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**Reward-related brain activity and behavior are associated with peripheral ghrelin levels in obesity**

**Running Title.** Ghrelin-related reward activity in obesity

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**Highlights**

- Obese subjects exhibited increased activation in brain areas related to self-control and self-referencing during processing of non-food reward.
- Fasting levels of circulating ghrelin were associated with reward processing, both at the behavioral and neurobiological levels.
- The associations of ghrelin with decision times and dlPFC activity suggest the potential involvement of ghrelin in the development of behavioral alterations at early stage of obesity**.**

ABSTRACT

**Background/Objectives:** While excessive food consumption represents a key factor in the development of obesity, the underlying mechanisms are still unclear. Ghrelin, a gut-brain hormone involved in the regulation of appetite, is impaired in obesity. In addition to its role in eating behavior, this hormone was shown to affect brain regions controlling reward, including the striatum and prefrontal cortex, and there is strong evidence of impaired reward processing in obesity. The present study investigated the possibility that disrupted reward-related brain activity in obesity relates to ghrelin deficiency.

**Subjects/Methods:** Fifteen severely obese subjects (BMI > 35 kg/m$^2$) and fifteen healthy non-obese control subjects (BMI < 30 kg/m$^2$) were recruited. A guessing-task paradigm, previously shown to activate the ventral striatum, was used to assess reward-related brain neural activity by functional magnetic resonance imaging (fMRI). Fasting blood samples were collected for the measurement of circulating ghrelin.

**Results:** Significant activations in the ventral striatum, ventromedial prefrontal cortex and extrastriate visual cortex were elicited by the fMRI task in both obese and control subjects. In addition, greater reward-related activations were present in the dorsolateral prefrontal cortex, and precuneus/posterior cingulate of obese subjects compared to controls. Obese subjects exhibited longer choice times after

repeated reward and lower circulating ghrelin levels than lean controls. Reduced ghrelin levels significantly predicted slower post-reward choices and reward-related hyperactivity in dorsolateral prefrontal cortices in obese subjects.

**Conclusion:** This study provides evidence of association between circulating ghrelin and reward-related brain activity in obesity and encourages further exploration of the role of ghrelin system in altered eating behavior in obesity.

*Key words: Obesity, Reward, Behavior, fMRI, Ghrelin*

## 1. INTRODUCTION

Abnormal feeding behavior resulting in weight gain and obesity has been linked to alterations in reward processing (Burger and Berner, 2014). In support of this, impaired reward-related brain responses have been repeatedly documented in overweight/obese subjects (Carnell et al., 2012; Pursey et al., 2014; Puzziferri et al., 2016). These alterations were found not only in food-related paradigms but also in more generalized experimental conditions, including monetary incentive tasks (Balodis et al., 2013). Monetary delay discounting, which is a bias towards smaller but earlier rewards, is greater in obese subjects pointing to an abnormal reward expectation and decision making in obesity (Price et al., 2016). These effects may be related to a deregulation of hormones that control reward-directed and food-consuming behaviors. In this perspective, a promising target for obesity research is the ghrelin-regulating system, which is involved not only in appetitive behavior but also in higher cognitive functions (Anderberg et al., 2016; Ralevski et al., 2018).

Ghrelin is a peptide that is mainly produced by neuroendocrine cells in the stomach mucosa. Secretion of ghrelin has a fluctuating character depending on circadian rhythms and food anticipation (Natalucci et al., 2005). Immediately after food consumption, ghrelin levels fall (English et al., 2002) and then gradually increase to reach a peak before the next meal is initiated. Once secreted, ghrelin is able to act on numerous peripheral tissues and the brain (Muller et al., 2015). Peripheral ghrelin can affect brain function via different pathways (Howick et al., 2017), including vagal or brain stem stimulation (Date, 2012), blood stream (Angelidis et al., 2010), passage across the blood-brain barrier via saturated transport system (Solomou and Korbonits, 2014), and transport *via* the cerebrospinal fluid (Uriarte et al., 2018). Ghrelin receptors are widely expressed in the brain, notably in the orexigenic neurons of hypothalamic nuclei (Muller et al., 2015) and in the hippocampus, pituitary, dentate gyrus, substantia nigra, dorsal/median raphe nuclei and ventral tegmental area (Andrews, 2011). Altogether, they contribute to the regulation of appetite, energy balance, olfaction, but also memory/learning, stress response, mood, and reward control (Abizaid, 2019; Goldstone et al., 2014; Howick et al., 2017; Malik et al., 2008; Perello and Dickson, 2015).

Studies in animals have shown that ghrelin activates the reward circuitry comprising of the ventral tegmental area, lateral hypothalamus and nucleus accumbens (Revitsky and Klein, 2013). At the clinical level, reductions in dorsolateral prefrontal cortex (dlPFC) activity and decreased self-control, supposed to be related to uncontrolled overeating (Holsen et al., 2012), have been reported in overweight patients with Prader-Willi syndrome (PWS) who also exhibit increased ghrelin levels. In healthy subjects, increased ghrelin levels have been related to increased sensitivity to reward and impulsivity/lower self-control (Ralevski et al., 2018). Deregulation of the ghrelin system may also contribute to the development of obesity (Abizaid, 2019). In obese subjects, not only fasting ghrelin levels are reduced proportionally to body mass index (BMI) (Tschop et al., 2001; Nonogaki, 2008), but also alterations in the circadian rhythm and postprandial inhibition of ghrelin secretion are reported (Yildiz BO et al., 2004). The patterns of phasic and tonic ghrelin regulations are altered (Foster et al., 2007) and ghrelin resistance is proposed as one of the major mechanisms of ghrelin system deficiency in obesity (Zigman et al., 2016). Central action of ghrelin in obesity may be also impaired due to different facets of ghrelin resistance (Zigman et al., 2016). Interestingly, normalization of blood levels of ghrelin after the weight loss is associated with improved brain sensitivity to ghrelin (Briggs et al., 2013).

The association of the ghrelin system with both reward response and appetite control/metabolic regulation suggests the potential role of decreased ghrelin levels in the development of brain reward system alterations and disrupted eating behaviors likely to promote the development of obesity. While altered reward-related neural activation in obesity has been frequently associated with changes in other energy balance regulating hormones, including leptin and insulin (Baicy et al., 2007; Farooqi et al., 2007; Kullmann et al., 2012), no studies have investigated the potential link between obesity-related ghrelin deficiency and disruptions in the brain reward system. The present study addressed this issue in a sample of severely obese subjects compared to healthy non-obese controls, using functional magnetic resonance imaging (fMRI) during a non-food related task known to induce reward-related cerebral activity. Behavioral responses and brain processing of reward outcome were investigated and their relationship with individual levels of ghrelin was assessed.

## 2. METHODS

### 2.1. Subjects

Fifteen severely obese subjects (BMI > 35 kg/m$^2$; 3 males and 12 females) and fifteen healthy non-obese control subjects (BMI < 30 kg/m$^2$; 2 males and 13 females) were included. All subjects were right-handed adults, free of any treatment with psychotropic effects, and had not eaten for the last two hours before the fMRI session. Additional exclusion criteria were age > 65 years old, history of brain damage or neurological disease, acute or chronic inflammatory disorders (other than obesity and obesity-related conditions), severe or uncontrolled medical conditions, psychiatric disorders, metallic implants and eating disorders (in non-obese control subjects). All subjects provided written informed consent and received compensation for their participation in the study. The study was approved by the Committee for the Protection of Persons (CPP) of Bordeaux.

### 2.2. Guessing task

We used a guessing-task paradigm that was previously shown to activate the ventral striatum, involved in reward processing (Capuron et al., 2012; Reuter et al., 2005). Subjects were presented with two hidden cards on the left and right sides of the screen and were asked to guess which of the two cards was "red" by pressing on a keypad with the index (left key) or middle finger (right key) (**Figure 1A, B**). The time window for the choice was fixed at 2s, after which the selected card was turned over and the outcome was displayed on the screen for another 2s. If the card was "red", the subject gained one Euro, if "black" one Euro was lost. The sequence of gains and losses was similar for all subjects. Unbeknownst to participants, the outcomes followed a noisy sinusoid with a slight upward trend (**Figure 1A**), as described elsewhere (Reuter et al., 2005). The net score, which was initially equal to 15 Euros, was always shown on the screen and updated each time the chosen card was revealed. The choice time was recorded for each trial. In total, there were 100 trials, and the total duration of the task was 6 min 40 sec. In contrast to previous studies using this paradigm, participants were informed that the task should be considered as a game and the amount of their compensation (related to their participation in the study) would not depend

of their gain or lost; furthermore, the number of trials was reduced by a factor of three (Reuter et al., 2005). These methodological adaptations did not impede significant ventral striatum reward response, as shown in **Figure 2**. All stimuli presentations and recordings of reaction time and response keys were performed with E-Prime 2.0 software (Psychology Software Tools, Sharpsburg, PA, USA).

Before the acquisition started, a practice session was performed outside the scanner room on a laptop computer under the supervision of the experimenter. An additional practice session was performed in the scanner using the MRI-compatible keypad (4-key response box). After practice, participants performed the task during the scanning session. The task was presented on a screen placed in front of the scanner bed and was monitored through a computer located outside the scanner room. Participants were able to see the screen through a mirror attached to the head coil. Choice time (CT), i.e. the time elapsed from the presentation of the cards on the screen to the volunteer's button press signaling the choice of one of the two hidden decks (left or right), was recorded as a behavioral parameter. We were particularly interested in decision-making processes related to further reward acquisition *after* a reward had already been received, as this is an experimental situation that could provide a model for the prolonged consumption of food in the obese. Accordingly, only CTs after winning trials were analyzed. Furthermore, CTs were grouped into two subsets according to the event-sequence, as this is known to affect cerebral responses in reward tasks (Akitsuki et al., 2003). When there was just one win before the current trial, response latency was classified as CT after short reward (CT1). When there were two or more wins in a row before the current trial, CT was considered as being within the context of long or sustained reward (CT2).

### 2.3. MRI acquisition

Scanning was performed on a 3T scanner GE Discovery MR750 with a 32 channels head coil. First, a high-resolution T1-weighted anatomical scan was acquired via a FSPGR sequence with the following parameters: TR=7.896 ms, TE=3.216 ms, TI=400 ms, FA=10°, FoV=288 $mm^2$, 200 sagittal slices with a 288x288 acquisition matrix, 1 $mm^3$ isotropic resolution, total acquisition time: 3 min 43 s. Then, two functional runs of T2*-weighted echo-planar imaging for measurement of the blood oxygen

level–dependent (BOLD) contrast were acquired, with the following parameters: TR=2000 ms, TE=30 ms, FA=80°, FOV=19.20 cm, 35 64×64 slices with a 3.5 mm thickness and a 0.5 mm between-slice gap (effective voxel size = 3x3x4 mm), with asset factor 2. Slices were prescribed with a negative pitch angle of 30 degrees relative to AC-PC horizontal plane, in order to minimize the ventro-medial BOLD signal dropout, as advised in (Weiskopf et al., 2007). During the first functional run (5 dummy scans + 264 dynamic scans; total duration 8 min 58 s), volunteers performed an attention task, whose results will be discussed and reported elsewhere. During the second functional run (5 dummy scans + 204 dynamic scans; total duration 6 min 58 s), subjects performed the guessing task described above.

### 2.4. Imaging preprocessing

MRI images were analyzed using the SPM software package (*SPM8 v4290; Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK; http//www.fil.ion.uce.ac.uk/spm*) implemented in MATLAB (Mathworks Inc., Sherborn, MA).

For each subject, motion correction to the first functional scan was performed with a six-parameter rigid-body transformation. No excessive motion was found using the criterion of >1 mm instantaneous shift. The structural image was co-registered to the average of the motion-corrected functional images, and then segmented into grey matter, white matter, and cerebrospinal fluid probability maps. The warping parameters obtained from the segmentation algorithm were used to spatially normalize the structural image to the Montreal Neurological Institute (MNI) standard brain space (output voxel size: 1x1x1 $mm^3$). These parameters were also applied to the functional images to bring them into the same MNI space (output voxel size: 3x3x3 $mm^3$), after which they were spatially smoothed with a Gaussian kernel with a FWHM of 6 mm.

### 2.5. Measurement of ghrelin circulating levels

In all participants, overnight fasting blood samples were collected between 8 am and 10 am for the measurement of circulating ghrelin (total). For logistic reasons, blood samples could not be collected

on the same day as the fMRI assessment in 60% of study participants. On average, these samples were collected within the 4 weeks preceding the fMRI scan in the obese group, and within the week preceding the scan in the group of healthy controls. After clotting, samples were centrifuged (1000g, 10 min, 4°C) and sera were stored at -80°C until the assay. Serum total ghrelin concentration was determined by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer specifications (EZGRT-89K, Millipore, Billerica, Massachusetts). Sensitivity was 50 pg/mL and intra- and inter-assay variability were ± 1.26% and ± 7.81%, respectively.

### 2.6. Statistical analysis

Participant characteristics, including age, gender, BMI, education level, and circulating levels of ghrelin were compared between the two groups using two-sample *t* tests or Chi-square tests using MATLAB (Mathworks Inc., Sherborn, MA). Two subjects from the control group and 1 subject from the obese group did not properly follow instructions for the performance of the guessing task during the MRI acquisition. Therefore, only data related to ghrelin levels and anatomical brain images were used in those subjects.

Cerebral BOLD responses during the fMRI task were assessed using a general linear model approach similar to the one described by Reuter and collaborators (Reuter et al., 2005). Briefly, for each subject, we set up a linear model with two regressors of interest corresponding respectively to the differential "*gain vs. loss*" response to the outcome (each outcome period modeled as 2 second-blocks), and "*net score*" (modeled with 4 second-blocks, with a height tracking the current net gain), both time-locked to the moment when the outcome was revealed (i.e., when the cards were turned up). The two regressors were convolved with the SPM canonical hemodynamic response function. The motion parameters estimated during the realignment phase were added as potential confounds to the model, which also included an autoregressive component of order 1 plus white noise, to account for the presence of autocorrelation in the residuals, and a basis set of discrete cosine functions modeling a high-pass filter with a cut-off period of 240 sec. A random-effects group analysis was then performed using two-sample F-test for identification of any significant “gain *vs.* loss” statistical effect in any of the two groups (obese

and/or control subjects). A similar analysis, but using a one-sample t-test, was conducted to assess the effect of interest separately within the obese and the control groups. Finally, a two-sample t-test was performed to compare the effect between the groups. An *undirected search* (whole-brain analysis) was performed looking for significant voxels with a $p<0.05$ family wise error rate (FWER), corrected for the whole brain volume. For the between-group and single group cluster level inferences, cluster-forming thresholds for the single-voxel significance were set at $p<0.001$, while the cluster-level threshold was 0.05 FWER with a cluster size threshold of $_kE>214$ voxels. We wanted to explore the link between individual amplitudes of reward-related cerebral activity, ghrelin levels and choice times. Since the dlPFC turned out to be more activated in the obese group compared to controls, we focused on this region of interest (ROI), extracting the eigenvariates of parameter estimates of the "gain *vs*. loss" contrast using a bilateral spherical ROI of 6 mm radius around the peak of the group effect in dlPFC. The resulting values were then used to test the main and interaction effects between variables of interest: reward-related brain activation, levels of ghrelin and behavior in groups of healthy volunteers and obese subjects with ANCOVA analysis in MATLAB. We calculated the effect size and reported it as Cohen's *d* for between-group comparisons and the partial Eta Squared value, $\eta 2p$, for ANCOVA analysis.

## 3. RESULTS

### 3.1. Characteristics of study participants

As shown in **Table 2**, there was no significant difference between obese subjects and non-obese participants in terms of age, gender and education level. The average weight and BMI were respectively 114.7 kg and 41.2 $kg/m^2$ in obese subjects *versus* 60.5 kg and 21.8 $kg/m^2$ in non-obese controls (all $p < 0.0001$). Circulating ghrelin levels were significantly lower in obese subjects compared to non-obese controls (375.2±47.6 pg/mL vs. 811.6±87.8 pg/mL; $df = 28$; $p < 0.001$, for both parametric t-test and non-parametric Mann-Whitney test, Cohen's $d = 1.6$).

### 3.2. Neuroimaging results

As expected, the fMRI task elicited significant activations in the ventral striatum (left and right sides) (**Figure 2A**), the extrastriate visual cortex and the ventromedial prefrontal cortex (**Table 2**) in both groups. While the peak voxels in the left and right ventral striatum showed a greater response in obese subjects compared to controls (see bar plot in **Figure 2A**), the group difference was not significant when controlling for the FWER across the whole-brain, or when using a small-volume-correction for the two ventral striatum clusters. When looking at the reward-related activity separately for each group, the obese subjects showed a larger extent of activation compared to controls (**Table 2 and Figure 2B**). A direct statistical comparison of the reward-related effect in the two experimental groups showed a greater activation of the left and right dlPFC and the precuneus/posterior cingulate in obese subjects compared to controls (**Table 2 and Figure 3**).

### 3.3. Behavioral performance during the fMRI task

Choice times after one subsequent win (successful guess), CT1, and choice times after two or more subsequent successful guesses, CT2, were compared between the two groups. No significant difference was found in CT1 (0.96s ± 0.05s in obese subjects *vs.* 0.92s ± 0.07s in controls). In contrast, choice times after two or more successive winning trials, i.e. CT2, were significantly longer in obese subjects compared to controls (1.17s ±0.07s *vs.* 0.94s ± 0.05s, df = 25, $p<0.05$, Cohen's $d = 0.8$) (**Figure 4A)**.

### 3.4. Relationships among reward-related brain activity, behavioral performance and ghrelin levels

ANCOVAs were performed to test for main and interaction effects among the variables of interest; i.e., reward-related brain activation, levels of ghrelin and behavior (choice time, CT), in obese patients and controls. The first ANCOVA, performed on the individual values for the reward-related dlPFC contrasts of parameter estimates (Win-Lose) with group as factor and ghrelin levels as covariate, revealed a strong effect of group, which was not surprising given that the dlPFC ROI had been identified

from the Obese vs. Controls contrast of the fMRI group analysis. Our comparison of interest, however, revealed a significant interaction between groups and ghrelin levels (**Figure 4B**; $F(1, 23)=5.17$, $p=0.033$), indicating that reward-related dlPFC activation was more strongly related to lower ghrelin levels in the group of obese subjects than in control subjects. A similar pattern was identified with the ANCOVA performed on choice times, with group as factor and ghrelin levels as covariate. Here also the slope of decreasing choice times with increasing ghrelin levels was steeper in obese subjects compared to controls, indicating that slower choice behavior was more strongly related to reduced ghrelin levels in the group of obese subjects (**Figure 4C**; Group X Ghrelin interaction: $F(1,23)=5.26$, $p=0.031$). Finally, the ANCOVA performed on the choice times, with group as factor and the values of reward-related dlPFC contrast of parameter estimates as covariate, showed a robust slowing of choice times with increasing reward-related dlPFC activation (**Figure 4D**; covariate effect: $F(1,23)=9.39$, $p=0.005$), with no significant group differences ($F(1,23)=1.01$, $p=0.33$), or interaction effects ($F(1,23)=0.18$, $p=0.68$).

## 4. DISCUSSION

This study assessed cerebral activity and behavioral response during a guessing task to identify abnormalities in reward outcome-related processing in severely obese subjects. Reward-related activations in the ventral striatum and ventromedial prefrontal cortex were observed in both obese and non-obese subjects. In obese subjects, group-specific reward-related activations were found in the bilateral dlPFC and precuneus/posterior cingulate. In both obese and non-obese subjects, reward-related activation in the dlPFC correlated with slower choice times for repeated reward. However, significant differences between groups were found with respect to the association of ghrelin with dlPFC activation and reward-related behavior.

The comparable ventromedial prefrontal cortex activation during winning trials in obese and control subjects, and the slight obesity-related trend for hyperactivation in the ventral striatum were unexpected in view of the well-documented alterations in reward system function in obesity (Volkow et al., 2011). This result may be due to the nature of the paradigm/incentive stimuli (money-labeled gaming

reinforcement task) used in the present study. In support of this possibility, alterations in reward-related brain activity in obesity have been shown to be task-dependent (Volkow et al., 2011) and, while activity in the ventral striatum can be triggered by both monetary and food reward, BMI was found to correlate only with the anticipatory food-reward processing (Simon et al., 2014).

The guessing task paradigm used in the present study produced a significant hyperactivity in the precuneus/posterior cingulate and dlPFC in obese subjects, suggesting the recruitment of a more extended brain network for the processing of reward-related information in obesity. This hypothesis is in line with previous reports indicating greater connectivity between reward network brain regions and regions of executive control, emotional arousal, and somatosensory networks in obese individuals (Gupta and Bhatia, 2008). The increased activation of the precuneus, a region of the default mode, found in the group of obese subjects recruited in the present study is consistent with previous reports documenting increased bilateral functional connectivity in the precuneus (Kullmann et al., 2012), together with greater precuneus activity at baseline and in response to food and non-food reward-related cues and during response inhibition and error processing (Bohon, 2017; Hsu et al., 2017; Opel et al., 2015; Tregellas et al., 2011; Zhang et al., 2015) in obese samples. Similarly, greater dlPFC activity and deficits in executive function have been well documented in obesity (Gautier et al., 2000; Holsen et al., 2012; Gentier et al., 2013). Interestingly, in the present study, increased dlPFC activity correlated with slower choice times after repeated (but not single) preceding gains/reward. In view of the greater dlPFC activation in obese subjects, this finding may indicate a greater engagement of effortful, self-control executive processes in rewarded decision-making context (Danner et al., 2012; Gentier et al., 2013), consistent with the known link between greater dlPFC activity and greater task difficulty (Manoach et al., 1997) or high-risk choice (Bembich et al., 2014). In that perspective, the observed overactivation of brain regions related to self-focusing, i.e., precuneus, (Cavanna and Trimble, 2006) and self-control, i.e., dlPFC, (Belfort-DeAguiar et al., 2018) could reflect an increased demand for self-control in rewarding circumstances in obese subjects. A potential alternative explanation is that the occurrence of a string of reward episodes captures more attentional resources in obese subjects, and thus slows down subsequent choices (Kube et al., 2017).

Reward-related over-activation in the dlPFC was associated with reduced circulating levels of ghrelin, and this association was stronger in obese subjects. This finding may be interpreted as somewhat surprising in view of previous results indicating increased neural responses to food pictures in brain areas involved in reward processing after ghrelin administration in healthy volunteers (Malik et al., 2008). Nevertheless, it may rely on brain processes differentially involved in reward anticipation and receipt (Oldham et al., 2018). In support of this, prefrontal involvement has been reported specifically at reward receipt, and not during reward anticipation. In line with this, obese subjects were found to exhibit dlPFC hyperactivity in response to food images only after meal (Holsen et al., 2012), which is temporally better aligned with reward receipt than anticipation and consistent with results from the present study where dlPFC hyperactivity was measured during reward receipt (gain *vs* loss trials). This result is also in line with data indicating reduced dlPFC activation in patients with Prader-Willi syndrome, characterized by hyperphagia and hyperghrelinemia, and increased dlPFC activity in obese subjects with moderate overeating and low ghrelin (Belfort-DeAguiar et al., 2018; Holsen et al., 2012).

Similar to the relationship found with the dlPFC, decreased ghrelin levels were found to significantly correlate with slower choice times, particularly in the obese group. This finding is consistent with the role of the ghrelin system in reward and decision-making processes. Given the recent experimental evidence for a role of ghrelin in impulsive behavior/choice (Anderberg et al., 2016), it is possible that this association relies on the effects of the ghrelin system on the central/brain regulation of processes related to self-control. In line with this, higher fasting ghrelin levels were recently found to be associated with greater reward sensitivity and reduced self-control/increased impulsivity (Ralevski et al., 2018). Alternatively, this association may also reflect increased stress-related processes or greater worry of unsuccessful outcome in obese subjects given the associations previously documented between ghrelin and increased susceptibility/altered response to stress (Sarker et al., 2013; Spencer et al., 2012).

Albeit they strongly support the link between the ghrelin system and reward-related and decision-making processes in obesity, results from the present study do not provide any information regarding the temporality and causality of events nor their role in the development of overweight/obesity. While previous studies addressing reward processing alterations have shown that hedonically driven food intake,

along with stress-induced comfort eating, may contribute to weight gain and obesity maintenance (Burger and Berner, 2014; Lee and Dixon, 2017), further large-scale longitudinal studies are needed to specifically address this issue.

This study presents certain limitations that future investigations should also overcome. Due to logistic constraints inherent to clinical research on patients (ghrelin measurement was part of the routine preoperative evaluation of obese patients), ghrelin levels were not measured on the same day of the fMRI acquisition in 60% of the participants. While this limitation should not critically impact results from the present study, based on data from prospective studies showing stable fasting ghrelin levels with time in obese subjects (Hanusch-Enserer et al., 2004) and healthy volunteers (Leidy et al., 2004), future investigations with concomitant measurement of ghrelin and brain activation are needed to comfort the present findings. The exclusive measurement of total ghrelin, and not the active acylated form of the hormone, represents another limitation of this study. Because of its high instability at room temperature, the exact measurement of the active form of ghrelin is highly challenging and requires specific measures/procedures for blood collection and processing (Hosoda and Kangawa, 2004) that were not planned in the present study. While complementary measurements of the active form of ghrelin are highly encouraged in future studies, we believe that assessment of total ghrelin remains highly relevant in the context of the present study since it was shown to be highly sensitive and reliable in multiple studies of obesity. In particular, total ghrelin, was found to correlate with BMI and disease severity in most cases of obesity (except Prader-Willi Syndrome) and to be reduced in hyperphagia and obesity (Nonogaki, 2008; Monti et al., 2006). These associations were not found with acylated ghrelin that seems to be more relevant with respect to metabolic abnormalities, notably in normal subjects (Pacifico et al., 2009). Another aspect that would merit further investigation is the relationship between ghrelin and insulin and/or other neuroendocrine factors, given the known interactions between these different factors and their possible impact on reward signaling (Baicy et al., 2007; Farooqi et al., 2007; Kullmann et al., 2012). In support of this, strong relationships exist between the ghrelin system and the insulin system [e.g. ghrelin and its receptors are expressed within the pancreas and β-cells and ghrelin alters insulin-dependent glucose metabolism in a direct and non-direct manner (Churm et al., 2017)], which has been shown to be involved in the regulation of reward processing in preclinical and clinical studies (Eisenstein

et al., 2015; Kleinridders and Pothos, 2019). Finally, another limitation of the study comes from the use of an ROI (dlPFC) identified from the group Win-Lose contrast for subsequent correlational analyses of brain activation with ghrelin and behavior. In this situation, a complete statistical independence may not be assured nor the possibility of additional associations with other brain structures be excluded. While these issues merit further investigation in future studies, they should not substantially influence the findings given that the ROI defined by a between-group test was used for assessing within-group relationships.

In conclusion, the present findings indicate an increased activation of brain areas related to self-control and self-referencing during processing of non-food reward in obese subjects. Moreover, fasting levels of circulating ghrelin, the hormone regulating energy balance and food consuming behavior, were associated with generalized reward processing, both at the behavioral and neurobiological levels. The associations of ghrelin with decision times and dlPFC activity suggest the potential involvement of this hormone in the obesity-related behavioral alterations**.**

**Conflict of Interest.** The authors declare no conflict of interest in relation to the work described.

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## FIGURE LEGENDS

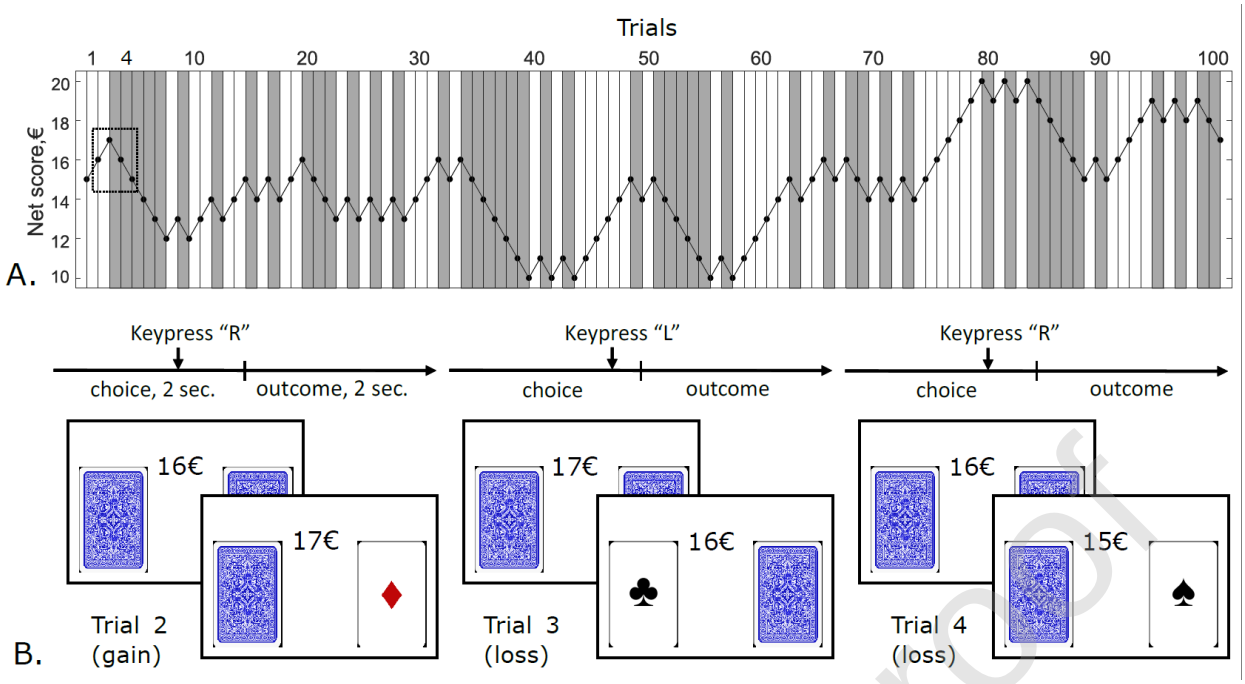


**Figure 1. Reward-task paradigm**

**Panel A:** Full timeline of the stimulus presentation design, which was identical across subjects**.** The net score is indicated by the noisy sinusoid, along with gain and loss trials (white and grey bars)

**Panel B:** Trial structure. Examples of trials 2-4 are shown. Red cards indicate gain, black cards indicate loss. Subjects could choose either left or right card, but the outcome in terms of win or loss was predefined (see **A**).

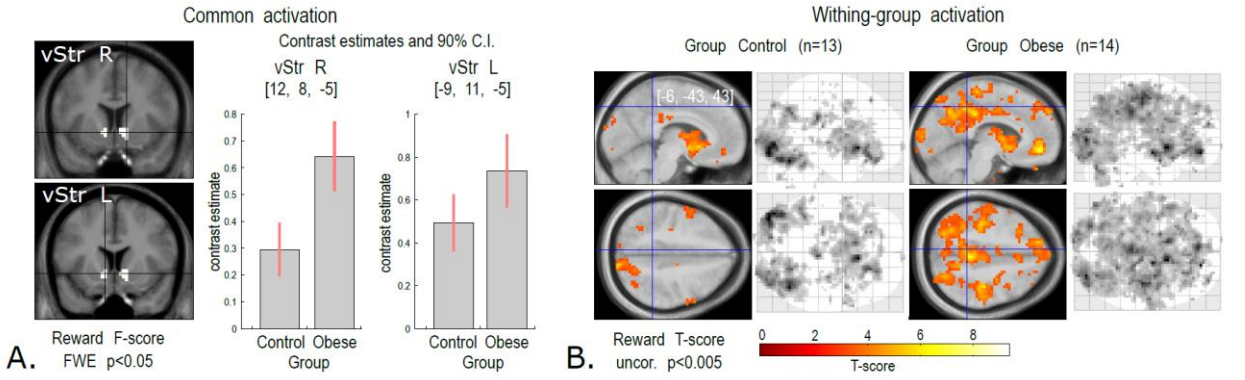


**Figure 2. Reward-related responses in obese subjects and non-obese controls**

**Panel A:** Reward-related response in the ventral striatum across both experimental groups, as the result of the two-sample (obese, N=14 or controls, N=13) "gain *vs.* loss" F-test.

**Panel B:** Group-wise reward-related activation. Note that the activation in obese subjects extends to precuneus/posterior cingulate and dorsolateral prefrontal cortex (dlPFC), unlike in non-obese controls (within-group activation).

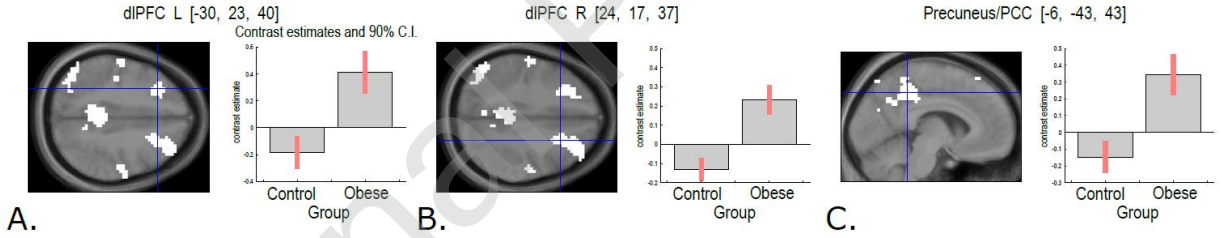


**Figure 3. Reward-related activity is greater in obese subjects**

The statistical contrast Obese (N=14) > Controls (N=13), thresholded for illustration purposes at cluster forming threshold p<0.005 is mapped onto the average of the individual subjects' normalized anatomical images (n=30). Significant clusters and respective peak contrast estimates are shown: (**A**) left dorsolateral prefrontal cortex, (**B**) right dorsolateral prefrontal cortex and (**C**) precuneus/posterior cingulate.

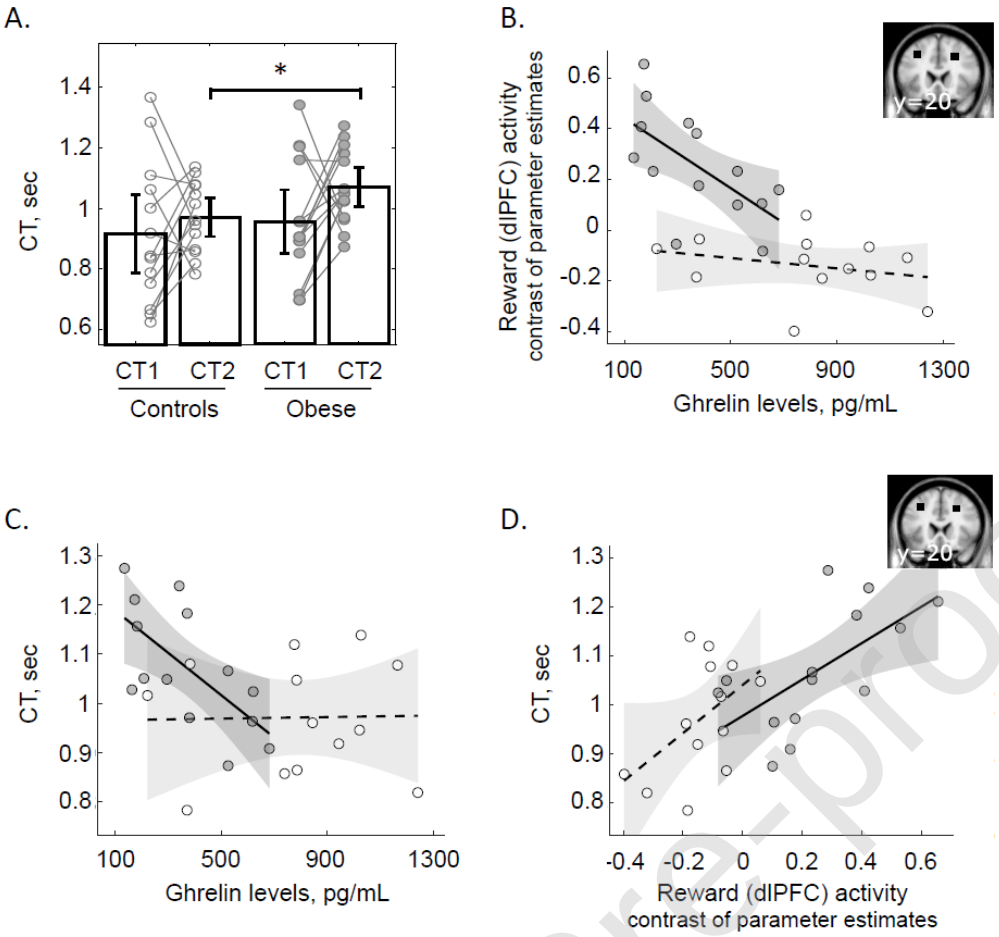


**Figure 4. Choice times and relationship with dorsolateral prefrontal cortex (dlPFC) activity and ghrelin levels in obese subjects and non-obese controls**

**Panel A**. Choice time (CT) group effect. A significant difference between groups was observed for choice time during repeated reward (CT2) but not during single reward (CT1); * $p<0.05$.

**Panel B**. Relationship between ghrelin levels and reward-related activity in the dlPFC. The parameter estimates of the "Gain vs. Loss" contrast were extracted from the bilateral dlPFC (i.e., the first eigenvariates of the individual contrast images within two spherical ROI of 6 mm radius centered on the peaks' coordinates of [24 17 37] and [-30 23 40] were extracted; as shown in a right upper corner). Main effects and interactions: Group: $F=14.6$, $p<0.001$, $\eta 2p=0.39$; Ghrelin: $F=9.4$, $p<0.01$, $\eta 2p=0.29$; Group x Ghrelin: $F=5.2$, $p<0.05$, $\eta 2p=0.18$.

**Panel C**. Relationship between choice time during repeated reward (CT2) and ghrelin levels. Corresponding CIs displayed as gray shadows. Main effects and interactions: Group: $F=5.9$, $p<0.05$, $\eta 2p=0.20$; Ghrelin: $F=4.9$, $p<0.05$, $\eta 2p=0.18$; Group x Ghrelin: $F=5.3$, $p<0.05$, $\eta 2p=0.19$.

**Panel D.** Relationship between choice time during repeated reward (CT2) and reward-related activity in the dlPFC. The parameter estimates of the "Gain vs. Loss" contrast were extracted from the bilateral dlPFC (i.e., the first eigenvariates of the individual contrast images within two spherical ROI of 6 mm radius centered on the peaks' coordinates of [24 17 37] and [-30 23 40] were extracted; as shown in a right upper corner). Main effect: dlPFC: $F=9.4$, $p<0.01$, $\eta 2p=0.29$.

○ control subjects, N=13 ● obese subjects, N=14

**TABLES**

**Table 1. Characteristics of study participants**

| | **Obese (N=15)** | **Normal-weight Controls (N=15)** | ***Difference P value*** |
|---|---|---|---|
| Age, years (SD) | 38.7 (9.4) | 37 (7.1) | 0.6 |
| Gender, men/women (%) | 3/12 (20, 80) | 2/13 (13.3, 86.7) | 0.6 |
| Body Mass Index (BMI), kg/m$^2$ | 41.2 (2.1) | 21.8 (2.6) | < 0.0001 |
| Weight, kg (SD) | 114.7 (9.1) | 60.5 (10) | < 0.0001 |
| Diploma[1] | 2.9 (0.96) | 3.4 (1.40) | 0.3 |
| Ghrelin levels, pg/mL (SD) | 375.2 (47.6) | 811.6 (87.8) | < 0.001 |

[1] Diploma was measured on a scale from 0 (no diploma) to 7 (PhD or MD).

Data are shown as mean (SD) or prevalence (%). The statistical significance of group differences (*p*-value) was assessed by Student t-tests (Chi-square test, for Gender).

**Table 2. Brain regions activated by the reward condition (winning trials > losing trials) in obese and non-obese controls**

| | | | Peak coordinates | | |
|---|---|---|---|---|---|
| **Brain Region** | **$k_E$** | **T-score** | **X** | **Y** | **Z** |
| ***Non-obese controls (cluster-forming threshold p<0.001)*** | | | | | |
| Ventral striatum | 317 | 9.65* | 9 | 17 | -5 |
| Orbitofrontal cortex | 107 | 7.40 | 21 | 29 | -14 |
| Extrastriate visual cortex (L) | 466 | 9.78* | -36 | -88 | -11 |
| Extrastriate visual cortex (L) | 40 | 6.73 | -39 | -46 | -23 |
| Extrastriate visual cortex (R) | 500 | 9.42* | 42 | -64 | -20 |
| Posterior cingulate cortex | 45 | 7.68 | 6 | -31 | 28 |
| Intraparietal sulcus | 76 | 6.94 | 21 | -61 | 34 |
| ***Obese subjects (cluster-forming threshold p<0.001)*** | | | | | |
| Ventral striatum + ventromedial prefrontal cortex + orbitofrontal cortex | 681 | 9.30* | 18 | 47 | -11 |
| Extrastriate visual cortex (R) | 720 | 8.78* | 30 | -88 | 4 |
| Extrastriate visual cortex (L) | 263 | 6.93 | -27 | -82 | -11 |
| S1(R) + precuneus + posterior cingulate cortex | 1516 | 7.84 | 39 | -22 | 52 |
| Intraparietal sulcus | 159 | 4.08 | -27 | -70 | 52 |
| S1(R) | 79 | 6.17 | 57 | -13 | 34 |
| Frontal operculum/premotor cortex | 93 | 4.01 | -42 | 2 | 13 |
| ***Obese subjects > non-obese controls (cluster-forming threshold p<0.001)*** | | | | | |
| Dorsolateral prefrontal cortex (R) | 81 | 6.28* | 24 | 17 | 37 |
| Dorsolateral prefrontal cortex (L) | 56 | 4.92 | -30 | 23 | 40 |
| Precuneus / posterior cingulate | 126 | 5.32 | -6 | -43 | 43 |

*Clusters where the T-score of the peak voxel is significant (FWER p<0.05, at the voxel level). The other clusters are significant at the cluster level, but not voxel level (FWER p<0.05, at the cluster level), $k_E$ is the cluster size in voxels. Obese subject, N=14; non-obese controls, N=13.