

ORIGINAL ARTICLE

Differences in response to food stimuli in a rat model of obesity: in-vivo assessment of brain glucose metabolism

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Food intake is regulated by factors that modulate caloric requirements as well as food's reinforcing properties. In this study, we measured brain glucose utilization to an olfactory stimulus (bacon scent), and we examined the role of food restriction and genetic predisposition to obesity on such brain metabolic activity. Zucker obese (Ob) and lean (Le) rats were divided into four groups: (1) Ob ad-libitum fed, (2) Ob food restricted (70% of *ad libitum*), (3) Le ad-libitum fed and (4) Le food restricted. Rats were scanned using μ -positron emission tomography and 2-[¹⁸F]-fluoro-2-deoxy-D-glucose under two conditions: (1) baseline scan (no stimulation) and (2) challenge scan (food stimulation, FS). FS resulted in deactivation of the right and left hippocampus. Ob rats showed greater changes with FS than Le rats (deactivation of hippocampus and activation of the medial thalamus) and Ob but not Le animals deactivated the frontal cortex and activated the superior colliculus. Access to food resulted in an opposite pattern of metabolic changes to the food stimuli in olfactory nucleus (deactivated in unrestricted and activated in restricted) and in right insular/parietal cortex (activated in unrestricted and deactivated in restricted). In addition, restricted but not unrestricted animals activated the medial thalamus. The greater changes in the Ob rats suggest that leptin modulates the regional brain responses to a familiar food stimulus. Similarly, the differences in the pattern of responses with food restriction suggest that FS is influenced by access to food conditions. The main changes with FS occurred in the hippocampus, a region involved in memory, the insular cortex, a region involved with interoception (perception of internal sensations), the medial thalamus (region involved in alertness) and in regions involved with sensory perception (olfactory bulb, olfactory nucleus, occipital cortex, superior colliculus and parietal cortex), which corroborates their relevance in the perception of food.

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Introduction

The importance of the internal environment in part via the hypothalamus in the regulation of body weight is well recognized.¹ There is also increasing interest on the importance of the external environment in modulating food intake as well as its contribution to the dramatic increases in obesity.² Food intake is regulated by multiple factors that

modulate not only nutrient and caloric requirements but also food's reinforcing properties, and thus changes in food palatability and availability are expected to affect its rate of consumption.³ Thus, environmental factors that have been associated with the increase in obesity include a wide expansion in food variety, increased food palatability, low cost and availability (particularly from energy-dense foods), larger food portions and finally increases in the exposure to food cues.⁴ It is also recognized that emotional variables such as stress and depression can also contribute to overeating.⁵ Of these, the contribution that the plethora of sensory cues (that are conditioned to elicit powerful responses involved in food craving) have on overeating is poorly understood.

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Measurements of regional brain glucose metabolism have been done with positron emission tomography (PET) and 2-[¹⁸F]-fluoro-2-deoxy-D-glucose (FDG) to investigate the regions of the brain that are involved in processing food stimuli in the human brain. One such study showed that visual and chemosensory exposure to an appetitive food stimulus led to significant whole brain activation that was most accentuated in the left parietal somatosensory cortex, left and right insula and left orbitofrontal cortex in food deprived subjects.⁴ In satiated subjects exposure to descriptors of highly desirable foods led to activation of the amygdala and orbitofrontal cortex when compared to activation with descriptors of neutral foods.⁶ A baseline comparison of regional brain glucose metabolism between obese (Ob) and normal weight subjects revealed that even without FS, Ob subjects had significantly higher metabolism in the parietal somatosensory cortex where the lips and tongue are represented, which was interpreted to reflect increased sensitivity of the regions that process food palatability.⁷

Studies in laboratory animals have also identified regional activation of the prefrontal cortex,⁸ amygdala⁹ and nucleus accumbens¹⁰ upon exposure to rewarding food stimuli. Here, we evaluate the regional brain metabolic responses to a food stimulus to assess the brain response to food reinforcers. In parallel, we also evaluated the effects of food restriction and genotype (Zucker Ob vs Zucker lean, Le) to evaluate the interaction between deficits in leptin receptor function and access to food in the response to FS. The Ob Zucker rat (fa/fa) has a leptin receptor deficiency (mutation in the leptin receptor gene) that prevents the expression of the long form of the leptin receptor¹¹ that is mainly responsible for its Ob phenotype. Leptin is an anorexigenic peptide involved in the modulation of food intake and energy balance and as these rats show impaired leptin signaling they become severely Ob by adolescence.¹²

For this purpose, we used small animal PET (μ PET) and FDG and a recently developed automatic method that applies the statistical parametric mapping (SPM) techniques traditionally used to analyze images of the human and the rat brains.¹³

We hypothesized that (1) the hippocampus and the prefrontal cortex would be engaged in the response to the food stimuli, (2) Ob rats would show a greater activation when exposed to the FS than Le rats and (3) food-restricted animals would have greater activation to the FS than unrestricted animals.

Materials and methods

Animals

Male adult Ob Zucker (fa/fa) ($N=20$) and Le ($N=20$) rats were purchased from Harlan (Indianapolis, IN, USA) and maintained on a 12 h dark/light cycle. Rats were divided into

four groups consisting of 10 rats in each group. Specifically (1) Ob rats with unrestricted (U) ad-libitum food access, (2) Ob rats with restricted (R) food access (70% of *ad libitum*), (3) Le U rats and (4) Le R rats. Rats were housed on a reverse 12 h light/dark cycle with lights off at 0700. All experiments were conducted in conformity with the National Academy of Sciences Guide for the Care and Use of Laboratory Animals¹⁴ and Brookhaven National Laboratory Institutional Animal Care and Use Committee protocols.

Behavior assessment: food intake and weight

Rats placed on restricted food access were given a daily amount of food limited to 70% of food levels consumed by similarly aged ad-libitum fed animals. Rats were fed a standard (Purina) laboratory rat chow and food intake was monitored daily at 1500. All rats were weighed every other day.

[¹⁸F] FDG scanning protocol

All animals were scanned at 6–7 months of age using FDG, which was synthesized at the Brookhaven National Laboratory Cyclotron. Each animal was fasted for 24 h before the scan. Blood was sampled from either the left or right lateral tail vein and glucose strip measurements were taken (True-Track smart system, CVS) before administration of FDG to ensure expected fasting blood glucose levels. Each animal was scanned twice and each scan was performed 2 weeks apart. Scan 1 was used as a control scan during which each animal received an intraperitoneal injection of approximately 0.8 mCi FDG and was immediately placed in a custom-built cage (50 × 30 × 30 cm), representing a novel environment. The floor and ceiling of the cage were made out of steel mesh. The floor was elevated three inches above the ground so that the olfactory stimulus (5 × 5 cm cotton gauze with bacon scent) could be placed underneath. Each rat was awake during the presentation of the olfactory stimulus for a period of 40 min during the FDG uptake. Rats were then anesthetized with a mixture of ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹) and placed in a stereotaxic head holder (David Kopf Instruments, Tujunga, CA, USA) in a prone position on the bed of the scanner. Scan 2 occurred 2 weeks later and was the same as scan 1 except that this time each animal was given daily 5 g of cooked bacon for 5 consecutive days prior to the 24 h fasting period. Figure 1 illustrates the sequence of the procedures. Rats in the restricted diet groups were also given the bacon but had this amount subtracted from their daily food amount.

MicroPET image acquisition

An R4 μ PET tomograph (Concorde CTI, Siemens, Knoxville, TN, USA) was used for FDG μ PET imaging. The μ PET R4 has a transaxial resolution of 2.0 mm full-width at half maximum, with a field-of-view (FOV) of 11.5 cm. Animals were placed in the center of the FOV and were scanned under a static

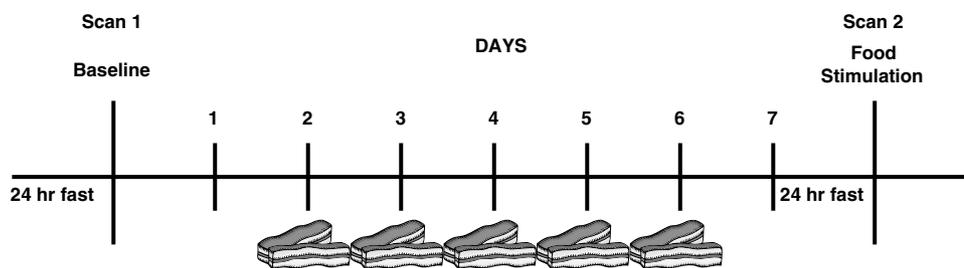


Figure 1 Experimental timeline. Animals were scanned at two time points: (1) before and (2) after exposure to a highly palatable food stimulus (bacon).

imaging protocol for 80 min using a ramp filter with cutoff at Nyquist frequency. After scanning, all images were corrected for photon emission. Initially, images were reconstructed using filtered back projection; however, to achieve higher pixel resolution, they were later reconstructed using the OSEM3D/MAP algorithm provided by Concorde CTI.

MRI image acquisitions

Four magnetic resonance imaging (MRI) brain images, (one from each group) were obtained on a 4 T Superconducting Magnet System using the proton-density pulse sequence with TE/TR = 20/2000 ms at 1 mm slice thickness (37 total slices). The matrix size was 256×256 points at a FOV of 4.0×4.0 cm². Rats were anesthetized with a mixture of ketamine (100 mg kg⁻¹) and xylazine (10 mg kg⁻¹) and placed in a custom in-house designed volume coil.

MicroPET image analysis

To obtain statistical parametric maps from different animals, these studies must be properly co-registered. For this purpose, all the images in the dataset were co-registered to a reference image (manually selected by the user). The registration algorithm makes use of normalized mutual information to find the rigid transformation that co-registers both images, and works in two multiresolution steps: At the first step (lower resolution), the whole reference image is used, whereas at the second step (higher resolution) the reference image is masked in such a way that only those pixels inside the brain are used to compute the cost function. To minimize registration errors, this process was repeated three times, selecting different reference images in every repetition. These three reference images are also registered against each other. Finally, by combining all these geometrical transformations, we can automatically detect any incorrect registration making use of consistency measures.¹⁵ Once the whole dataset is properly registered, an FDG PET template image is created by averaging all co-registered images. Ob and Le Zucker rats have very different metabolisms and such differences in metabolism could underlie observed glucose uptake levels. As this posed a potential confound in data interpretation, we assessed the regional metabolic change of each animal relative to its global

activity. This prevented the characterization of metabolic changes being attributed to differences in animal metabolism. It also prevented misleading glucose uptake effects due to injected dose and weight differences between animals.

Images were analyzed using the SPM2 software package. Two separate analysis of variance (ANOVA) models were used. The first defined four different groups that corresponded to Ob and Le rats with and without food restriction (U and R) during the baseline condition, and thus examined differences in metabolism between these groups prior to bacon exposure. The second defined the same four groups, examining the difference in the activation patterns between the images obtained prior to and after FS (Scans 2 and 1). Images were subtracted after intensity normalization to 100 by the proportional scaling method. After estimation of the statistical model, an F contrast was applied to reveal the effects of interest. These effects were overlaid on the previously generated MRI brain images to get a more accurate representation of the areas of activation. An uncorrected *P*-value of 0.001 was used as threshold to determine statistical significance for both ANOVA models.

Results

Body weight and food intake

A one-way ANOVA revealed significant main effects in body weight ($F = 24.44$; $DF = 3, 99$; $P < 0.004$). Ob U rats showed significantly greater weight than Le R ($t = 8.42$; $P < 0.001$), Le U ($t = 5.57$; $P < 0.001$) and Ob R ($t = 4.69$; $P < 0.001$). Similarly, Le U and Ob R showed significantly greater weight compared to Le R, ($t = 2.85$; $P = 0.0054$) and ($t = 3.72$; $P < 0.001$), respectively). Mean weights for all groups were Ob U, 725.02 ± 13.7 g; Le U, 470.66 ± 4.09 g; Ob R, 527.15 ± 15.5 g; Le R, 326.36 ± 4.31 g (Figure 2a).

A one-way ANOVA showed significant main effects ($F = 96.97$; $DF = 1, 49$; $P < 0.001$) for food intake. Ob rats had higher food intake levels compared to Le ($t = 9.85$; $P < 0.001$). Ob rats consumed on average an additional 8 g of food per day than Le U rats (34% more) (Figure 2b).

FDG brain μ PET image analysis

Differences in metabolism prior to FS between obese and lean and restricted and unrestricted. Le U rats showed the greater

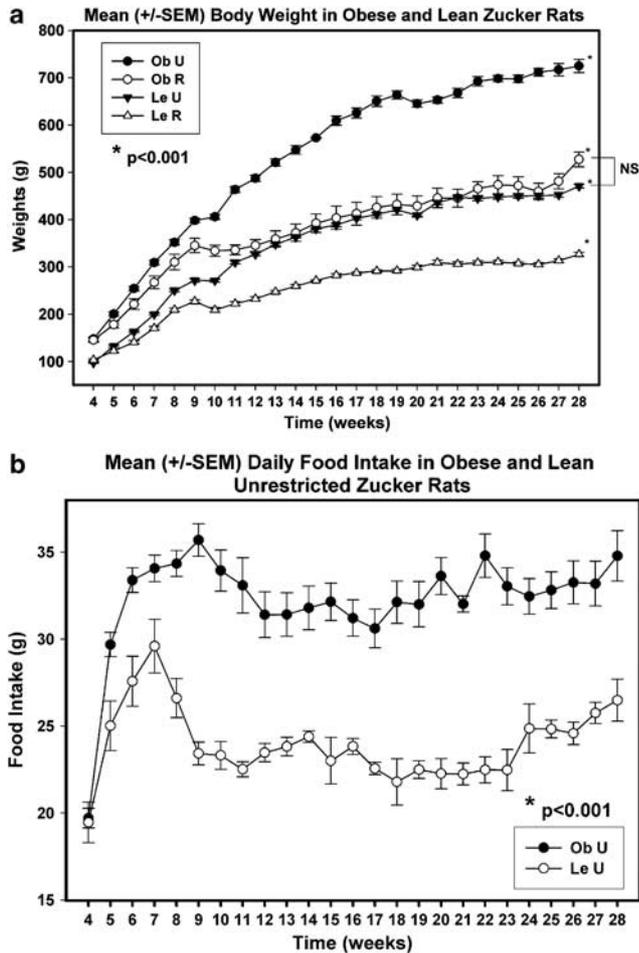


Figure 2 (a) Weekly mean (\pm s.e.m.) body weight (g) in obese unrestricted (Ob U), lean unrestricted (Le U), obese restricted (Ob R) and lean restricted (Le R) rats from 4 to 28 weeks of age. (b) Weekly mean (\pm s.e.m.) food intake (g) in Ob U and Le U rats from 4 to 28 weeks of age.

metabolic activation in the occipital cortex when compared to the other groups (Figure 3a; Table 1). No significant differences were found between the other groups.

Differences in metabolism induced by FS across the groups. The SPM activation results for the two-way ANOVA are shown in Figure 3b. FS resulted in significant deactivation of right and left hippocampus and of a region where the superior colliculus is located. These effects were significant in the Ob R and in the Le U animals but not in the Ob U and in the Le R (Table 2, Figure 4a).

Overall the magnitude of the changes was greater in Ob than in lean subjects (Figure 4b). The effects were significant in left hippocampus, frontal cortex, medial thalamus and in a region where the superior colliculus is located (Table 3, Figure 4b). In Ob animals, deactivation of the left hippocampus and activation of the medial thalamus were significantly greater than in lean animals. Also Ob animals

but not the lean animals showed deactivation in the frontal cortex whereas the Ob animals showed activation in the area of the superior colliculus, the lean ones showed deactivation.

Differences in the changes induced by FS between restricted and unrestricted animals. Overall, the magnitude of the changes was greater in restricted than in unrestricted animals. The restricted animals had significantly greater activation in right and left olfactory nucleus, medial thalamus and right hippocampus. In contrast, unrestricted animals showed greater activation in the insular/parietal cortex and in the olfactory bulb (Table 4, Figure 4c). The interaction by food access conditions revealed an opposite pattern of metabolic changes in these regions. The left and right olfactory nucleus were deactivated in the unrestricted but activated in the restricted animals; the olfactory bulb, the right insular cortex and the right parietal cortex were activated in the unrestricted but deactivated in the restricted animals. In addition in restricted but not in unrestricted conditioning activated medial thalamus and right hippocampus (Figure 4c).

Discussion

Brain glucose utilization

Our results showed significant changes in several brain regions that have been associated with responses to food or food-related stimuli in clinical and preclinical studies upon exposure to a familiar olfactory food stimulus (Figures 4a, b and c). Among these, the hippocampus, superior colliculus and occipital cortex showed the most significant metabolic change. Other regions that showed significant changes included the olfactory bulb, anterior olfactory nucleus, medial thalamus, frontal cortex, insula and somatosensory parietal cortex. Overall, the lateralized response to the food stimuli in the Le U rats (right > deactivation than left) is reminiscent of the lateralized responses to food stimuli reported in the human brain.¹⁶ Both, food access and genotype significantly affected pattern of responses to the conditioned food stimulus, and greatest differences were observed in hippocampus, superior colliculus and occipital cortex.

Hippocampus. Exposure to the bacon stimulus resulted in a significant decrease in metabolism in the hippocampus in Ob U (26 and 27% in right and left hippocampus, respectively) and an even greater response in Le U rats (74 and 38% in right and left hippocampus, respectively) when compared with the baseline condition. The greater response of the hippocampus in the Le U than in the Ob U may possibly reflect the effects of leptin in modulating responses to familiar food stimuli. Indeed, the hippocampus expresses large concentrations of leptin receptors¹⁷ and leptin modulates the excitability of hippocampal neurons¹⁸ facilitating learning.¹⁹ There are several reports of volumetric

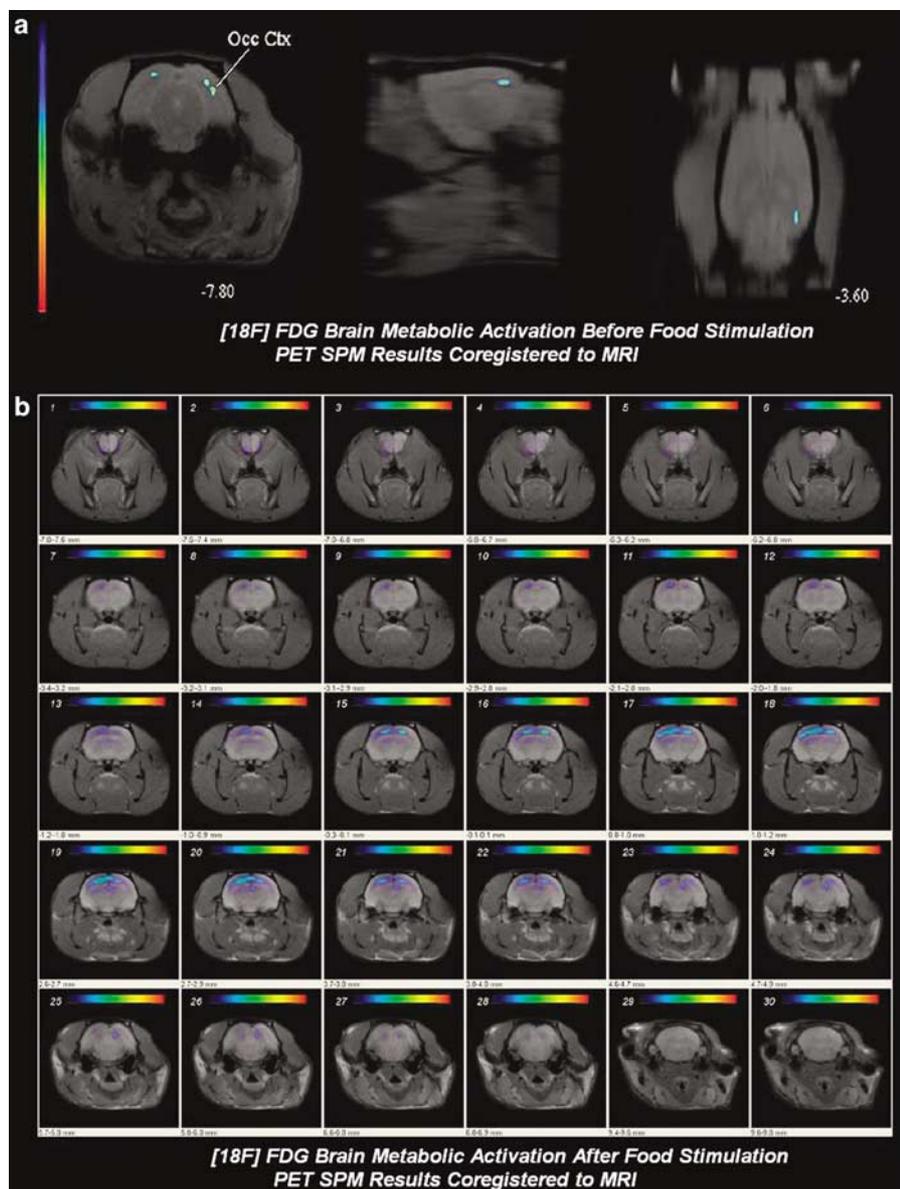


Figure 3 Representative statistical parametric mapping (SPM) images co-registered to magnetic resonance imaging (MRI) and generated with the two analysis of variance (ANOVA) models described above. Images were fused using the Amide v. 0.8.15 software package. (a) Regional metabolic difference as a percentage of brain global activity at baseline. Only activation was in the occipital cortex. (b) Regional metabolic change as a percentage of brain global activity food stimulation (FS). Activated regions: medial olfactory bulb, anterior olfactory nucleus and frontal cortex (1–6), frontal cortex (7–12), hippocampus, insular/parietal cortex, medial thalamus (13–18), hippocampus, occipital cortex, superior colliculus, medial thalamus (19–24) and superior colliculus (25–28).

Table 1 Greatest regional brain metabolic activation: baseline effects

Brain structure	Cluster level (K_E)	Strain effects	F-value	Z-score	P-value	Stereotaxic location x, y, z (mm)
Right occipital cortex	5	Le U>Ob U Le U>Ob R Le U>Le R	10.53	3.32	<0.001	-5, 11, -5

Abbreviations: Le, lean; Ob, obese; R, restricted; U, unrestricted. Metabolic activation under baseline conditions (before FS) in the four experimental groups. Pairwise comparisons and statistical results of our one-way ANOVA model implemented through the SPM2 software package. Stereotaxic location refers to distance (mm) of the specific structure from the ear bar.

Table 2 Greatest regional brain metabolic activation: interaction effects of strain and diet

Brain structure	Cluster level (K_p) ^a	Interaction effects	T-value	Z-score	P-value	Stereotaxic location <i>x, y, z (mm)</i>
Right hippocampus	65	Le R>Le U Ob R<Ob U Ob R<Le R Ob U>Le U	4.97	3.81	<0.001	1, 11, -2
Left hippocampus	65	Le R>Le U Ob R<Ob U Ob R<Le R Ob U>Le U	4.73	3.69	<0.001	-3, 11, 0
Left superior colliculus/occipital cortex	65	Le R>Le U Ob R<Ob U Ob R<Le R Ob U>Le U	4.05	3.31	<0.001	-3, 11, -4

Abbreviations: Le, lean; Ob, obese; R, restricted; U, unrestricted. Pair-wise comparisons and statistical results of the interactions between the factors strain and diet within the two-way ANOVA model implemented through the SPM2 software package. Stereotaxic location refers to distance (mm) of the specific structure from the ear bar. ^aThe three regions above fall within one major cluster.

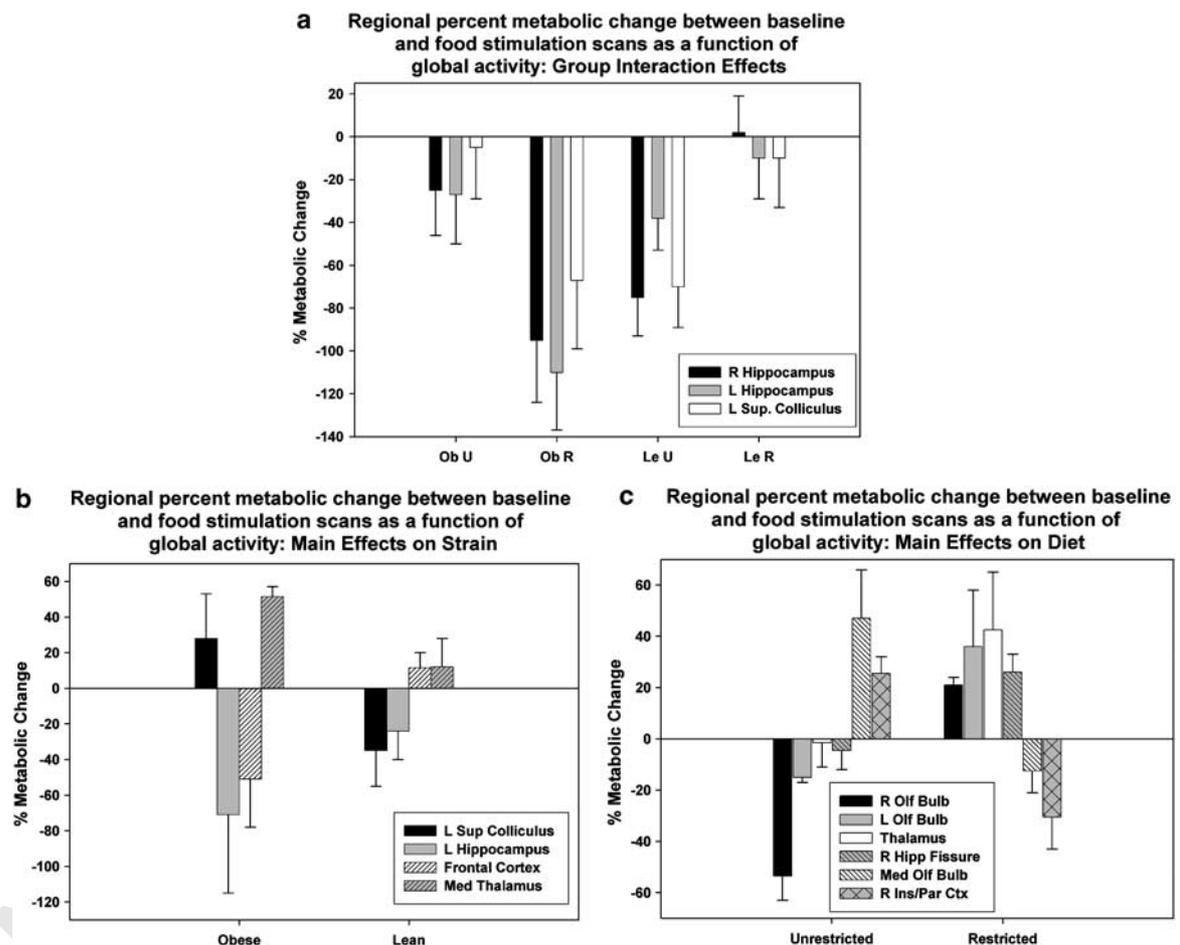


Figure 4 (a) Two-way analysis of variance (ANOVA) pair-wise interaction (a) effects. Regional percent metabolic change (food stimulation-baseline scan) relative to the global value. The y axis shows the difference between food stimulation and the baseline scan in a relative scale in which 100 equals the global mean and represents a measurement of the effect size. (b) Two-way ANOVA effects on strain. Regional percent metabolic change (food stimulation-baseline scan) relative to the global value. The y axis shows the difference between food stimulation and the baseline scan in a relative scale in which 100 equals the global mean and represents a measurement of the effect size. (c) Two-way ANOVA effects on diet. Regional percent metabolic change (food stimulation-baseline scan) relative to the global value. The y axis shows the difference between food stimulation and the baseline scan in a relative scale in which 100 equals the global mean and represents a measurement of the effect size.

Table 3 Greatest regional brain metabolic activation: effects of strain

Brain structure	Cluster level (K_E)	Strain effects	T-value	Z-score	P-value	Stereotaxic location x, y, z (mm)
Left hippocampus	1	Le > Ob	3.85	3.19	<0.001	-3, 11, 0
Thalamus	2	Ob > Le	3.28	2.83	<0.002	2, 9, -2
Frontal cortex	6	Le > Ob	3.14	2.73	<0.003	0, 11, 4

Abbreviations: Le, lean; Ob, obese; R, restricted; U, unrestricted. Strain-specific pair-wise comparisons and statistical results of our two-way ANOVA model implemented through the SPM2 software package. Stereotaxic location refers to distance (mm) of the specific structure from the ear bar.

Table 4 Greatest regional brain metabolic activation: effects of diet

Brain structure	Cluster level (K_E)	Diet effects	T-value	Z-score	P-value	Stereotaxic location x, y, z (mm)
Right olfactory bulb	10	R > U	4.84	3.75	<0.001	0, 8, 8
Superior colliculus	8	U > R	4.28	3.44	<0.001	-2, 9, -5
Left olfactory bulb	5	R > U	3.99	3.28	<0.001	-3, 9, 8
Thalamus	5	R > U	3.60	3.04	<0.001	-1, 9, -2
Medial olfactory bulb	5	U > R	3.27	2.82	0.002	0, 9, 10
Insular/parietal cortex	12	U > R	3.19	2.76	<0.003	6, 9, 1
Right hippocampus	3	R > U	2.87	2.54	<0.005	4, 8, -1

Abbreviations: R, restricted; U, unrestricted. Diet-specific pair-wise comparisons and statistical results of the two-way ANOVA model implemented through the SPM2 software package. Stereotaxic location refers to distance (mm) of the specific structure from the ear bar.

and cell density differences between the left and right hippocampus (reviewed in Lister *et al.*²⁰) but the significance of this lateralization as it relates to food stimuli is unclear.

Food restriction in the Ob rats accentuated the decrease in hippocampal metabolism between the two scans. Specifically, Ob R rats showed a respective decrease of 95 and 110% in right and left hippocampus (compared to 26 and 27% in Ob U) (Figure 4a). The accentuated decrease in hippocampal activity observed in Ob R rats compared to their ad-libitum counterparts may reflect an adaptation to compensate for unmet food intake requirements imposed by the food restriction. In contrast in the Le rats, food restriction decreased the differences between FS and baseline showing a 2% increase and a 10% decrease in right and left hippocampus (compared to decreases of 74 and 38% in Le U) (Figure 4a; Table 2). The small difference in the hippocampal response between FS and baseline in the Le R rats in contrast to the large response in the Ob R suggests that the hippocampus is a key structure in processing food stimuli and that leptin is one of the signals that modulates this response as a function of access to food. Therefore, there is increasing evidence of importance of leptin in processing food stimuli.²¹ Evidence of hippocampal involvement in food-related behaviors has also been reported. To start with, the neuroanatomical connections of the hippocampus allow it to receive information from areas of the brain involved in satiety and hunger signals including the arcuate nucleus in the hypothalamus,²² the nucleus tractus solitarius, which receives afferents from the vagus²³ that are thought to regulate food intake²⁴ and from the insula, which processes internal perception of hunger signals²⁵ and in concert with the hippocampus is thought to be involved in novel taste learning.²⁶ Furthermore, the hippocampus is directly linked to the nucleus accumbens which is a brain region involved

in reward.²⁷ The hippocampus in addition to leptin receptors also expresses receptors for other neuropeptides involved in regulating food intake such as insulin and cholecystokinin,²⁸ and finally, two recent clinical studies showed that gastric stimulation and distention activated the hippocampus.^{29,30} This suggests the possibility of functionally relevant connections between peripheral organs that is important in food-intake regulation such as the stomach and the hippocampus.

In birds, hippocampal size is positively correlated with food-hoarding behavior,³¹ possibly through spatial memory function (remembering the location of food hoards).³¹ In humans and rodents, normal hippocampal function is implicated in memories of food or of the rewarding consequences of eating.³² If this function is disturbed, these memories and the environmental cues that retrieve them, may have increased power to evoke appetitive responses that are instrumental in obtaining and consuming food.³² Indeed, impaired hippocampal functioning in rats has been linked to a reduction in the ability to use information provided by interoceptive energy state signals, an increase in appetitive behavior, food intake, weight gain, as well as heightened general behavioral activity in environments that are strongly associated with food.³² Leptin receptors in the hippocampus appear to be involved in memory function;¹⁷ direct administration of leptin into the hippocampus facilitates long-term potentiation and improves memory processing in mice.³³ Insulin receptors are also expressed in large concentrations in the hippocampus and are believed to be involved in memory function, possibly through their regulation of glucose transport.³⁴ Accordingly, glucose administration improves memory in both humans and animals.³⁵ Finally, obesity and obesity-related diabetes mellitus are both associated with leptin and insulin

resistance as well as memory impairment,³⁶ and Ob Zucker rats, which are both leptin receptor deficient and insulin resistant, have been found to have impaired spatial learning and memory.³⁶ Our results suggest that the Ob Zucker rat in addition to impairment of spatial memory³⁶ may also have impairments in conditioned learning, which involves a different type of memory.³⁷ The responses to FS in the Ob Zucker rats differed in hippocampus, which suggests that abnormal leptin receptor function underlie these changes. To our knowledge, there are no published behavioral studies on conditioning responses in Zucker rats. On the basis of our findings, we hypothesize that conditioning responses to food and also others reinforcers may be disrupted in Ob Zucker rats.

Superior colliculus. There were significant differences in metabolism between FS and baseline in the superior colliculus between the groups. Specifically, Ob U and Le R rats showed a respective decrease of 5 and 10% of global activity between FS and baseline conditions whereas Ob R and Le U rats showed a decrease of 67 and 70%, respectively (Figure 4a; Table 2). In Ob rats, food restriction accentuated the decrease in the superior colliculus observed in ad-libitum-fed Ob rats, while in Le rats the metabolic response was attenuated by food restriction. It is unclear as to why we see such a differential pattern in the response of the superior colliculus between the two strains. The superior colliculus receives projections from retinal ganglion cells, the primary visual cortex and also shares a direct projection with the substantia nigra.³⁸ Some have argued that the superior colliculus is the decision center for reactions to novel, salient and/or moving stimuli in the peripheral visual field.³⁸ Specifically, its functions have been described to include the detection and localization of stimuli,³⁹ as well as the organization of orienting reactions toward or away from such stimuli.³⁹ Other studies point to an involvement of the superior colliculus in multisensory integration.⁴⁰ That is, the outer layers of the superior colliculus are primarily associated with the detection of visual stimuli while deeper layers involve the detection of combinations of visual, tactile and auditory stimuli.⁴⁰ Thus, the limited spatial memory performance reported in Ob Zucker rats could reflect not only deficits in learning and memory (hippocampus) but also impaired visuospatial detection and disrupted ability for detecting salient stimuli (superior colliculus).

The following are limitations of this study: (1) here we report on relative and not on absolute metabolic measures. This precludes us from assessing potential differences in regional metabolism at baseline between strains. Nonetheless, this method highlights regional differences in brain responses. (2) The limited spatial resolution of our in-vivo imaging approach does not allow us to assess within the hippocampus the regions that may be most affected by food deprivation condition or genotype. (3) In this study, we did not evaluate brain activation in response to unconditioned FS (rats receiving the olfactory stimulus for the first time)

and therefore we cannot exclude the possibility that the difference in the responses between the experimental groups are affected by this.

Here we show that food stimuli generate robust activation responses in the rodent brain that are influenced by access to food and leptin receptor function. The differences were most prominent in the hippocampus, a brain region that is increasingly being recognized important in food behaviors, and the superior colliculus that appears to modulate the saliency value of reinforcers and is therefore likely to mediate the enhanced value of food reinforcers as a function of deprivation. These findings have therapeutic implications as they suggest that interventions to restrict food access are likely to have different responses as functions of genetic diversity and that a better understanding of this interaction is likely to lead to tailored interventions that maximize success in weight gain regimes.

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