and BOLD activity in the left and right VTA and left hypothalamus, as well as the strength of the differences between hypophagic MDD and two other groups in the correlations between acylated ghrelin AUC and BOLD activity in the right hypothalamus (see Table 4). Additionally, differences between groups in slopes for the relationship between BOLD activity to high-calorie vs. low-calorie food stimuli and ghrelin AUC are displayed in Table S2 (Supplementary Material).

Exploratory analyses

Separate independent t-tests were conducted to identify potential between-group differences in mean BOLD response to high-calorie food > low-calorie food stimuli in designated ROIs, independent of ghrelin levels. These analyses revealed no significant group differences between the hyperphagic MDD and hypophagic MDD groups (see Supplementary Material, Table S3 for differences between HC and MDD groups). Additionally, we examined relationships between BOLD activity to high-calorie food vs. object stimuli and acylated ghrelin AUC in the MDD groups, which did not reveal any significant positive relationships between BOLD activity and acylated ghrelin AUC in the hyperphagic group (Supplementary Material, Table S4). Acylated ghrelin AUC was negatively related to BOLD activity in the VTA in the hypophagic group (see Supplementary Material, Table S4). Independent of acylated ghrelin AUC, there were several regions in which hyperphagic MDD, hypophagic MDD, and HC groups differed in BOLD response to high-calorie food vs. object stimuli (Supplementary Material, Table S5).

Discussion

The current study was designed to investigate the pathophysiology of trait features of opposing appetite phenotypes in MDD. Specifically, we investigated the relationship between peripheral ghrelin levels and BOLD response to high- (vs. low-) calorie food stimuli in individuals with hyperphagic MDD and hypophagic MDD, in remission and healthy controls. Our data reveal significant differences between groups in the association between postprandial change in acylated ghrelin and brain activity in key homeostatic and reward regions with rich distribution of GSHR-1a receptors (Guan et al., 1997; Zigman et al., 2006), with strong positive relationships unique to the hyperphagic MDD group.

Specifically, in individuals with hyperphagic MDD, a greater change in acylated ghrelin was associated with increased BOLD response to high-calorie food in the bilateral VTA and left hypothalamus, regions known to play a key role in the regulation of hedonic and homeostatic food intake. This positive association was absent in individuals with hypophagic MDD, who instead showed a negative association between changes in acylated ghrelin and BOLD response to high-calorie food in the right hypothalamus. Importantly, these brain-hormone associations were found in absence of discrepant appetitive ratings, as the groups did not differ on their self-reported appetite. Additionally, postprandial acylated ghrelin levels were significantly greater in the hypophagic MDD group compared to the hyperphagic MDD and healthy control groups. Taken together with the absence of an acute depressive episode, these findings suggest that the association between the brain-hormone systems might be one of the pathophysiological trait features differentiating hyperphagic MDD and hypophagic MDD phenotypes.

In general, current findings of associations between peripheral ghrelin levels and BOLD response to a specific contrast (high- vs. low-calorie food) are consistent with earlier studies showing that ghrelin is associated with an increase in preference for palatable foods in animals and humans (Monteleone et al., 2012; Skibicka et al., 2012). In addition to enhancing preference for palatable foods, ghrelin increases motivation to obtain rewarding food by exerting its actions at the level of the VTA (Egecioglu et al., 2010; King et al., 2011; Skibicka et al., 2011). Specifically, ghrelin is thought to increase dopaminergic signaling in the VTA through inducing changes in the expression of dopamine receptors (Skibicka et al., 2012), acting on the primary ghrelin receptor (i.e., GHS-R1a) expressed in dopaminergic neurons (Abizaid et al., 2006), and increasing dopamine release (Abizaid et al., 2006; Jerlhag et al., 2007b). While this study was not designed to make inferences on the molecular mechanisms underlying the effects of ghrelin in the VTA, the positive correlation between acylated ghrelin levels and BOLD response in the VTA in the hyperphagic MDD group is in line with additional data in preclinical models which suggest ghrelin levels surge as a compensatory response to chronic psychosocial stress but also increase food intake both during and following acute stress (Lutter et al., 2008), a phenotype which parallels hyperphagic MDD in humans.

Conversely, the hypothalamus is a key region regulating homeostatic signaling of energy balance (Schneeberger et al., 2014). In the current study, changes in acylated ghrelin was negatively correlated with BOLD response in the right hypothalamus in the hypophagic MDD group, implying the typical association between ghrelin and homeostatic responses to food is reversed in hypophagic MDD, a group which demonstrates lowered appetite in an MDD episode. These findings are consistent with extant data in animal models of stress-related hypophagia/anorexia, in which disruption of the AgRP system is associated with greater body weight loss following social isolation (Denis et al., 2015), discordance between ghrelin levels and AgRP expression in the hypothalamus (Boersma et al., 2016), and downregulation of AgRP response to acute emotional stress (Liu et al., 2007), and suggest that hypophagic MDD might stem in part from maladaptive AgRP signaling. Of note, this system has also been implicated in the pathophysiology of anorexia nervosa (Dardennes et al., 2007; Vink et al., 2001) and shares an overlapping behavioral phenotype (depression, chronic stress, appetite/weight loss) with hypophagic MDD. The convergence of

directionality of these ghrelin-BOLD associations mapping to opposing appetite phenotypes in MDD (hyperphagic: positive relationship; hypophagic: negative relationship) provides preliminary evidence that these phenotypes might evolve in part through altered ghrelinergic signaling in homeostatic and hedonic food reward regions. In addition to replicating current findings, future studies should examine potential genetic and molecular pathways linking appetite and ghrelin in MDD, which will foster the development of targeted therapeutics.

Previous studies in healthy individuals found positive associations between *fasting* levels of unacylated ghrelin and BOLD responses while viewing food stimuli vs. blurred control images. For example, in our previous investigation involving healthy individuals, we found that increased levels of fasting acylated ghrelin was associated with increased BOLD responses while viewing high-calorie foods vs. objects in the right amygdala, right hippocampus, right anterior insula and right orbitofrontal cortex (Holsen et al., 2014), while Kroemer and colleagues reported similar positive associations in healthy controls between fasting ghrelin and BOLD activity to food vs. control (scrambled) stimuli in the caudate, pallidum, midbrain, amygdala, anterior cingulate cortex and right hypothalamus (Kroemer et al., 2013). However, this same study reported no correlations between BOLD response to food stimuli and ghrelin levels *following a caloric load* (i.e., glucose tolerant test) in healthy controls (Kroemer et al., 2013). In the current study, our primary focus was on associations between BOLD response and postprandial change (i.e., multiple measurements over time) in acylated ghrelin in response to a mixed meal in individuals with MDD. However, for illustrative purposes, we computed correlations between ghrelin AUC and BOLD activity [in ROIs (VTA, hypothalamus) found to be significant in the MDD groups] in the healthy control group; however, these analyses did not yield any significant associations. In this manner, our results appear to be consistent with Kroemer and colleagues. Of note, the nature of the opposing direction of these associations (positive for fasting ghrelin, absent for postprandial ghrelin AUC) in healthy individuals is likely driven by the measurement approach for ghrelin, as fasting ghrelin levels play a role in orienting towards and approaching food cues in an individual's environment (Muller et al., 2015), and thus positive associations with reward-related brain activity is congruous with this role. The volume of change in ghrelin in response to a meal, on the other hand, incorporates the dynamic fluctuations in ghrelin, including decreases, such that attenuated ghrelin AUC levels in healthy control subjects might indicate an insufficient satiety response, and thus might be associated with a higher level of BOLD activity in regions associated with reward. Of note, evidence from a series of elegant studies in healthy individuals suggests larger postprandial reductions in *total* ghrelin are associated greater postprandial attenuation of BOLD response in the midbrain, amygdala, pallidum, hippocampus, and insula to a milkshake (Sun et al., 2015; Sun et al., 2014), supporting the role of ghrelin dynamics in satiety-related changes in brain activity in key mesolimbic regions.

Additionally, BOLD data for the primary analyses in the current study were derived from a contrast of high-calorie vs. low-calorie food (rather than food vs. non-food stimuli), which represents a more stringent and tightly-controlled contrast as the only varying aspect of the stimuli is the reward value of the food, and thus might more conclusively indicate reward signaling specific to palatable food. In general, our current findings are in line with previous clinical and preclinical studies as we demonstrate an interaction between ghrelin signaling

and neural activity in response to palatable food. In exploratory analyses, relationships focused on the high-calorie food vs. object contrast revealed no significant positive relationships in the hyperphagic MDD group, although a negative relationship in the VTA in the hypophagic MDD group was noted. The lack of consistency between relationships might be related to the specificity of the high- vs. low-calorie food contrast, which should in theory be more tightly related to ghrelin, as opposed to high-calorie food vs. objects, which represents a more general contrast.

Independent of ghrelin levels, the two remitted depressed groups differed from the healthy control group in the BOLD response to high-calorie food in regions of the mesolimbic reward circuitry in exploratory analyses. Specifically, compared to healthy controls, the hyperphagic MDD group exhibited greater BOLD response to high-calorie (vs. low-calorie) food in the left caudate, and lower BOLD response in the bilateral amygdala. The hypophagic MDD group exhibited greater BOLD response to high-calorie food in the bilateral VTA, and lower BOLD responses in the left amygdala. Similar trends emerged from between-group analyses of the high-calorie food vs. objects contrast. A recent study demonstrated increased BOLD responses to food stimuli (vs. objects) in mesolimbic reward circuitry in acutely depressed patients with heightened appetite compared to acutely depressed patients with lower appetite and to healthy controls (Simmons et al., 2016). Depressed patients with heightened appetites had greater activity in the orbitofrontal cortex, ventral striatum, ventral pallidum and putamen, while there were no differences in these regions between the depressed patients with lowered appetite and the healthy controls. The depressed patients with lowered appetite on the other hand, had lower BOLD responses in the caudal anterior and mid-insula compared to healthy controls and acutely depressed patients with heightened appetite. Current results complement these findings, extending the focus to remitted patients and the interaction between the most potent appetite-stimulating hormone, ghrelin, and neural responses to palatable food. Furthermore, our data are in line with previous studies reporting altered reward processing in patients with remitted MDD in response to monetary (Dichter et al., 2012; Pechtel et al., 2013) and food-related (McCabe et al., 2009) rewards. Taken together, our data extend previous reports by providing evidence that dysfunction in mesolimbic signalling and interactions with neuropeptide modulators of this circuitry as potential factors which distinguish appetite phenotypes in remitted MDD.

To our knowledge, our data are the first to demonstrate alterations in postprandial acylated ghrelin levels in a hypophagic MDD patients, however, one study measured fasting plasma total ghrelin and acylated ghrelin in depressed patients with weight loss as one of their symptoms, but did not find differences on ghrelin levels between depressed patients and healthy controls (Gimenez-Palop et al., 2012). Similarly, there were no differences found in fasting acylated ghrelin between acute and remitted MDD patients, and healthy subjects (Matsuo et al., 2012). Also, total ghrelin levels did not differ between depressed patients and healthy controls (Kluge et al., 2009; Schanze et al., 2008). Overall, ghrelin levels seem not to be different in depressed vs. healthy controls, however, the findings used different measurement approaches (i.e., total/acylated ghrelin, fasting/non-fasting and measurements at a single time point) which complicates comparison with our current finding of hypophagic MDD subjects exhibiting greater changes in acylated ghrelin levels compared to hyperphagic MDD and healthy controls. Important to note is that our results were found in

absence of possible confounders of ghrelin levels, such as differences in BMI and energy consumption (i.e., total kcal consumed during the meal) at the day of the visit.

One limitation of our study is the unbalanced sample size between groups, with a relatively small sample of individuals with hyperphagic MDD. Smaller sample sizes do reduce the generalizability of our results. The BMI range of our participants did not reflect individuals in the underweight ($\text{BMI} < 18.5 \text{ kg/m}^2$) range, which limits generalizability to the wider MDD population. Future work is needed to address this limitation, given the variance in ghrelin secretion and sensitivity across BMI ranges (Wren, 2008), and the fact that obesity and depression has been reciprocally linked to each other (Luppino et al., 2010). Ghrelin has been suggested as a critical mediator in the link between stress-induced eating and depressive mood (Schellekens et al., 2012). Although the exact neuroendocrine mechanisms by which ghrelin modulates the interaction between mood changes and alterations in appetite/food-related reward is unknown, there is accumulating evidence that ghrelin plays an important role in the pathophysiology of both depression as obesity (Schellekens et al., 2012). Further, some of our participants were using antidepressant medication at the time of the study, which may have affected ghrelin levels and neural activity. However, current use of medication did not differ between the hypophagic and hyperphagic MDD groups, and there were no significant differences in ghrelin levels in participants currently on vs. off medication (data not shown). Atypical antipsychotic medications may impact appetite changes during a prolonged period of treatment, including when taken during a depressive episode. Data were not collected in this study regarding use of atypical antipsychotics during past acute depressed phases in participants with hyper- and hypophagic MDD. Future studies would benefit from larger, equal samples of unmedicated individuals of both genders with hyperphagic and hypophagic MDD. Finally, these initial findings of within-group relationships between ghrelin and BOLD activity to palatable food cues could inform future studies designed to directly examine interactions between group (hyperphagic MDD, hypophagic MDD, HC) and directionality in these relationships.

Effective treatment of depression is complicated by the heterogeneity of the disorder, with individuals exhibiting symptoms associated with hyperphagic MDD experiencing more deleterious effects. Our findings contribute to further refining biologically-based endophenotypes of depression based on neurohormonal factors associated with reward processing in individuals with remitted MDD with varying appetitive phenotypes (Insel et al., 2010). This may facilitate the development of more precise and effective treatments for opposing appetite phenotypes in depression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Ghrelin is associated with increased BOLD response to food in hyperphagic MDD.
- Ghrelin is associated with decreased BOLD response to food in hypophagic MDD.
- Findings give insight into trait features differentiating appetite phenotypes in MDD.
- Ghrelin-reward system interaction is relevant for developing therapeutic targets.

Figure 1. Relationship between BOLD response to high-calorie vs. low-calorie food stimuli and acylated ghrelin AUC in hyperphagic MDD, hypophagic MDD, and healthy controls. Positive relationships between acylated ghrelin AUC and BOLD response to high-calorie vs. low-calorie food in hyperphagic MDD subjects in (A) the left and right ventral tegmental area (VTA) and (B) left hypothalamus. (C) Negative relationship between acylated ghrelin AUC and BOLD response to high-calorie vs. low-calorie food in hypophagic MDD subjects in the right hypothalamus. Voxels surviving the statistical threshold [identified using small volume correction (initial voxel-wise height threshold: $p < 0.05$ uncorrected) and reported as significant if they additionally met the peak-level threshold of $p < 0.05$, FWE-corrected] are projected on a single-subject T1, centered at $t_{\max}$. Within-group associations between ghrelin AUC and extracted % signal change values in each ROI are visualized in corresponding scatterplots below each ROI, with data markers and fit lines stratified by group according to legend.

Table 1.

Demographics and clinical characteristics

	Group			Between-Group Comparisons ¹
	Healthy control	Hypophagic MDD	Hyperphagic MDD	
	Mean ± SD	Mean ± SD	Mean ± SD	
Age	28.0 ± 4.1	28.8 ± 5.4	30.2 ± 6.1	HC vs Hypo: $t(34)=0.519$, $p=0.607$ HC vs Hyper: $t(26)=1.132$, $p=0.268$ Hypo vs Hyper: $t(26)=-0.611$, $p=0.546$
Handedness (right)	15 (83%)	16 (89%)	9 (90%)	HC vs Hypo: $\chi^2(1)=0.232$, $p=0.630$ HC vs Hyper: $\chi^2(1)=0.233$, $p=0.629$ Hypo vs Hyper: $\chi^2(1)=0.008$, $p=0.927$
BMI	29.1 ± 8.7	28.3 ± 10.3	30.6 ± 7.3	HC vs Hypo: $t(34)=-0.252$, $p=0.802$ HC vs Hyper: $t(26)=0.471$, $p=0.642$ Hypo vs Hyper: $t(26)=-0.631$, $p=0.534$
% IBW	126.2 ± 35.8	121.5 ± 37.7	130.3 ± 28.0	HC vs Hypo: $t(34)=0.377$, $p=0.708$ HC vs Hyper: $t(26)=0.311$, $p=0.758$ Hypo vs Hyper: $t(26)=-0.638$, $p=0.529$
Age at symptom onset		17.2 ± 7.2	19.2 ± 5.2	Hypo vs Hyper: $t(26)=-0.784$, $p=0.440$
Duration of illness		11.7 ± 5.3	12.0 ± 8.3	Hypo vs Hyper: $t(26)=-0.130$, $p=0.898$
% in partial remission		4 (22%)	2 (20%)	Hypo vs Hyper: $\chi^2(1)=0.019$, $p=0.891$
Months in remission		26.9 ± 22.0	38.3 ± 66.6	Hypo vs Hyper: $t(26)=-0.669$, $p=0.509$
Current psychotropic medication ²		8 (44%)	6 (60%)	Hypo vs Hyper: $\chi^2(1)=0.622$, $p=0.430$
BDI total	1.8 ± 3.7	6.2 ± 6.2	8.1 ± 7.5	HC vs Hypo: $t(34)=-2.623$, $p=0.013$ HC vs Hyper: $t(26)=3.021$, $p=0.006$ Hypo vs Hyper: $t(26)=-0.716$, $p=0.480$
STAI - Trait Anxiety	29.5 ± 8.2	41.6 ± 9.9	40.5 ± 8.2	HC vs Hypo: $t(34)=-4.005$, $p<0.001$ HC vs Hyper: $t(26)=3.399$, $p=0.002$ Hypo vs Hyper: $t(26)=0.302$, $p=0.765$
STAI - State Anxiety	26.1 ± 6.2	34.3 ± 8.6	33.4 ± 9.1	HC vs Hypo: $t(34)=-3.325$, $p=0.002$ HC vs Hyper: $t(26)=2.540$, $p=0.017$ Hypo vs Hyper: $t(26)=0.271$, $p=0.789$
SHAPS	50.3 ± 4.9	48.1 ± 4.3	49.8 ± 4.7	HC vs Hypo: $t(34)=1.409$, $p=0.168$ HC vs Hyper: $t(26)=-0.251$, $p=0.804$ Hypo vs Hyper: $t(26)=-0.958$, $p=0.347$
Modified SHAPS ³	39.6 ± 4.0	37.7 ± 3.3	39.0 ± 3.6	HC vs Hypo: $t(34)=-1.550$, $p=0.130$ HC vs Hyper: $t(26)=-0.366$, $p=0.717$ Hypo vs Hyper: $t(26)=-0.985$, $p=0.333$
TFEQ – Cognitive restraint ⁴	11.0 ± 1.8	11.1 ± 2.4	11.5 ± 2.5	HC vs Hypo: $t(25)=-0.074$, $p=0.942$ HC vs Hyper: $t(19)=0.519$, $p=0.610$ Hypo vs Hyper: $t(24)=-0.447$, $p=0.659$

	Group			Between-Group Comparisons ¹
	Healthy control	Hypophagic MDD	Hyperphagic MDD	
	Mean ± SD	Mean ± SD	Mean ± SD	
TFEQ – Disinhibition ⁴	8.4 ± 3.1	8.4 ± 2.9	10.3 ± 2.5	HC vs Hypo: t(25)=−0.010, <i>p</i> =0.992 HC vs Hyper: t(19)=1.553, <i>p</i> =0.137 Hypo vs Hyper: t(24)=−1.744, <i>p</i> =0.094
TFEQ – Susceptibility to hunger ⁵	6.8 ± 2.3	7.4 ± 2.3	6.3 ± 1.9	HC vs Hypo: t(26)=−0.678, <i>p</i> =0.504 HC vs Hyper: t(20)=−0.575, <i>p</i> =0.572 Hypo vs Hyper: t(24)=1.283, <i>p</i> =0.212
Kcal consumed during meal	652 ± 78.5	583 ± 133.1	592 ± 164.9	HC vs Hypo: t(34)=1.898, <i>p</i> =0.066 HC vs Hyper: t(26)=1.309, <i>p</i> =0.202 Hypo vs Hyper: t(26)=−0.162, <i>p</i> =0.872
% of meal consumed	96 ± 7.0	84 ± 14.2	88 ± 20.3	HC vs Hypo: t(34)=3.265, <i>p</i>=0.003 HC vs Hyper: t(26)=1.577, <i>p</i> =0.127 Hypo vs Hyper: t(26)=−0.608, <i>p</i> =0.548

¹ Significant results bolded.

² In total, 8 Hypophagic MDD participants were taking the following medications at the time of the study: bupropion and citalopram (n=1), lithium, fluvoxamine, quetiapine (n=1), fluoxetine (n=2), bupropion (n=1), sertraline (n=1), bupropion and escitalopram (n=1), venlafaxine (n=1). In total, 6 Hyperphagic MDD participants were taking the following medications at the time of the study: bupropion and citalopram (n=1), citalopram (n=1), duloxetine and methylphenidate hydrochloride (n=1), venlafaxine (n=1), quetiapine and lamotrigine (n=1), bupropion (n=1).

³ Excluding items 4, 6 and 10.

⁴ Data available for n=11 HC, n=16 hypophagic MDD.

⁵ Data available for n=12 HC, n=16 hypophagic MDD.

Table 2.

Between-group differences in acylated ghrelin AUC and appetite ratings

	Group			Between-Group Comparisons ¹
	Healthy control	Hypophagic MDD	Hyperphagic MDD	
	Mean ± SD	Mean ± SD	Mean ± SD	
Acylated ghrelin AUC	8835.8 ± 1184.2	10580.8 ± 2826.4	8730.0 ± 2479.6	F(2)=3.49, <i>p</i>=0.039, $\eta^2=0.140$ HC vs Hypo: Mean Diff=-1745.0, <i>p</i>=0.024 HC vs Hyper: Mean Diff=105.8, <i>p</i> =0.905 Hypo vs Hyper: Mean Diff=1850.8, <i>p</i>=0.042
Desire to eat sweet foods ² (pre- to post-meal)	-3.3 ± 3.3	-1.6 ± 3.2	-4.5 ± 2.3	Main effect of time: F(1)=42.9, <i>p</i> < 0.001, $\eta^2=0.500$ Interaction: F(2)=3.09, <i>p</i> =0.056, $\eta^2=0.126$
Desire to eat fatty foods ² (pre- to post-meal)	-3.7 ± 2.8	-2.4 ± 2.8	-4.3 ± 2.1	Main effect of time: F(1)=73.1, <i>p</i> < 0.001, $\eta^2=0.630$ Interaction: F(2)=1.90, <i>p</i> =0.162, $\eta^2=0.081$
Hunger (pre- to post-meal)	-6.3 ± 2.2	-4.6 ± 3.3	-6.4 ± 1.6	Main effect of time: F(1)=208.3, <i>p</i> <0.001, $\eta^2=0.829$ Interaction: F(2)=2.52, <i>p</i> =0.092, $\eta^2=0.105$

¹Significant results bolded.²Desire to eat sweet and fatty food was assessed on a reversed scale (i.e., scores ranged from 0: very much desire for sweet food to 10: not at all desire for sweet food). Hunger was assessed on a scale ranging from 0: not hungry at all to 10: never been more hungry.

Table 3.

Relationships between BOLD activity to high-calorie vs. low-calorie food stimuli and acylated ghrelin AUC in *a priori* ROIs

ROI	Ke	x	y	z^1	Positive relationship ² , t_{max} (voxel level FWE-corrected p)
Hyperphagic MDD					
Left VTA	3	-3	-13	-11	3.69 (0.012)
Right VTA	3	3	-13	-11	2.91 (0.023)
Left Hypothalamus	13	-9	-10	-11	4.37 (0.045)
Right Hypothalamus	14	3	-10	-11	3.25 (0.132)
Left/Right NAcc					NSC ³
Left/Right Amygdala					NSC
ROI	Ke	x	y	z^1	Negative relationship ² , t_{max} (voxel level FWE-corrected p)
Hypophagic MDD					
Left/Right VTA					NSC
Left Hypothalamus	5	-6	-1	-14	2.50 (0.155)
Right Hypothalamus	6	9	-1	-14	3.45 (0.042)
Left NAcc	4	-9	11	-8	2.06 (0.220)
Right NAcc					NSC
Left Amygdala	1	-15	-4	-14	2.07 (0.372)
Right Amygdala					NSC

¹Coordinates are presented in MNI space.

²Clusters identified using small volume correction within an anatomical ROI (initial voxel-wise height threshold: $p < 0.05$ uncorrected) are reported (in **bold**) as significant if they additionally met the peak-level threshold of $p < 0.05$, FWE-corrected.

³NSC = No suprathreshold clusters at $p < 0.05$, uncorrected.

Table 4.

Between-group differences in correlations between BOLD activity to high-calorie vs. low-calorie food stimuli and acylated ghrelin AUC

ROI	Pearson's correlation coefficient, r^I			Between-Group Comparisons ^I
	Healthy control	Hypophagic MDD	Hyperphagic MDD	
Left VTA	-0.193	0.132	0.793	HC vs. Hypo: Z=-0.900 HC vs. Hyper: Z=-2.79 Hypo vs. Hyper: Z=2.07
Right VTA	-0.142	0.348	0.717	HC vs. Hypo: Z=-1.39 HC vs. Hyper: Z=-2.28 Hypo vs. Hyper: Z=-1.18
Left Hypothalamus	0.225	0.012	0.840	HC vs. Hypo: Z=0.59 HC vs. Hyper: Z=-2.17 Hypo vs. Hyper: Z=2.64
Right Hypothalamus	-0.200	-0.653	-0.373	HC vs. Hypo: Z=1.58 HC vs. Hyper: Z=0.41 Hypo vs. Hyper: Z=-0.850

^IZ and r-values calculated for illustrative purposes only.