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Article

Ghrelin Modulates Brain Activity in Areas that Control Appetitive Behavior

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SUMMARY

Feeding behavior is often separated into homeostatic and hedonic components. Hedonic feeding, which can be triggered by visual or olfactory food cues, involves brain regions that play a role in reward and motivation, while homeostatic feeding is thought to be under the control of circulating hormones acting primarily on the hypothalamus. Ghrelin is a peptide hormone secreted by the gut that causes hunger and food consumption. Here, we show that ghrelin administered intravenously to healthy volunteers during functional magnetic resonance imaging increased the neural response to food pictures in regions of the brain, including the amygdala, orbitofrontal cortex, anterior insula, and striatum, implicated in encoding the incentive value of food cues. The effects of ghrelin on the amygdala and OFC response were correlated with self-rated hunger ratings. This demonstrates that metabolic signals such as ghrelin may favor food consumption by enhancing the hedonic and incentive responses to food-related cues.

INTRODUCTION

The presence of food, and the anticipation of pleasure it could provide, are potent triggers to feeding. This hedonic feeding behavior can be described as nonhomeostatic in that it occurs in the absence of nutritional or caloric deficiency. While nonhomeostatic feeding may have once provided an adaptive advantage to humans, in our plentiful environment it is likely a significant cause of obesity and its associated morbidity. Homeostatic feeding regulation mediated by the hypothalamus is well described (Saper et al., 2002); however, factors other than internal energy status also influence food intake. For instance, nutrient consumption is significantly influenced by external cues such as visual food stimuli. In animals, the behavioral response to such stimuli is mediated by specific neurons in the orbitofrontal cortex (OFC), amygdala, and striatum (Holland and Gallagher, 2004; Rolls, 1994), which form part of a mesolimbic reward system that is implicated in motivated behaviors (Cardinal et al., 2002). It has been suggested that while the hypothalamus primarily regulates the homeostatic drive to eat, these other neural circuits integrate environmental and emotional factors to control the "hedonic" drive. Nonetheless, to influence behavior, homeostatic signals may access reward-related brain areas.

Ghrelin is a 28 amino acid peptide synthesized in the gastrointestinal tract that acts as a homeostatic signal involved in the brain-gut regulation of feeding (Kojima et al., 1999). Ghrelin administration increases food intake and adiposity in animals (Nakazato et al., 2001; Tschop et al., 2000). The preprandial rise and postprandial fall in plasma ghrelin levels in humans suggest that it is a hunger signal that promotes meal initiation (Cummings et al., 2001). Administration of ghrelin to lean and obese subjects significantly increases energy consumed from a free-choice buffet, relative to placebo (Druce et al., 2005; Wren et al., 2001). Overall, acute and chronic nutritional states seem to influence endogenous levels of the peptide.

It is well established that ghrelin activates the hypothalamic NPY/AgRP orexigenic pathway (Nakazato et al., 2001), where ghrelin receptors are densely concentrated. However, ghrelin also has specific effects on many brain regions implicated in reward and motivation, including the ventral tegmental area (VTA), nucleus accumbens, amygdala, and hippocampus (Abizaid et al., 2006; Carlini et al., 2004; Diano et al., 2006). The VTA and hippocampus express ghrelin receptors (Zigman et al., 2006), and direct injections into these regions as well as the amygdala lead to measurable changes at the neuronal and behavioral levels. Hence, it is possible that, in addition to its role as a metabolic signal for nutrient intake, ghrelin may modulate the incentive and hedonic aspects of ingestive behavior.

Here, we present evidence that ghrelin influences the responsiveness of brain regions involved in processing food cues in humans. Using functional magnetic resonance imaging (fMRI), we measured the cerebral response to food and non-food (scenery) images following single-blinded ghrelin infusions (1 μg/kg) (Figure 1). Twenty nonobese subjects were tested 3 hr after ingestion of a standardized meal. Twelve subjects viewed pictures before and after ghrelin administration (control/ghrelin group), and eight subjects viewed the same pictures in two identical blocks without receiving ghrelin (control/control group). All subjects were told they might receive ghrelin. Ghrelin increased the response to food pictures in amygdala, OFC, insula, visual areas, and striatum. These regions encode the salience and the hedonic and incentive value of visual cues. This effect likely accounts for the ability of ghrelin to trigger and promote feeding.

RESULTS

Biochemical Data

All subjects had normal blood glucose prior to the scan. In the group that received ghrelin there was a significant increase in

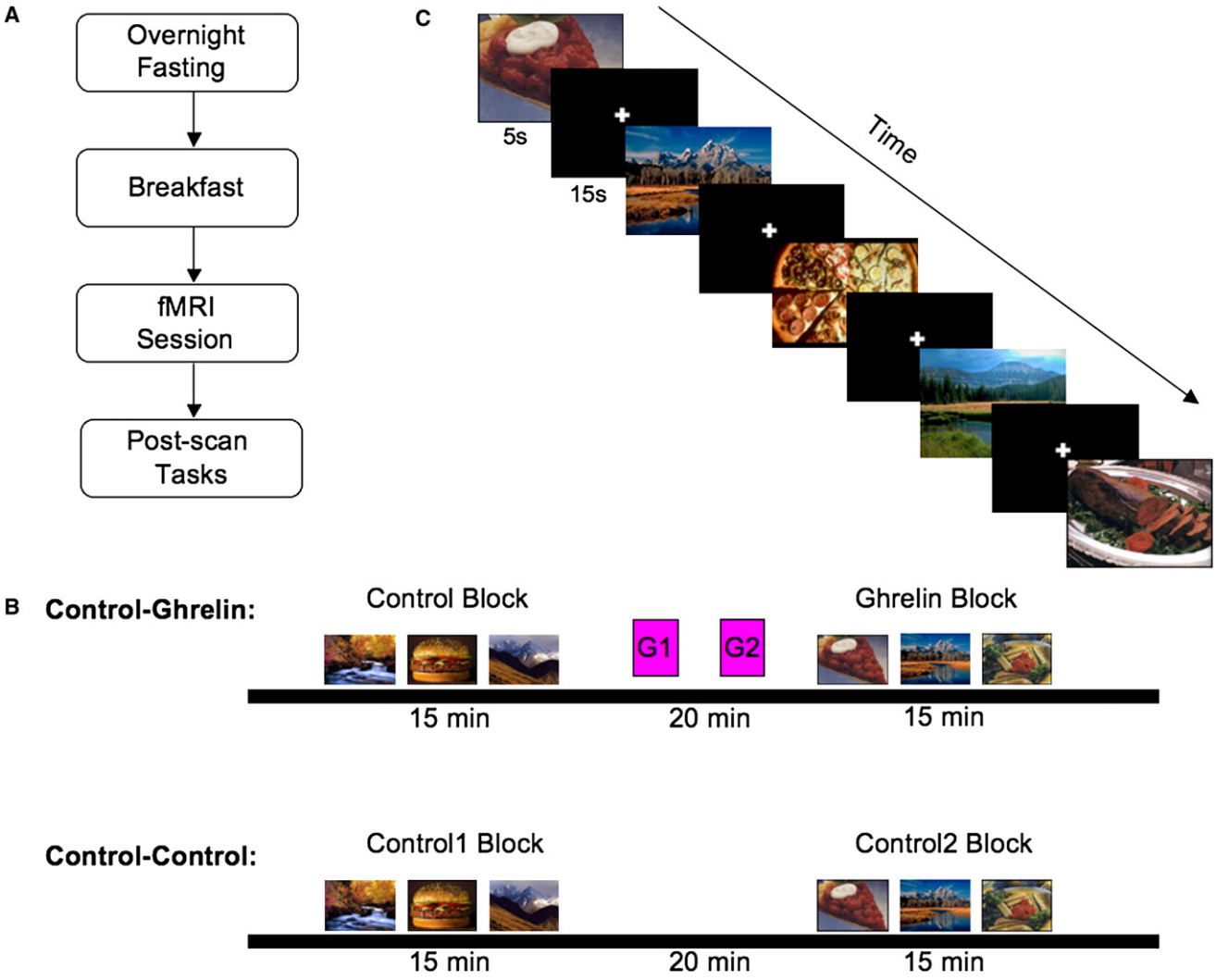

Figure 1. Overview of the Protocol

(A) The fMRI session was 3 hr post-breakfast.

(B) Three 5 min functional runs with images were presented during each of the two blocks. In the ghrelin study, two ghrelin infusions (G1 and G2, 0.5 μg/kg each over 1 min, 15 min apart) were administered between the blocks. Subjects did not know whether or when ghrelin would be administered via the intravenous. The control study was identical except that no ghrelin was administered. Visual analog scales (VAS) assessing mood and appetite were administered at four time points.

(C) Each run comprised 15 stimuli (half food, half scenes). Images were presented for 5 s followed by a 15 s fixation cross. Food and scenery images were presented randomly.

plasma growth hormone (pre-scan ± standard deviation [SD]: 1.0 ± 1.2 μg/l; post-scan: 62.7 ± 16.6 μg/l; $p < 0.001$), which is an expected consequence of the ghrelin infusions. In the control/control group there was also a significant increase in plasma growth hormone, but the effect was much smaller than in the ghrelin group (pre-scan: 0.14 ± 0.08 μg/l; post-scan: 4.99 ± 4.24 μg/l; $t = 3.02$, $p = 0.02$). Insulin levels did not change in either the control/ghrelin group (pre-scan: 37.0 ± 21.7 pmol/l; post-scan: 30.2 ± 16.2 pmol/l; $p = 0.24$) or the control/control group (pre-scan: 33.2 ± 12.30 pmol/l; post-scan: 23.5 ± 12.18 pmol/l; $t = 1.71$, $p = 0.13$).

Behavioral Data

In the control/ghrelin group there was a significant increase in the subjective ratings (mean ± standard error of the mean [SEM]) for hunger and borderline increases for irritable and nauseous in the ghrelin relative to the control condition (hunger: control: 5.5 ± 0.6, ghrelin: 8.3 ± 0.4, $t = 4.91$, $p < 0.001$; irritable: control: 3.0 ± 0.5, ghrelin: 4.2 ± 0.7, $t = 2.74$, $p = 0.02$; nauseous: control: 1.3 ± 0.5, ghrelin: 2.4 ± 0.8, $t = 2.68$, $p = 0.02$; bored: control: 4.1 ± 0.6, ghrelin: 5.1 ± 0.6, $t = 1.45$, $p = 0.18$).

In the control/control group, however, the subjective rating for hunger did not change between the two blocks (hunger: control 1: 5.9 ± 0.68, control 2: 6.4 ± 0.83, $t = 1.08$, $p = 0.32$). There were increases of borderline significance in the subjective ratings for irritable and bored in the second relative to the first block of images (irritable: control 1: 3.5 ± 0.83, control 2: 5.0 ± 1.22, $t = 2.38$, $p = 0.05$; bored: control 1: 4.9 ± 0.66, control 2: 6.5 ± 0.86, $t = 3.36$, $p = 0.01$). Nausea levels did not change (nauseous: control 1: 1.9 ± 0.63, control 2: 2.97 ± 0.94, $t = 1.44$, $p = 0.19$).

The food pictures were presented to the subjects a second time, after the scan outside the scanner. Food items presented in the ghrelin condition were more often recognized than those displayed in the control condition (mean ± SD: 88.8% ± 7.3% compared to 81.8% ± 10.8%, t = 2.90, p = 0.01). There was no difference in the hedonic rating of the pictures viewed in the ghrelin versus the control condition (t = 0.73, p = 0.48). Note that there was no measurement of hedonic rating at the time of scanning, however, so we cannot say whether ghrelin affected the perceived pleasantness of the food pictures during the scan.

In the control/control group there was no difference in the hedonic ratings of the pictures viewed in the two control conditions (t = 0.81, p = 0.45) nor any difference in the recognition of food items presented in the two control blocks (control 1: 84.1% ± 3.3%, control 2: 84.2% ± 5.3%, t = 0.026, p = 0.98).

Neuroimaging Data: Control/Ghrelin Group

Neural activation associated with food stimuli was examined via subtraction of the scenery response (Table 1). In both the control and ghrelin states, visual areas in the parietal and occipital cortex were activated. However, the amygdala (bilaterally), right hippocampus, and left pulvinar were more responsive to food than scenery pictures only during the ghrelin condition. The anterior and mid-dorsal insula were also activated bilaterally in the ghrelin condition (Figure 2, Table 1). Extraction of the blood oxygen level-dependent (BOLD) effect sizes from peak voxels identified in this contrast confirmed these findings (Figure 3). There was a statistically significant effect of ghrelin on the response in reward-related regions (bilateral amygdala, left OFC, right substantia nigra (SN)/VTA, left caudate, and right hippocampus), anterior insular cortex (bilateral mid-dorsal and ventral insula), and visual areas (including pulvinar and fusiform gyrus).

A t map of the food minus scenery contrast for all scans (ghrelin and control combined) was also generated. Significant activation was detected in bilateral caudolateral OFC, piriform cortex (olfactory area), and ventral pallidum, in addition to the aforementioned areas (Table 2).

To ensure that ghrelin did not alter the response to scenery pictures, scenery images were contrasted to the blank screen stimulus. Bilateral activation was observed in several occipital areas, namely the cuneus, fusiform, lingual, and middle occipital gyri, as well as in the pulvinar and parahippocampal gyrus. Activation was not different between the ghrelin and control conditions.

To determine whether the ghrelin effect observed here could play a role in promoting feeding we correlated the fMRI signal (the effect size from the general linear model) in the food minus scenery contrast with self-report measures. We found that the increase in activation due to ghrelin correlated with self-reported hunger during the ghrelin scans in bilateral amygdala, left OFC, and left pulvinar ($p < 0.05$, Figure 4). Ghrelin's effect on amygdala activation was also correlated with its effect on the left OFC ($p = 0.06$, Spearman's correlation) and the left pulvinar ($p = 0.03$, Figure 5). Finally, right insula activation correlated positively with recognition scores for the ghrelin pictures ($p = 0.05$).

Table 1. Food Minus Scenery Contrast for Ghrelin and Control Conditions

		Ghrelin				Control			
Region		t stat	x	y	z	t stat	x	y	z
Orbitofrontal cortex	L	3.82	−36	30	−6				
Inferior/middle frontal gyrus (6/44)	R	5.26	50	6	30	5.43	48	10	30
	L	4.75	−52	2	32	5.28	−44	4	32
Precentral gyrus	L	3.52	−50	4	4				
Amygdala	R	4.92	20	−10	−8				
	L	4.48	−18	−10	−16				
Hippocampus	R	4.13	32	−10	−30				
Insula (anterior)	R	4.24	42	8	−6				
	L	3.84	−34	16	10	4.2	−34	20	8
Insula (mid)	R	4.23	42	−6	10				
	L	5.92	−36	−12	14				
Caudate	L	3.59	−8	−2	12				
Cuneus	R	4.68	20	−100	4				
	L					4.77	−16	−100	−2
Fusiform gyrus	R	7.22	42	−70	−12	5.67	46	−72	−12
	L	8.64	−50	−66	−10	7.14	−34	−80	−14
Pulvinar	L	4.27	−16	−36	2				
Lingual gyrus	R	5.18	18	−98	−4				
	L	3.6	−10	−96	−12				
Inferior parietal lobule	L	5.36	−42	−48	58	6.21	−46	−38	50
Middle occipital gyrus	R	7.3	32	−90	6	4.91	38	−84	2
	L	6.05	−26	−92	14	6.85	−48	−74	−6
Superior occipital gyrus	L	5.53	−26	−76	30				
Superior parietal lobule	R	4.9	28	−58	56	5.4	32	−62	56
	L	6.36	−20	−66	48	5.56	−32	−58	56

All peaks listed at $p < 0.001$ uncorrected with a minimum cluster extent of 100 mm^3. For the visual areas the extent of activation was quite large. When there is more than one peak within one functional region, only the most statistically significant peak is listed. The x, y, z refer to the coordinates in Montreal Neurological Institute space.

Neuroimaging Data: Control/Control Group

In order to explore the possibility that the results in the group that received ghrelin were due to order effects, we subsequently recruited an additional eight subjects who underwent the same paradigm with the exception that they only received normal saline rather than ghrelin. The two scanning blocks are referred to as control 1 and control 2. Neural activation associated with food stimuli was examined via subtraction of the scenery response. Regions belonging to significant clusters ($p < 0.05$, corrected for multiple comparisons) were identified. In control 2, several visual areas including the bilateral fusiform and occipital gyrus and left inferior parietal lobule were activated. Only the left inferior occipital gyrus was activated in the control 1.

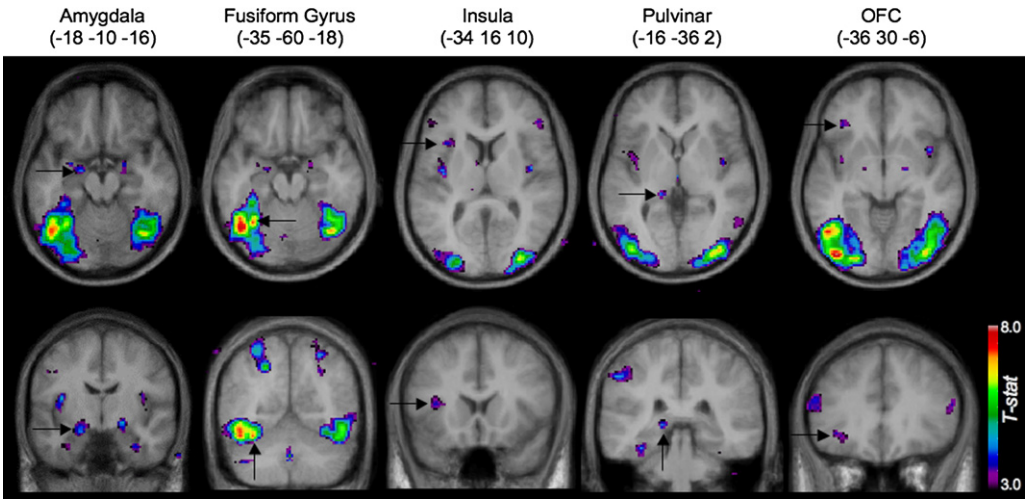

Figure 2. Statistical Maps

Representative t maps for amygdala, fusiform gyrus, insula, pulvinar, and OFC regions. All images are from the food minus scenery contrast, ghrelin condition (Table 1). The t maps are thresholded at t > 3. Arrows indicate the peak locations for each region.

Importantly, no significant activation in the amygdala, insula, pulvinar, hippocampus, caudate, or OFC was observed in either control conditions, even when lowering the threshold to t = 2.5 (p = 0.005 uncorrected). Extraction of BOLD effect sizes using peak voxel coordinates identified in the control/ghrelin group confirmed that there was no difference in the neural activation between controls 1 and 2 in the aforementioned regions (all p > 0.1). A t map of the food minus scenery contrast for all scans (control 1 and control 2 combined) was also generated. Activation was detected in visual areas, including bilateral fusiform gyrus, and left insula (Table S2 available online). The peak voxel coordinates observed in the fusiform gyrus and insula were also used to extract the BOLD signal effect sizes in each of the two control conditions. Again, paired t tests showed no difference between the two blocks (Figure S3), confirming that the effects observed in the ghrelin group were not due to the order of conditions.

Finally, we generated a t map of the interaction effect between the ghrelin/control group and the control/control group. There was a significantly greater increase in BOLD response (food minus scenery) in the control/ghrelin than the control/control group in the OFC, bilateral anterior insula, left mid-insula, left pulvinar, right SN/VTA, and bilateral fusiform (Table S3). There were no areas showing a greater increase in the control 2 minus control 1 blocks than in the ghrelin minus control blocks.

DISCUSSION

The cerebral response to food cues following ghrelin administration was increased in multiple areas, including the amygdala, insula, OFC, and striatum, implicated in reward processing and appetitive behavior (Figure 3, Table 1). Moreover, self-reports of hunger were significantly increased in the ghrelin versus the control condition and correlated positively with the ghrelin-induced increase in cerebral activity in the amygdala, OFC, and pulvinar (Figure 4). Finally, food pictures shown in the ghrelin condition were more easily recalled than those shown in the control condition. Importantly, these neural and behavioral changes were not observed in the double control experiment.

The brain regions reactive to ghrelin in this investigation play a role in the hedonic and incentive evaluation of visual stimuli. The amygdala is responsive to most biologically relevant stimuli and is crucially involved in the coordination of appetitive behaviors (Baxter and Murray, 2002; Cardinal et al., 2002; Holland and Gallagher, 2004). It is thought that the amygdala signals the current hedonic value of a stimulus or object, via interactions with the OFC (Holland and Gallagher, 2004), and that it increases the salience of biologically relevant stimuli by interacting with posterior visual areas (LaBar et al., 2001), such as the pulvinar and fusiform gyrus. Consistent with this model, we found that ghrelin's effect on left amygdala activation correlated with its effect on left OFC and left pulvinar activation (Figure 5).

Numerous studies in animals have shown that activity in amygdala and OFC signals the current appetitive value of a food or food cue (Baxter and Murray, 2002; Holland and Gallagher, 2004). Human imaging studies have confirmed this. When the hedonic/motivational value of an olfactory or visual cue is modulated using pleasant or unpleasant verbal labels (de Araujo et al., 2005), or by feeding an associated food to satiety (Gottfried et al., 2003), activity in amygdala and OFC, at coordinates close to the ones reported here, varies with pleasantness. The response of the OFC to food ingestion also decreases as a food is fed to satiety and its pleasantness decreases (Kringelbach et al., 2003; Small et al., 2001). The OFC and amygdala also mediate the anticipation and receipt of a taste reward (O'Doherty et al., 2002) and are additionally involved in the hunger-enhanced memory of food cues (Morris and Dolan, 2001). Correlated increases in the activity in the OFC and amygdala would therefore be expected in conjunction with an increase in hunger, as demonstrated here (Figure 4), and presumably food consumption.

The anterior insula was also ghrelin responsive. This structure, lying beneath the frontal operculum, includes the primary

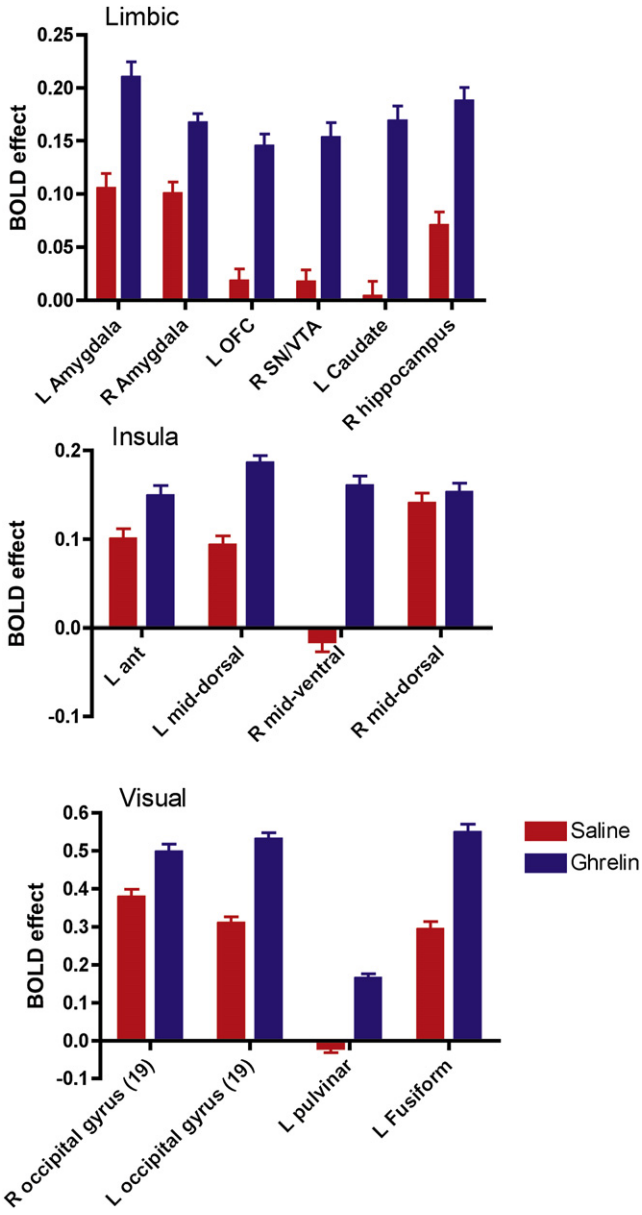

Figure 3. Ghrelin Effect

Bar graph showing the BOLD effect (parameter estimates from the general linear model of food pictures minus scenery pictures) in the ghrelin and control conditions for different regions identified in the categorical analysis. Error bars represent the SD of the general linear model. All comparisons show a significant effect of ghrelin ($p < 0.0001$, two-tailed), except for R occipital gyrus ($p = 0.0006$) and R mid-dorsal insula (not significant). Abbreviations and MNI coordinates: amygdala (right: 20, −10, −8; left: −18, −10, −16); OFC: orbitofrontal cortex (−36, 30, −6); SN/VTA: substantia nigra, ventral tegmental area (8, −16, −10); caudate (−8, −2, 12); hippocampus (32, −10, −30); Ins: insula (left anterior: −34, 16, 10; left mid-dorsal: −36, −12, 14; right mid-ventral: 42, 8, −6; right mid-dorsal: 42, −6, 10); occipital gyrus (right: 40, −67, −15; left: −51, −66, −10); left pulvinar (−16, −36, 2); left fusiform (−35, −60, −18).

Table 2. Food Minus Scenery Contrast: Ghrelin and Control Conditions Combined

Region		t stat	x	y	z
DLPFC	L	5.1	−52	34	18
DLPFC	R	3.89	54	30	18
OFC*	L	4.23	−28	30	−10
OFC*	R	3.81	25	28	−12
Insula (anterior)	L	5.31	−34	20	8
Inferior frontal gyrus	R	6.39	48	8	30
Medial frontal gyrus	L	4.13	0	8	54
Piriform cortex*	R	4.24	34	6	−14
Cingulate gyrus	L	4.71	0	4	40
Inferior frontal gyrus	L	6.38	−46	4	32
Insula (mid-dorsal)	R	5.13	42	−6	10
Precentral gyrus	L	4.42	−56	−6	42
Ventral pallidum	R	4.77	18	−10	−8
Ventral pallidum*	L	4.42	−24	−12	−10
Parahippocampal gyrus	R	4.55	36	−28	−22
Inferior parietal lobule	L	6.7	−46	−38	50
Inferior parietal lobule	R	4.15	32	−44	44
Superior parietal lobule	L	7.33	−32	−60	58
Superior parietal lobule	R	6.79	28	−60	56
Superior parietal lobule	L	6.29	−22	−66	50
Fusiform gyrus	R	8.85	38	−68	−14
Middle occipital gyrus	L	10	−50	−70	−10
Middle occipital gyrus	R	7.81	44	−74	−10
Middle occipital gyrus	L	5.03	−28	−74	28
Superior occipital gyrus	R	3.41	30	−80	26
Inferior occipital gyrus	R	8.25	40	−82	−8
Inferior occipital gyrus	L	11.22	−40	−86	−8
Inferior occipital gyrus	R	7.89	32	−90	0
Inferior occipital gyrus	L	7.13	−28	−92	12
Inferior occipital gyrus	L	7.95	−34	−94	0
Cuneus	R	5.13	24	−98	2
Lingual gyrus	L	5.08	−16	−98	−8
Lingual gyrus	R	5.03	16	−98	−2
Cuneus	L	4.86	−18	−100	−2

All $p < 0.05$ corrected except *$p < 0.001$ with a cluster size > 100 mm^3. BA: Brodmann area. OFC: orbitofrontal cortex. DLPFC: dorsolateral prefrontal cortex.

gustatory and visceral sensory cortex and participates in several feeding-related functions (Scott and Plata-Salaman, 1999). In human imaging studies the insula responds to the taste of food (O'Doherty et al., 2002; Small et al., 2001) and to visual cues such as food pictures (LaBar et al., 2001; Simmons et al., 2005; St-Onge et al., 2005) and restaurant menus (Hinton et al., 2004), and this response varies with the subject's desire to eat (Hinton et al., 2004; Small et al., 2001; Tataranni et al., 1999). Experiments with insula-lesioned rats show that the insular cortex functions in recalling changes in incentive value based on motivational state (Balleine and Dickinson, 2000). Therefore, like the amygdala and OFC, the anterior insula is involved in anticipation of food rewards and hedonic evaluation of food stimuli. The role of the anterior insula in incentive memory could account for the positive correlation between ghrelin-induced insular activation and subsequent recognition scores for food pictures in our study.

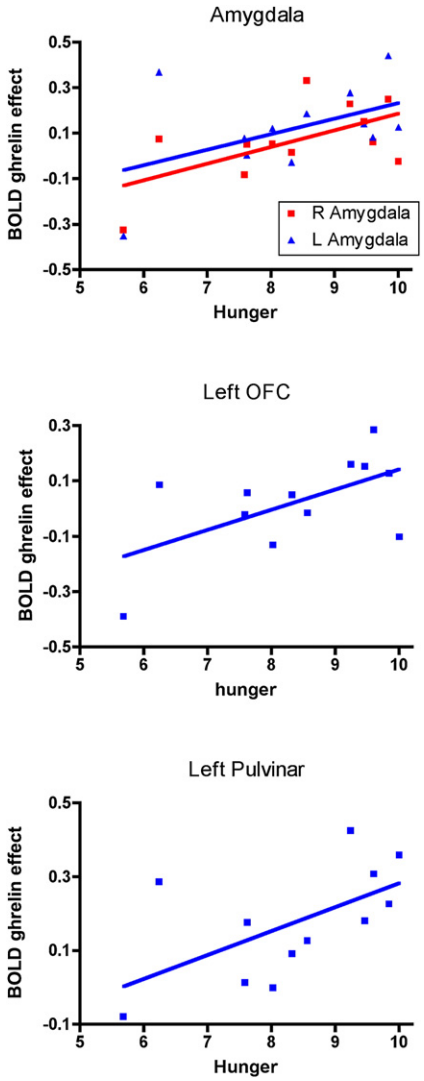

Figure 4. Hunger Effect

Correlation between mean self-rating of hunger during the ghrelin scans and the change in BOLD effect due to ghrelin (i.e., difference in parameter estimates of food minus scenery for the ghrelin and control scans at the peak voxel of this region). All regressions are $p < 0.05$ except for left amygdala ($p = 0.12$).

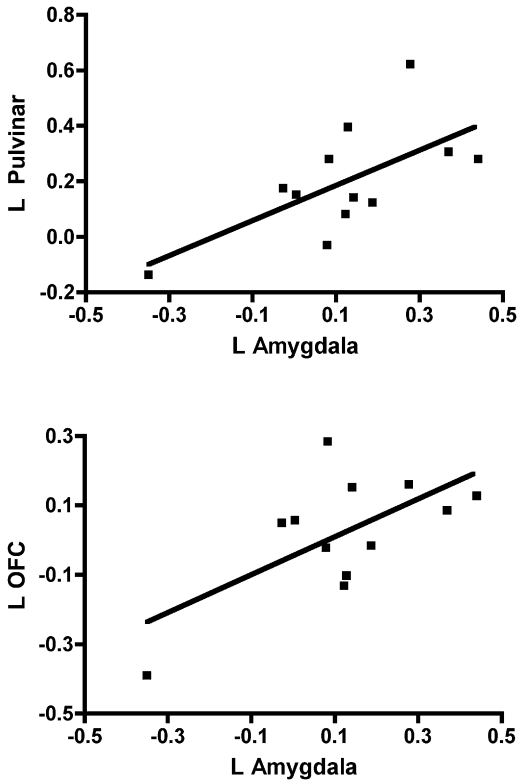

Figure 5. Correlations with Amygdala

Change in food minus scenery effect size (ghrelin minus control) for the left amygdala (x axis) and the left pulvinar and left OFC. Data were extracted from the peak coordinates in the subtraction analyses. Correlations were assessed using Spearman's rho. The p values for the correlations were 0.03 (pulvinar) and 0.06 (OFC).

Ghrelin also increased the response to food pictures of brain areas involved in visual processing, attention, and memory. The pulvinar and fusiform gyrus are specifically involved in focused visual attention (Kastner et al., 2004; Petersen et al., 1985; Vuilleumier and Driver, 2007), and fMRI experiments show that the increased salience of behaviorally relevant or emotionally arousing visual stimuli is mediated by an interaction of amygdala, fusiform, and pulvinar (Vuilleumier and Driver, 2007; Morris et al., 1997). We also found a ghrelin effect on the hippocampus, a structure that, along with the amygdala, is well known to be involved in memory formation (LaBar and Cabeza, 2006; McGaugh, 2004). Previous fMRI studies have shown activation of these two regions in response to food cues during the hunger state (LaBar et al., 2001; Morris and Dolan, 2001; St-Onge et al., 2005). Moreover, in animals, ghrelin regulates hippocampal spine synapse density and long-term potentiation (Diano et al., 2006) and enhances spatial learning and memory (Carlini et al., 2002, 2004).

Finally, two dopaminergic regions, the striatum and SN/VTA, were also modulated by ghrelin. These form the core of a reward network involved in the processing of feeding-related stimuli (O'Doherty et al., 2002; Small et al., 2001, 2003; Tataranni et al., 1999; Volkow et al., 2002) and setting the motivational or incentive properties of food cues (Berridge and Robinson, 1998). Local injections of ghrelin into the rodent VTA promote locomotor activity, striatal dopamine release, and feeding (Abizaid et al., 2006; Jerlhag et al., 2007), while systemically administered ghrelin causes VTA dopamine neuron firing and simultaneous feeding behavior (Abizaid et al., 2006).

Ghrelin therefore appears to modulate the response to food cues of a neural network involved in the regulation of feeding and, most importantly, in the appetitive response to food cues. This appetitive response has several components: attention, anticipation of pleasure, motivation to eat (hunger), consumption, and memory for associated cues. Brain regions implicated in all of these functions were modulated by ghrelin. How ghrelin acts on the brain is not known, but several potential mechanisms have been identified. First, peripheral ghrelin may act on ghrelin receptors in the gut, which then relay information to the brain via the vagus nerve (Date et al., 2002), although this pathway is not

necessary since total vagal deafferentation does not abolish the orexigenic effects of peripherally administered ghrelin (Arnold et al., 2006). This suggests that circulating ghrelin also acts directly on the brain. A likely region mediating this effect is the hypothalamus, where ghrelin increases the firing rate of NPY/AgRP neurons in the arcuate nucleus (Nakazato et al., 2001). These neurons in turn project directly and indirectly to the VTA and amygdala (Kelley, 2004; Saper et al., 2002), where they act to regulate feeding behavior. Circulating ghrelin may also act directly on the dopamine system. There are ghrelin receptors in the VTA (Zigman et al., 2006) and peripheral ghrelin increases VTA dopamine neuron firing, an effect that is blocked by intra-VTA administration of a ghrelin receptor antagonist (Abizaid et al., 2006). Abizaid et al. also provide evidence that ghrelin increases the VTA response to appetitive stimuli. The effect of ghrelin on the amygdala could be direct, as the amygdala contains ghrelin-positive axon terminals (Cowley et al., 2003), or indirect via the hypothalamus or the VTA, which sends dopaminergic projections to the amygdala (Moore and Bloom, 1978). Note, however, that direct injection of ghrelin into the amygdala failed to increase food intake in one study (Carlini et al., 2004). Finally, the anatomical distribution of ghrelin receptors on presynaptic sites suggests that the hormone acts mostly as a neuromodulator, enhancing the response of neurons that control feeding (Cowley et al., 2003). Thus, while ghrelin itself may not directly initiate feeding, it likely enhances the appetitive response to food cues, as shown here.

We describe the effects of an orexigenic hormone, but two recent fMRI studies have examined hormones that reduce food intake. Leptin, when administered to two young individuals with congenital leptin deficiency, reduced the neural response to food pictures in the ventral striatum (Farooqi et al., 2007), an area associated with reward processing. We did not find a ventral striatal response to food pictures in our study, although two functionally related regions, the SN/VTA and dorsal striatum, were sensitive to ghrelin. Note that our results are not inconsistent with those of Farooqi et al. since our subjects presumably had normal leptin levels, which appear to suppress the ventral striatal response to food pictures. Indeed, other fMRI studies have similarly failed to show ventral striatal activation in response to food pictures in healthy subjects (LaBar et al., 2001; Simmons et al., 2005). Another study measured the brain response to an infusion of PYY (Batterham et al., 2007), which is anorexic when administered systemically. Despite the differences in experimental paradigms, there was considerable overlap between the regions identified in that study and ours, possibly because PYY and ghrelin act on the same hypothalamic neurons (although with opposite effects). The left caudolateral OFC, SN/VTA, and left insula all showed a modulatory effect of PYY infusion.

Our results can also be compared to findings in Prader-Willi syndrome, a condition characterized by obesity, severe hyperphagia, and persistent elevations in ghrelin levels. In an fMRI study, comparison of Prader-Willi patients to lean control subjects demonstrated an abnormally elevated response to food pictures, following a meal, in the amygdala, OFC, insula, parahippocampal gyrus, and fusiform (Holsen et al., 2006). Our results suggest that this represents an effect of ghrelin, which remains elevated after eating in these patients.

A few limitations must be addressed. First, it was not possible to counterbalance the control and ghrelin conditions, as ghrelin administered during the first block would have still had effects during a subsequent control block. We therefore performed a control experiment (control/control group) to confirm that the effects attributed to ghrelin were not merely due to scan order. This second group of subjects was recruited after the first study was completed, and their data were analyzed separately; however, the same scanner and analysis software were used. We also provide data from a separate experiment that did not have the potential confounding effect of order and that confirms our findings (see Supplemental Data).

Second, we failed to see any hypothalamic activation in our imaging data. The hypothalamus is densely populated with ghrelin receptors (Howard et al., 1996) and plays a crucial role in ghrelin-induced feeding behavior (Nakazato et al., 2001). It is possible that its small size may have impeded the detection of a change in BOLD signal. Note, however, that our study identified brain regions responding to food pictures. It is very likely that the hypothalamus affects the response of other brain areas to food pictures without itself displaying a change in neuronal firing when subjects view the pictures. There are also intrinsic limits to the fMRI method that must be taken into account. The spatial resolution of roughly 6 mm does not permit us to identify the specific nuclei of the amygdala modulated by ghrelin. Moreover, signal dropout in the medial OFC means that we cannot exclude an effect in this region. Indeed, a study using positron emission tomography, which does not suffer from signal loss in the OFC, demonstrated that a large part of the medial OFC was involved in the appetitive response to chocolate ingestion (Small et al., 2001), along with the other regions identified in the current study. We may therefore have underestimated the spatial extent of the ghrelin effect in the OFC. Although we attribute the effects measured here to ghrelin, it is important to note that ghrelin causes increased secretion of growth hormone, ACTH, cortisol, and prolactin (Arvat et al., 2001), all of which may also act on the brain. Finally, since only males were included in this investigation, comparable studies in females must be pursued as there may be gender differences in food-related neural processing (Uher et al., 2006).

EXPERIMENTAL PROCEDURES

Materials

Pharmaceutical-grade human ghrelin ($C_{149}H_{249}N_{47}O_{42}$, molecular weight (MW) = 3370.9) was purchased from CLINALFA, a subsidiary of Merck Biosciences AG (Laufelfingen, Switzerland). Manufactured according to GMP regulations, the peptide was sterile and pyrogen free. The hormone was lyophilized in individual 100 μg glass vials and intended for intravenous infusion to human subjects.

Subjects

Twenty healthy medication-free normal weight male subjects were recruited. Twelve subjects participated in the control/ghrelin part of the study (mean age ± SEM, 24.1 years ± 1.1; body mass index, 22.2 ± 0.5). Eight took part in the control/control study in which no ghrelin was administered (mean age, 23.2 yrs ± 1.3; body mass index, 22.3 ± 0.7). All were right-handed with normal or corrected-to-normal vision. Exclusion criteria included one or more of the following: history of neurologic or psychiatric illness, body mass index > 25.9 or < 19, tobacco use, diabetes, gastrointestinal or eating disorders, food allergies, vegetarianism, and/or contraindications for MRI scanning.

The Dutch Eating Behavior Questionnaire (Van Strien et al., 1986), the Three Factor Eating Questionnaire (Stunkard and Messick, 1985), the Eating Attitudes Test (Garner et al., 1991), and the eating-related section of the Structured Clinical Interview for DSM-IV Screening Module (First et al., 1995) were used to exclude potential subjects with abnormal eating behavior. This research protocol was approved by the Montreal Neurological Institute Research Ethics Board as well as by the Therapeutic Products Directorate of the Canadian government. Prior to the experiment, subjects were given a description of the paradigm and provided written informed consent.

Experimental Paradigm

All subjects underwent a single fMRI session at the Montreal Neurological Institute. On testing day, participants ate a standard test breakfast provided by us (125 ml orange juice, 42 g cheddar cheese, 2 slices toasted bread: 1 white and 1 whole wheat, 15 ml strawberry jam, 10 ml butter, 1 cup coffee with 20 ml 2% milk and 1 sachet white sugar) following a 12 hr overnight fast. Breakfast was taken at either 8 a.m. (n = 10) or 10 a.m. (n = 10), in our cafeteria, accompanied by one of the investigators. All subjects consumed the entire breakfast and finished eating within 30 min. Visual analog scales rating hunger and mood were completed both before and after breakfast.

The imaging study was initiated 3 hr after the standardized breakfast to ensure that subjects were neither full nor hungry and lasted approximately 65 min. Ghrelin levels are at a nadir at this time (Cummings et al., 2001). Prior to subjects' placement in the scanner, an intravenous catheter was inserted into a left forearm vein and kept permeable with a slow infusion of normal saline. Following a high-resolution structural scan, the functional scanning began. The functional protocol was divided into two blocks (Figure 1). The first block entailed three 5 min functional runs (runs 1–3). During each run, 15 images (7–8 food, 7–8 scenery) were presented in random order. Subjects were instructed to focus their attention on the stimuli. Each picture was shown for 5 s followed by a 15 s dark screen with a central fixation cross. A total of 45 images were displayed (22 food, 23 scenery). At the start and end of the block, subjects answered questions regarding their mood and appetite (e.g., how hungry are you right now?) on a 10 point visual analog scale. Responses were recorded using an MRI-compatible mouse-like device. Images and questions were displayed on a projector screen using Presentation software (version 9.60, Neurobehavioral Systems, CA, USA). Food and scenery pictures had been previously matched for visual appeal. The mean pleasantness ratings on a scale of 1–9 were, for food, 6.54 (SD: 1.55) and, for scenery, 6.57 (SD: 1.48).

Following the first image acquisition block there was a 20 min period for ghrelin infusion during which no stimuli were presented. Subjects in the control/ghrelin group received two ghrelin infusions (0.5 μg/kg in normal saline infused over 60 s each time) approximately 13 min apart, in single-blinded fashion. Subjects in the control/control group did not. Prior to scanning subjects had been told they might or might not receive ghrelin during the scan but not when this would occur if it did. The ghrelin was administered via the intravenous tubing from outside the scanner by an investigator who was not visible to the subjects. There was no change in the flow rate or temperature of the intravenous solution during ghrelin infusion.

The second block was identical to the first, consisting of three 5 min functional runs, except that different stimuli were used (23 food, 22 scenery). Questions regarding mood and appetite were again administered at the beginning and end of the block. All subjects viewed the same set of images. Pictures were presented in random order and no stimuli were repeated. Blood samples were collected just before the scanning started and as soon as it ended to quantify glucose, insulin, and growth hormone levels.

Finally, two post-scan tasks were administered to the subjects on a personal computer approximately 30 min later. First, subjects were shown all 45 food images that they had viewed in the scanner intermixed with 26 novel food images and were asked to state whether or not they had seen each image while in the scanner. This recognition task was performed to ensure that subjects were paying attention to the images during the scan. Second, they were asked to rate the images on a scale of 1 to 9 (1 = "extremely dislike" and 9 = "extremely like").

Imaging Parameters

Functional imaging data were acquired on a 1.5T Siemens Vision MRI scanner equipped with a quadrature radiofrequency head coil. Head motion was minimized with a vacuum cushion. First, high-resolution T1-weighted anatomical images were obtained. Thereafter, T2* weighted images with BOLD contrast were acquired. Thirty-two 4 mm thick slices that covered the whole brain were collected using the following parameters: T_R: 3 s, T_E: 40 ms, FOV: 256 mm, flip angle: 90°, and voxel size: 4 × 4 × 4 mm. The functional session consisted of six runs of 5 min (three control and three ghrelin, or three control 1 and three control 2), each consisting of 100 volumes per run. Food and scenery pictures were projected onto a screen in the scanner room and viewed through a mirror mounted on the head coil. Scanning time and stimulus presentation were synchronized by a trigger signal from the scanner at the beginning of every run. Two dummy images were taken at the onset of each sequence and discarded to reduce non-steady state effects.

Data Analysis

Functional images were spatially smoothed with a 6 mm Gaussian filter and motion corrected prior to statistical analyses. A general linear model was designed using separate regressors for food and scenery pictures, consisting of boxcar functions convolved with a standard hemodynamic response function. Regional brain activation was determined by calculating a contrast of food minus scenery and computing effect and standard deviation at each brain voxel for each individual. These parametric images were transformed into Montreal Neurological Institute space (Collins et al., 1994) and a group analysis was performed using a mixed effects statistical model. The software package *fmristat* was used to conduct the statistical analysis (Worsley et al., 2002). The basic method is to calculate a t statistic from the effect size and standard deviation of the general linear model for each individual. The t value at each voxel is a measure of the likelihood that there was greater BOLD signal in response to the food than the scenery pictures at that location in the brain. Thus a t map is generated. This map is then thresholded in order to correct for multiple comparisons based on the search volume (the entire brain), the amount of smoothing applied, and the degrees of freedom. Here we corrected for multiple comparisons by only listing brain regions containing clusters of voxels with $p < 0.001$ and a volume greater than 100 ml. This effectively reduces the risk of false positives to less than 1 in 20 (i.e., $p < 0.05$) for the experiment. Significant peaks are listed in the tables along with the t values and the locations of the peaks, expressed in Montreal Neurological Institute coordinates based on the stereotaxic atlas of Talairach and Tournoux (Talairach and Tournoux, 1988).

To confirm the significance of the ghrelin effect we performed an analysis of the interaction between group and condition. We did this by generating a t map of the following effect: ([ghrelin − control] − [control 2 − control 1]). Finally, we also created a t map of the response to scenery pictures minus the response to the blank screen to ensure that ghrelin did not have a nonspecific effect on attention or arousal. We compared activation to the scenery pictures in the ghrelin and control states.

The effect sizes from the general linear model were also extracted from the peak voxels of areas of significant activation to food pictures, so that the ghrelin and control conditions could be compared and correlated with behavioral data. Behavioral and hormonal data were analyzed using SPSS (SPSS Inc., IL, USA). A paired t test was used to compare these measures in the ghrelin and control conditions.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures, three figures, and three tables and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/7/5/400/DC1/.

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