Importance of Prefrontal Inhibitory Circuitry in Hunger and Satiation:
The Case of Prader-Willi Syndrome vs. Simple Obesity

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ABSTRACT (word limit: 300; current word count: 286)

Background: To date, the majority of research on obesity has been primarily clinical (i.e., eating behavior, adiposity measures), or on peripheral appetite-regulatory peptides (i.e., leptin, ghrelin). However, recent functional neuroimaging research has demonstrated that reward circuitry regions which are associated with appetite-regulatory hormones are also involved in the development and maintenance of obesity. Prader-Willi syndrome (PWS), characterized by hyperphagia and hyperghrelinemia reflecting multi-system dysfunction in inhibitory and satiety mechanisms, serves as an extreme model of simple (non-PWS) obesity (OB).

Objective: The current study was designed to investigate subcortical food motivation circuitry and prefrontal inhibitory circuitry functioning in response to food stimuli before and after eating in PWS compared with OB. We hypothesized that groups would differ most significantly in prefrontal regions associated with cognitive control [i.e., dorsolateral prefrontal cortex (DLPFC)] after eating.

Design and Participants: Fourteen individuals with PWS, 14 BMI- and age-matched individuals with OB, and 15 age-matched healthy-weight controls (HWC) viewed food and non-food images before (pre-meal) and after (post-meal) eating a 500 kcal meal. Using SPM8, group contrasts were tested for hypothesized food motivation circuitry regions: hypothalamus, nucleus accumbens (NAc), amygdala, hippocampus, orbitofrontal cortex, medial PFC, and DLPFC.

Results: Compared with OB and HWC, PWS demonstrated higher activity in reward/limbic regions (NAc, amygdala) and lower activity in hypothalamus and hippocampus, in response to food (vs. non-food) pre-meal. Post-meal, PWS exhibited higher subcortical activation (hypothalamus, amygdala, hippocampus) compared to OB and HWC, while OB showed significantly higher activity in cortical inhibitory regions [DLPFC, orbitofrontal (OFC)] versus PWS and HWC.
Conclusion: In PWS compared with obesity per se, results suggest hyperactivations in subcortical reward circuitry and hypoactivations in cortical inhibitory regions after eating, which contributes to understanding the neural substrates of food motivation in obesity.

KEYWORDS: obesity, DLPFC, inhibition, fMRI, brain activity, Prader-Willi syndrome
INTRODUCTION

In response to rising rates of obesity over the past two decades, recent research has consistently identified brain circuitry involved in basic hunger and satiation and reward processing in obesity. Functional MRI (fMRI) studies comparing obese and healthy-weight individuals generally indicate hyperactivation in the striatum1-2, amygdala2, hippocampus1-2, medial prefrontal cortex (mPFC)2-3, anterior cingulate cortex2-3, and insula1-2 in response to food stimuli pre-meal, and in the hypothalamus4 and mPFC3 after eating. Increasingly, the focus of fMRI studies on eating behaviors, weight gain, and obesity has highlighted dysfunction in regions involved in cognitive self-control, such as the dorsolateral prefrontal cortex (DLPFC)5. Greater DLPFC activation was associated with higher levels of self-control/restraint during food-related decision-making in healthy-weight dieters6 and in response to tasting a sweet rewarding food in healthy-weight and obese adolescent girls7. However, hyperactivation in DLPFC in response to visual food images was also reported in obese compared to healthy-weight children8-9. This suggests that for obese individuals, decision-making in the presence of food stimuli, especially after eating, may require significantly greater top-down control from DLPFC to counteract hyperactivity of subcortical food reward circuitry. Few studies have examined whether the ability to recruit the DLPFC for inhibitory control of eating behavior is related to excessive overeating and weight outcomes in individuals with obesity.

Prader-Willi syndrome (PWS), characterized by extreme hyperphagia and obesity, is a contiguous gene syndrome affecting one in 20,000 live births10 which results from the lack of expression of several imprinted genes in the 15q11-q13 region from the paternal chromosome 1511. Around age 2 years, individuals with PWS begin displaying an insatiable appetite that, if left unchecked, leads to obesity by early to middle childhood12. Consequences of unattended
hyperphagia in PWS include maintenance of over 200% ideal body weight (in 1/3 of the PWS population) and occasional stomach rupture. In direct contrast to individuals with simple obesity (OB; i.e., obesity not related to PWS), the ratio of adiposity to lean mass is elevated and total and resting energy expenditure decreased in PWS. Although peripheral leptin levels are similar in PWS and OB, fasting ghrelin levels are over four times higher in PWS. Behaviorally, individuals with PWS consume more food and eat for a longer period of time than those with simple obesity, suggesting possible disruption of the basic mechanisms of satiation. Additionally, higher-level cognitive control over eating behaviors (“hyperphagic drive”) is also disrupted in PWS and directly linked to extreme obesity within the PWS population, suggesting dysfunction in multiple processes involved in hunger, eating behavior, and weight gain in PWS.

A growing literature aimed at investigating the neural substrates of hyperphagia has yielded important findings that parallel the behavioral phenotype in PWS. Although some differences between individuals with PWS vs. healthy-weight controls have been observed during a fasting state, the most striking abnormalities in food reward circuitry appear following food intake. Specifically, post-meal hyperactivation in response to various food stimuli (visual images, glucose administration) was reported in the hypothalamus, nucleus accumbens, amygdala, hippocampus, medial PFC, orbitofrontal cortex (OFC), and insula, providing evidence of dysfunction in reward circuitry implicated in satiation. However, most of these studies have employed small sample sizes, examined either pre-meal or post-meal brain activation, and made comparisons only to healthy-weight controls, limiting the interpretation of these PWS findings with regard to understanding the development of simple obesity.

Collectively, previous studies on eating behavior, body composition, appetite-regulatory peptide levels, and neural substrates of hyperphagia in PWS indicate the potential of this genetic
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syndrome to serve as an extreme model of obesity. However, despite a recent increase in
functional neuroimaging studies on obesity, especially related to the role of prefrontal inhibitory
networks involved in dietary restraint, there have been no published neuroimaging reports directly
comparing individuals with simple obesity and PWS. Our overarching hypothesis was that the
absence of top-down control (operationalized as hypoactivation of DLPFC and orbitofrontal
cortex) in combination with hyperactivation of subcortical regions (such as the hypothalamus,
ventral striatum (i.e., nucleus accumbens), amygdala, and anterior hippocampus), may be lead to
the phenotypic characteristics of hyperphagia and morbid obesity seen in Prader-Willi syndrome.
The current study was designed to investigate subcortical food motivation circuitry and prefrontal
inhibitory circuitry functioning in response to food stimuli before and after eating in a relatively
large sample of individuals with PWS compared with simple obesity. We specifically
hypothesized that the most substantial differences between groups would be seen after eating in
prefrontal regions associated with cognitive control.

SUBJECTS AND METHODS

Subjects. This study was approved by the Human Subjects Committees at the University of
Kansas and University of Rochester Medical Centers. All applicable institutional and
governmental regulations concerning the ethical use of human volunteers were followed during
this research. Written informed consent was obtained from parents and assent was obtained from
14 individuals with Prader-Willi syndrome (PWS) (12 F/2 M; 2 Type 1 Deletion, 8 Type 2
Deletion, 4 UPD), 14 individuals with simple obesity (9 F/5 M; OB group), and 15 typically
developing, healthy weight control subjects (9 F/6 M; HWC group). Diagnosis of PWS was
confirmed through chromosomal and DNA molecular analysis as previously described\textsuperscript{27}. Groups
were matched on gender (Kruskal-Wallis ANOVA; n.s.), age [mean age (in years) ± sd: PWS =
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24.3 ± 11.3; OB = 25.0 ± 10.3; HWC = 23.1 ± 9.7; all t-tests n.s.] and handedness (all right-handed). The HWC group had a significantly lower BMI [mean BMI (in kg/m²) ± sd = 21.2 ± 2.8] than both PWS (mean BMI = 32.1 ± 7.8; HWC vs. PWS: t = 4.96/p<0.01) and OB (mean BMI = 32.4 ± 3.5; HWC vs. OB: t = 9.57/p<0.01) groups. PWS and OB groups did not differ in BMI (t = .14, n.s.).

Concomitant psychotropic medications in the PWS group included (number of subjects in parentheses): buspirone (1), clonazepam (1), divalproex (2), escitalopram (1), fluoxetine (1), fluvoxamine (1), lorazepam (1), quetiapine (1), risperidone (1), topiramate (1), sertraline (1), and ziprasidone (1). Additionally, one PWS participant was being treated for hypothyroidism. All participants were free from current growth hormone treatment, history of appetite suppressant use, and history of neurological illness.

Three-Factor Eating Questionnaire (TFEQ). Eating behavior was measured using a modified version of the TFEQ28. The TFEQ assesses degree of dietary restriction [“How often are you (is your child) dieting in a conscious effort to control your (his/her) weight?”], eating disinhibition [“Do you (Does your child) eat sensibly in front of others and splurge alone?”], and hunger level [“How often do you (does your child) feel hungry?”] in a questionnaire format. For the purposes of this study, only the 13 initial items on this questionnaire were used. These questions ask individuals to rate their behavior on a 4-point scale (with lower ratings indicating lower dietary restriction, lower eating disinhibition, and lower hunger levels). For individuals with PWS, parents or guardians completed the TFEQ on their child/ward. Individuals in the OB and HWC groups completed a self-report version of the TFEQ.

fMRI acquisition. Scanning was performed on either a 3 Tesla Siemens Allegra or Trio scanner (Siemens, Erlangen, Germany) fitted with a quadrature head coil. Participants’ heads were immobilized with head cushions. Most subjects were scanned at the University of Kansas Medical
Center (n = 38; Allegra) with the five remaining subjects (all PWS) scanned at the University of Rochester Medical Center (URMC; Trio). (Cary, did we conduct reliability studies across scanners? Do we have a response to this if a reviewer asks? We could run analyses by site just to ensure consistency across sites.) One anatomical and two functional sequences were run in each scanning session (i.e., pre-meal and post-meal). T1-weighted anatomical images were acquired using 3D MP-RAGE sequences. At KUMC, coronal orientation, repetition time/echo time (TR/TE) = 23/4 ms, flip angle = 8°, field of view (FOV) = 256 mm, matrix = 256 x 192, slice thickness = 1 mm were used. At URMC, sagittal orientation, TR/TE = 20/4 ms, flip angle = 15°, FOV = 256 mm, matrix = 256 x 256, slice thickness = 1 mm were used. Similar parameters were used at each site for fMRI studies. Single shot gradient echo planar imaging (EPI) fMRI scans were acquired in 43 contiguous coronal slices [TR/TE = 3000/40 ms, flip angle = 90°, FOV = 192 mm, matrix = 64 x 64, slice thickness = 3 mm (0.5 mm skip), in-plane resolution = 3 x 3 mm, 130 data points]. At URMC, a 36 ms echo time was used for the EPI fMRI scans. We calculated that one effect of a shorter TE (36 ms) is an increase in the signal-to-noise ratio (SNR) by ~7% based on the typical T2* in cortical gray matter, compared with the SNR at TE = 40 ms. The other effect is a decrease of task-induced BOLD signal change by ~10% compared with that at TE = 40 ms. Since the fMRI contrast-to-noise ratio (CNR) is proportional to the product of SNR and BOLD signal changes, the change of the CNR at TE = 36 ms was estimated at ~3% reduction compared to that at TE = 40 ms. Therefore, it was expected that the overall effect of the TE difference was not significant and within the range of the experimental variations. Moreover, given the rarity of the population of individuals with PWS and the need for larger samples than previously acquired, we believe the compromise of slightly different acquisitions was justified.
Experimental paradigm. Participants viewed pictures of food, animals, and Gaussian-blurred low-level baseline control images during two scanning sessions; one after fasting for four hours (pre-meal condition) and one immediately after eating a small uniform meal (post-meal condition) that was standardized for total number of calories [Kcal = 500], as well as macro- and micronutrient content. The order of sessions (pre-meal, post-meal) was counterbalanced across subjects. All subjects fasted for 4 hours prior to eating in the pre-meal condition. In the post-meal condition, the meal was consumed outside the scanner and the scan begun within 15 minutes of completing the meal.

Activation paradigm. Visual stimuli of two categories (food and blurred baseline control images) were obtained from LaBar and colleagues. Due to the mental and chronological age of some of the participants in this study, the comparison stimuli were animals to keep participants attentive to the task and to control for general familiarity. All images for the animal category were obtained from professional stock CD-ROMs and matched to food and blurred control images on brightness, resolution, and size. Each image was presented one time only to each subject during the fMRI scanning.

Each functional scan involved three repetitions of each block for stimulus condition type (i.e., food, animal), alternated between blocks of blurred images. Visual stimuli were projected through 3D limited view goggles (Resonance Technology, Inc., Northridge, California) controlled by the stimuli-generating computer program (NeuroSTIM, Neuroscan, El Paso, TX). Stimulus presentation time was 2.5 seconds, with an interstimulus interval (ISI) of 0.5 seconds. Within each of the two functional scans there were 13 blocks of stimulus presentation; within each block, 10 images were presented. The order of category presentation was counterbalanced across subjects.

To ensure that participants were attending to the stimuli being presented, they were instructed to remember images for a memory test following the scanning session. From each of the
food and animal groups, approximately 50% of the images used in the scanning session were
chosen for recall and interspersed with novel distracter images from the same category.
Participants completed a recognition memory test outside the scanner, immediately following each
scanning session. Participants were instructed to press one key if they had seen the image in the
scanner (old) and another key if they had not seen the image (new). Recognition memory task data
for 1 PWS subject were excluded due to technical errors during data acquisition.

**fMRI data analysis.** fMRI data were preprocessed using Statistical Parametric Mapping (SPM8
(Wellcome Department of Cognitive Neurology, 2008) and using custom routines in MATLAB
(Mathworks, Inc., 2000). Processing commenced with realignment and correction for bulk-head
motion. Images for each subject were spatially normalized using nonlinear volume-based spatial
normalization techniques within SPM. The template used by SPM is the standard brain template
developed at the Montreal Neurological Institute (MNI). Images were then spatial smoothed with a
Gaussian filter (6mm at FWHM). Finally, well-established artifact detection tools
(http://web.mit.edu.ezp-prod1.hul.harvard.edu/swg/software.htm) were used to identify and
exclude outliers in the global mean image time series and movement parameters. Outliers were
defined as: >3.8mm translational movement, >.05 radians rotational movement, and 1.40 standard
deviations away from the global mean image. Of the original n=15 participants/group, two subjects
(1 PWS, 1 OB) were excluded due to excessive movement, resulting in the final sample size of
n=43. In addition, behavioral evidence of diminished attention due to excessive sleepiness resulted
in discard of that run. From each group, runs that met these criteria were discarded (PWS: 5 runs;
OB: 2 runs; HWC: 2 runs).

Following preprocessing, statistical analysis was performed at the single-subject level
using SPM. SPM treats each voxel’s BOLD time series according to a general linear model. Each
epoch of trials was modeled using a boxcar function convolved with a canonical hemodynamic
response function. Specific comparisons of interest (food versus non-food, separately for pre-meal and post-meal) were tested using linear contrasts, and SPM maps were created based on these contrasts. These contrast values (estimates of the mean signal change at each voxel) were used in statistical analyses.

Voxel-wise analyses: Results from the individual subject level were submitted to a second level analysis in which subjects were treated as a random effect. Independent sample t-tests were used to compare the size of a particular effect between groups (PWS vs. OB; PWS vs. HWC; OB vs. HWC). Given our hypotheses about specific brain regions, we used an approach in SPM which limits voxel-wise analyses to voxels within our a priori ROIs. Anatomically-defined regions of interest included the hypothalamus, nucleus accumbens, amygdala, hippocampus, OFC, mPFC, and DLPFC. False positives were controlled using a voxel-wise height threshold (p<0.05 uncorrected) and an extent threshold that jointly resulted in a cluster-level false-positive level of p<0.05, corrected for multiple comparisons within the search volume using family-wise error (FWE) correction. Anatomic borders of hypothesized regions were defined using a manually segmented MNI-152 brain. These borders were then implemented as overlays on the SPM8 canonical brain using the Wake Forest University (WFU) PickAtlas toolbox for SPM.

Anatomical-ROI analyses: After identifying clusters within the ROIs, these anatomic overlays were used on the statistical maps of each individual to acquire signal change values across specific ROIs. Values indicated the degree of change in MR signal detected between the negative and neutral conditions and are expressed in terms of percent signal change (PSC). Average PSC values (beta weights averaged across all voxels within an anatomical region) were obtained for each ROI using the REX toolbox for SPM. The PSC values were used to calculate effect sizes (ES) for the difference between PWS and OB groups. The formula for calculating ESs
was: \( ES = \frac{\text{PWS group mean (food – non-food PSC)} - \text{OB group mean (food – non-food PSC)}}{\text{standard deviation of PSC value of the whole sample}} \).

RESULTS

Behavioral Data

Group comparisons on TFEQ scores were conducted in order to test whether groups differed in eating behavior and general level of hunger. Mean TFEQ scores for individuals with PWS (PWS: 2.98 ± 0.41) were significantly higher than the OB group (OB: 2.45 ± 0.29; \( t = 3.95/p < 0.01 \)) and HWC group (HWC: 2.22 ± 0.22; \( t = 6.38/p < 0.01 \)). The OB group had significantly higher mean TFEQ scores than the HWC group (\( t = 2.39/p < 0.05 \)). These results suggest a significant linear increase in hunger level, disinhibition, and dietary restraint behaviors in the comparison of HWC, OB, and PWS groups. Performance on the recognition memory test was above chance for all groups (\( p \) values <0.01), confirming that subjects were properly attending to visual stimuli during the scanning session. OB and HWC performed significantly better than PWS on recall of both food and non-food stimuli (\( p \) values <0.01), likely related to a lower mean IQ level in PWS.

fMRI Data

[Table 1 here]

The main contrasts of interest for this study focused on direct comparisons of PWS and OB in activations of hypothesized brain regions in response to food vs. non-food stimuli before and after eating. During pre-meal, PWS exhibited significantly greater activations than OB in the nucleus accumbens and amygdala, uncorrected for multiple comparisons (Table 1). In contrast to PWS, OB showed greater activation in response to food versus non-food stimuli pre-meal in the
hypothalamus and hippocampus. Effect sizes for group differences pre-meal (calculated from percent signal change values within each ROI) ranged from 0.13 (hippocampus) to 0.66 (nucleus accumbens; Table 1).

Comparison of PWS and OB post-meal indicated greater activations in PWS in the hypothalamus, amygdala, and hippocampus (Table 1; Figure 1). Conversely, OB exhibited greater activations post-meal in DLPFC [Brodmann Area (BA) 46] and OFC (BA 11; Figure 1). Group differences in DLPFC were significant FWE-corrected for multiple comparisons. Effect sizes for group differences post-meal ranged from 0.16 (hippocampus) to 0.95 (DLPFC).

In comparisons of OB and PWS with healthy weight children, OB exhibited greater activations in response to food versus non-food stimuli than HWC in the hypothalamus, amygdala, mPFC, and OFC during the pre-meal condition, and post-meal in the hypothalamus and DLPFC (Table 2). Conversely, HWC displayed greater activation than OB post-meal in OFC.

Finally, PWS exhibited persistent hyperactivation compared to HWC in the hypothalamus, amygdala, hippocampus pre-meal and post-meal, and in mPFC pre-meal (Table 3). There were no regions in which HWC displayed greater activation than PWS either pre-meal or post-meal.

**DISCUSSION**

Converging evidence from the growing literature on neural substrates of abnormal food intake and obesity has generally implicated somewhat overlapping but distinct neural circuits related to hunger/satiation, reward, and self-control. Results from the current study extend these findings suggesting unique patterns of brain activity in these regions in two groups of individuals with different types of obesity: one group with a genetic syndrome and phenotype that includes
extreme overeating (Prader-Willi syndrome), the other with simple (idiopathic) obesity.

Specifically, we report hyperactivations in response to visual food stimuli in individuals with PWS compared to BMI-matched OB subjects in subcortical regions (hypothalamus, amygdala, hippocampus) unaffected by appetitive state, with hyperactivation in amygdala both before and after meal consumption. More strikingly, individuals with PWS displayed significant hypoactivity in cortical inhibitory regions (posterior/lateral OFC, DLPFC) post-meal. The brain activation patterns distinguishing these groups map well onto the differences observed between PWS and OB in eating behavior (extreme hyperphagia versus moderate overeating), energy expenditure (very low versus moderately low), and appetite-regulatory peptide levels (hypergherlinemia versus low ghrelin), and thus support the utility of PWS as a model of extreme obesity.

Pre-meal subcortical hyperactivation has been documented previously in OB\textsuperscript{2} and PWS\textsuperscript{22,33} in comparison to healthy-weight controls, and post-meal in OB\textsuperscript{4} and in PWS\textsuperscript{24,33-34}. These regions (i.e., hypothalamus, amygdala, hippocampus), which are densely populated with ghrelin receptors\textsuperscript{35,41 42-45 45-48}, are involved in basic hunger and satiation signaling\textsuperscript{35}, reward and orientation to/approach behaviors related to food\textsuperscript{36-39}, and emotionally-modulated memory processes involved with food\textsuperscript{40}, respectively. Our results replicate these findings and extend them to demonstrate that subcortical reward circuitry hyperactivation in response to food stimuli is a hallmark of obesity in general and disorders of obesity, i.e., PWS, unaffected by appetitive state.

Further, the most striking finding in this study relates to post-meal differences between PWS and simple OB in cortical inhibitory regions, DLPFC and OFC, with significant effect sizes in the range of \( 3/4 \) to one full standard deviation from the mean. The DLPFC is well-established as a critical inhibitory region, associated with suppression of motor responses\textsuperscript{46}, and higher-level cognitive processes such as self-control in goal-directed behavior and decision-making\textsuperscript{47-48}, including issues involving food intake. Evidence for this role includes greater DLPFC activation in
response to: meal consumption in successful dieters compared with a group of obese individuals who did not diet\textsuperscript{49}, obese children\textsuperscript{9} and adult males\textsuperscript{50} in comparison to healthy-weight counterparts in response to food pictures\textsuperscript{9} or satiation\textsuperscript{50}, high-self-controllers vs. non-self-controllers during self-control trials\textsuperscript{6}, and tasting palatable food\textsuperscript{7} for individuals with high dietary restraint scores. Further, genetic variability related to subtle differences in behavioral profiles in PWS\textsuperscript{51} was significantly associated with differential activation of DLPFC post-meal\textsuperscript{33}, suggesting a genetic basis for abnormal cortical inhibitory control by DLPFC in obesity. Recent work suggested that failure of DLPFC inhibition in PWS may result from abnormalities in GABA\textsubscript{A} receptors in the frontal cortex\textsuperscript{52}, likely related to deletion of GABRB3, GABRA5, and GABRG3 genes from the ~6-Mb PWS region of chromosome 15\textsuperscript{11}. Our work to further refine the brain phenotype in PWS will help direct molecular genetics studies to identify additional genes and polymorphisms on chromosome 15 associated with specific brain abnormalities, which in turn may contribute to understanding genes associated with brain circuitry implicated in simple (non-PWS) obesity.

Taken together, these findings suggest that hyperactivation of DLPFC post-meal in response to food stimuli is associated with either the greater ability or heightened need to inhibit food-related behaviors and intake, reflecting the necessity of additional top-down inhibition in the presence of high-reward food stimuli. In light of these results, we suggest that hyperactivation of DLPFC in OB versus PWS post-meal reflects successful recruitment of this important inhibitory self-control region in individuals who overeat moderately, and unsuccessful activation of DLPFC in PWS, contributing to hyperphagia and excessive overeating. Our findings suggest that the ability to recruit DLPFC in simple obesity is a qualitative difference with PWS and distinct from the quantitative differences in hyperactivity of subcortical regions in OB and PWS.

PWS is a genetic syndrome associated with intellectual disability, with deficits in abstract reasoning and executive functioning, domains also governed substantially by DLPFC. Thus, it is
possible that general cognitive deficits could have driven group differences in brain activity in this region. However, PWS hypoactivation in DLPFC was unrelated to memory for food items post-meal \((r=0.2)\), suggesting that DLPFC deficits in PWS were not accounted for by global intellectual deficits. In fact, DLPFC activation and TFEQ scores were correlated in PWS \((r=0.44)\), suggesting a link between deficits in this area and food-related behaviors.

In addition to hypoactivation of DLPFC in the current study, individuals with PWS exhibited lower activation post-meal compared with OB in left posterior-lateral OFC, a region associated with evaluation of simple stimuli (such as food images) in the context of negative reinforcement leading to behavior changes\(^{55}\). OFC hypoactivation in response to food stimuli has previously been associated with higher BMI\(^8,56\), including in individuals with fewer striatal dopamine receptors\(^57\). Further, in obesity, dysfunction in the amygdala’s modulation of OFC was reported\(^58\), and in PWS, OFC volume was specifically decreased compared with healthy controls\(^59\).

We hypothesize that concurrent dysfunction in OFC and DLPFC in PWS significantly impaired the ability to effectively inhibit food intake during states of low appetite (post-meal), when food should not be consumed, and to learn appropriate responses based on negative experiences with food when satiated (i.e., physical discomfort, parental reprimand). We would argue that subcortical hyperactivation combined with cortical hypoactivation contributes importantly to the phenotype of excessive hunger, uncontrollable food seeking behavior, and hyperphagia in PWS as distinct from simple obesity, in which more intact functioning in these regions results in a less extreme behavioral profile.

In the current investigation, we replicated several previous findings from studies using similar fMRI paradigms in the comparison of PWS versus HWC\(^{22-23,33}\) and OB versus HWC\(^2-3,8\). Given that, to date, our study includes the largest sample in an fMRI study of BMI-matched PWS and OB groups, inconsistencies between previous studies may be resolved given increased...
statistical power of our tests. We did not attempt to match meal sizes to each subject’s BMI and corresponding caloric homeostatic needs. We developed our meal size according to the restricted diets that are characteristic for individuals with PWS, which may have influenced the level to which each individual felt satiated, affecting patterns of activation. However, given that our OB and PWS groups were matched on BMI, this was unlikely to be a confounder. Our sample was not matched on IQ, and the PWS group likely had a significantly lower mean IQ than the other groups. However, behavioral results indicated greater-than-chance accuracy on the recognition memory test in all groups, suggesting that all subjects were able to perform the task. Finally, the original design of this study did not include extensive neurobehavioral testing in the OB and HWC groups, thus we were unable to fully explore the relationships between brain activity and other cognitive/behavioral functioning which may contribute to understanding differences between PWS and simple obesity.

There are significant sex differences in obesity, and several of our hypothesized brain regions are sexually dimorphic. Given that the majority of our sample consisted of female participants, especially in the PWS group, it was not possible to conduct an analysis of sex differences. However, we conducted analyses separately for females and males and results were similar. Current work is investigating sex differences in brain circuitry in obesity.

In summary, findings in this study demonstrate dysfunction in dual circuits regulating food reward and intake in individuals with simple obesity compared with Prader-Willi syndrome, a model of extreme obesity. Given a low-appetitive state (post-meal), PWS compared to simple obesity demonstrated hyperactivations in the hypothalamus, amygdala, and hippocampus, subcortical regions associated with hunger and food motivation, and hypoactivations in DLPFC and OFC, cortical inhibitory regions involved in self-control during food-related decision-making. These findings provide evidence of distinct neural patterns that correspond with group differences
in eating behavior (degree of overeating) despite similar BMI levels, and suggest neural pathways that can be targeted in future studies of the treatment of obesity and related conditions.

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CONFLICT OF INTEREST

The authors have no financial, consultational, institutional, or other conflicts of interests to declare regarding this study.


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Table 1. Regions reaching significance for the between-group analysis (PWS vs. OB) contrast between food and non-food categories.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Contrast</th>
<th>Region of Interest</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Z-score</th>
<th>Uncorrected p-value</th>
<th>Voxel-level $P_{\text{FWE-corr}}$</th>
<th>Effect size of group difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pre-meal</strong></td>
<td>PWS &gt; OB</td>
<td>Nucleus accumbens</td>
<td>R</td>
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<td>0.081</td>
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<td>Hypothalamus</td>
<td>L</td>
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<td>Hippocampus</td>
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<td>-26</td>
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<td>0.006</td>
<td>0.154</td>
<td>0.13</td>
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<tr>
<td><strong>Post-meal</strong></td>
<td>PWS &gt; OB</td>
<td>Hypothalamus</td>
<td>R</td>
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<td>5</td>
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<td>OB &gt; PWS</td>
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</table>

1 Coordinates are presented in Talairach space

2 Voxel-wise Z-score significance level $p<0.05$ uncorrected for multiple comparisons within a hypothesized ROI; ROIs listed represent regions of significantly activated clusters within the apriori hypothesized ROI

3 FWE rate (family-wise error rate) used for SVC (small volume correction): Voxel-level significance level (FWE-corrected within the search volume of interest)

4 ES (Effect sizes) = standard deviations calculated as: differences between food versus non-food percent signal changes in PWS vs. OB; differences are divided by standard deviation of percent signal change value of the whole sample
Table 2. Regions reaching significance for the between-group analysis (OB vs. HWC) contrast between food and non-food categories.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Contrast</th>
<th>Region of Interest</th>
<th>Hemisphere</th>
<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z¹</th>
<th>Z-score</th>
<th>p-value ²</th>
<th>Uncorrected p-value ²</th>
<th>Voxel-level p_{FWE-corr} ³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-meal</td>
<td>OB &gt; HWC</td>
<td>Hypothalamus</td>
<td>L</td>
<td>19</td>
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<td>-9</td>
<td>-3</td>
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<td>0.002</td>
<td>0.048</td>
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<tr>
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<td>OB &gt; HWC</td>
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¹ Coordinates are presented in Talairach space

² Voxel-wise Z-score significance level p<0.05 uncorrected for multiple comparisons within a hypothesized ROI; ROIs listed represent regions of significantly activated clusters within the apriori hypothesized ROI

³ FWE rate (family-wise error rate) used for SVC (small volume correction): Voxel-level significance level (FWE-corrected within the search volume of interest)
Table 3. Regions reaching significance for the between-group analysis (PWS vs. HWC) contrast between food and non-food categories.

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<th>Voxels</th>
<th>x</th>
<th>y</th>
<th>z$^1$</th>
<th>Z-score</th>
<th>Uncorrected p-value$^2$</th>
<th>Voxel-level $P_{\text{FWE-corr}}$</th>
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</thead>
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<tr>
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FIGURE LEGEND

Figure 1. Comparison of PWS and OB groups for the food > non-food contrast in the Post-Meal condition. Regions demonstrating greater activation in the OB group compared to the PWS group (OB > PWS) include the left OFC (A) and left DLPFC (B). Greater activation in the PWS vs. OB group was seen in the right amygdala (C), right hypothalamus (D), and left hippocampus (E). Activation overlaid on the SPM8 single-subject T1 template in the coronal view. Bar graphs depicting average percent signal change in each group for corresponding ROIs are displayed below each ROI image.