Midbrain Response to Milkshake Correlates with Ad Libitum Milkshake Intake in the Absence of Hunger

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Abstract

There is now widespread agreement that individual variation in the neural circuits representing the reinforcing properties of foods may be associated with risk for overeating and obesity. What is currently unknown is how and whether brain response to a food is related to immediate subsequent intake of that food. Here we used functional magnetic resonance imaging (fMRI) to test whether response to a palatable milkshake is associated with subsequent *ad libitum* milkshake consumption. We predicted that enhanced responses in key reward regions (insula, striatum, midbrain, medial orbitofrontal cortex) and decreased responses in regions implicated in self-control (lateral prefrontal and lateral orbitofrontal cortex) would be associated with greater intake. We found a significant positive association between response to milkshake in the periaqueductal gray region of the midbrain and *ad libitum* milkshake intake. Although strong bilateral insular responses were observed during consumption of the milkshake this response did not correlate with subsequent intake. The associations observed in the midbrain and orbitofrontal cortex were uninfluenced by ratings of hunger, which were near neutral. We conclude that midbrain response to a palatable food is related to eating in the absence of hunger.

Keywords

Neuroimaging; Feeding; Flavor; Obesity; Midbrain; Periacqueductal gray; PAG; Decision making; Dopamine; Insula; Cannabinoid; Opiod; Pain

The hedonic properties of food can drive the motivation to eat beyond homeostatic energy needs (Kenney, 2011; Zheng et al., 2009). In an early neuroimaging study of feeding we showed that response to chocolate consumption in the insula/operculum, midbrain, striatum, and medial orbitofrontal cortex decreased as motivation to consume the chocolate decreased,

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whereas response in the lateral orbitofrontal cortex and lateral prefrontal cortex increased (Small et al., 2001). We suggested that these two circuits governed meal initiation and meal termination, respectively.

Since this time functional neuroimaging studies have elucidated brain response to food pictures, odors and flavors across differing internal states (Cornier et al., 2007; Frank et al., 2010; Führer et al., 2008; Goldstone et al., 2009; La Bar et al., 2001; Malik et al., 2008; O'Doherty et al., 2000; Siep et al., 2009), body weights (Rothemund et al., 2007; Stice et al., 2008; Stoeckel et al., 2008), eating phenotypes (e.g. restraint, food addiction) (Beaver et al., 2006; Demos et al., 2011; Lowe et al., 2009b) and as a function of prospective weight gain (Stice et al., 2008; Stice et al., 2010) or loss (Cornier et al., 2012). Enhanced responses in regions thought to represent the reinforcing properties of foods, such as the insula/ operculum, midbrain, medial orbitofrontal cortex, striatum and insula are associated with higher body mass index (BMI) (Rothemund et al., 2007; Stice et al., 2008; Stice et al., 2010; Stoekel et al., 2008). In contrast, reduced responses in regions critical for inhibitory behavior and self-control, such as the lateral orbitofrontal cortex and lateral prefrontal cortex (Hollmann et al., 2012; Hare et al., 2011) are often associated with higher BMI (Batterink et al., 2010; Volkow et al., 2009). There is also evidence for reduced grey matter density in lateral prefrontal cortex in obesity (Pannacciulli et al., 2006). Consequently, an important theme to emerge is that heightened reward responses, coupled with decreased inhibitory control, increase risk for overeating and weight gain (Del Parigi et al., 2003; Small, 2009; Volkow and Wise, 2005). What is less understood is how brain response to a food relates to subsequent intake of that food. Batterham and colleagues showed that the neural activity recorded during an fMRI scan could predict subjects' subsequent caloric intake (Batterham et al., 2007). However the scanning paradigm was unrelated to the test meal as no food or food-related cues were presented during scanning. This is an important issue because the relationship between brain response to a food and its subsequent intake is presumably an important mediator of the relationship between brain response to food and body weight.

The aim of the current fMRI study was to address this gap in the literature by identifying regions in which response to a palatable and energy-dense milkshake is associated with the immediate subsequent intake of that milkshake in non-deprived individuals who self-report being neither hungry nor full. We reasoned that there would be automatic engagement of circuits coding the appetitive aspects of the stimulus as well as circuits representing inhibitory control in the face of consuming a palatable food. An overarching prediction was that greater responses in brain regions representing the reinforcing properties of foods would be positively associated with intake while reduced responses in regions representing response inhibition and self-control would be negatively associated with intake.

Methods

Subjects

Twenty-three right-handed nonsmokers, taking no daily medication and having no known history of loss of consciousness, chemosensory impairment, neurological, or psychiatric disorders, gave written informed consent to participate in our study that was approved by Yale University School of Medicine Human Investigation Committee. Two subjects were excluded because of excessive movement during scanning, which was defined as greater than 2mm movement in any direction. An additional subject was excluded because assessment of *ad libitum* milkshake consumption indicated that this person drank 1028 gams of milkshake, which corresponds to 3.07 standard deviations more than the mean of other subjects. We therefore excluded the data point, but note that including the subject in the group analyses does not influence the results reported below. The results of the study are therefore based on the remaining 20 subjects (10 women, 10 men, mean age 27.0 ± 6.2

years, mean BMI 25.8 ± 4.0 (range = 20.8 to 35.7), with an Edinburgh Handedness Inventory score (1) ranging between 55–100).

Stimuli and delivery

Stimuli included Nestle's chocolate milkshake and a tasteless baseline solution. The tasteless solution consisted of 12.5 mM KCl and 1.25 mM NaHCO3 in distilled water. Four different concentrations were presented (25%, 50%, 75%, and 100% of the original concentration) and subjects were instructed to select the dilution that tasted most like nothing. The chosen concentration was used as the tasteless stimulus. Seventeen subjects chose the 25% solution, two chose the 50% solution, and one chose the 75% solution. Liquids were delivered as 1 ml of solution over 4 s using a gustometer system described previously (22). In brief, this system consists of computer controlled syringe pumps connected to an fMRI-compatible custom designed gustatory manifold via 25-foot lengths of beverage tubing (Saint-Gobain Performance Plastics). The manifold is mounted on the headcoil and liquids dripped from the manifold stylus onto the tongue.

Experimental Design

Subjects participated in one fMRI training session and one fMRI scanning session conducted on separate days. Subjects were instructed to arrive feeling neither hungry nor full and to abstain from eating or drinking anything (except water) for 1 h before both sessions. No other dietary instructions were provided. However, subjects rated their hunger upon arrival and these ratings were intended to exclude subjects reporting subjective hunger or fullness within 20 points of neutral. No subjects were excluded based upon this criterion. All scans were performed between 11 am and 3 pm. For the 10 women, the average days since the first day of their menstrual cycle was 14.6 but the range was large (2–24 days).

Training Session

Upon arrival to the training session, subjects first selected their tasteless solution. Next, after being inserted into the mock scanner bore, subjects provided a series of ratings using a mouse to move a curser to a desired location along a line on a computer screen. Subjects started by rating feelings of hunger and fullness using 20 mm visual analog scales (VAS) with the following labels: at the left anchor (−100), "I am not hungry at all" or "I am not full at all"; at the midpoint (0), "neutral"; and at the right anchor (+100), "I have never been more hungry" or "I have never been more full". Stimulus intensity was measured using the general Labeled Magnitude Scale (gLMS; Green et al., 1996), a 100 mm vertical line scale anchored by the labels "no sensation" at the bottom and the label "strongest imaginable sensation" at the top. In between these anchors the following words were quasi-logarithmically spaced in ascending order: "barely detectable", "weak", "moderate", "strong", and "very strong". Subjects rated pleasantness using a 100 mm Labeled Hedonic Scale (Lim, 2009) anchored by "most disliked imaginable" at the bottom anchor point (−100), "neutral" in the center (0), and "most liked imaginable" at the top anchor point (100). Importantly, this scale, which produces ratio-like data and minimizes ceiling effects was created by placing descriptors at locations corresponding to direct estimation of perceptual and psychological magnitude made by the subjects. Therefore perceptual units are not equal. For example 44 corresponds to "like very much". Another important feature of the scale is that it assesses liking across all possible sensations. For reference, in the original paper French fries received a liking rating of 26. Ratings of wanting to eat were measured using a VAS scale labeled as follows: at the left anchor, "I would never want to eat this"; at the middle point, "neutral"; and at the right anchor, "I would want to eat this more than anything".

After completing the ratings, subjects underwent a single mock run, which served to familiarize them with the procedure. During the mock and real runs, stimuli were delivered according to a long event-related design (Small et al., 2003; Small et al., 2004). Either a milkshake or a tasteless solution was presented to the subject for 4 s. After a 3–10 s wait, the milkshake was followed by a 4 s tasteless rinse. Before the onset of the next trial, there was a 3–10 s wait. Subjects were instructed to hold the solution in their mouths until they had received the full amount of the liquid and then to swallow during the wait. A single mock run, with a duration of 5 min 54 s and consistent of 11 presentation of each stimulus, was performed. After the mock run, subjects were asked to provide a second set of ratings.

After the mock scan, height and weight were measured using a Detecto 439 Mechanical Scale (footwear and coats removed) and BMI was calculated [BMI= weight (kg)/height $(m)^2$]. Eating style was then measured with the Three Factor Eating Questionnaire (TFEQ) (Stunkard and Messick, 1985) and the Power of Food Scale (Lowe et al., 2009).

fMRI Session

Before and after the fMRI scan, subjects provided their internal state and stimulus ratings. Once inside the fMRI scanner, subjects performed one anatomical run and two functional runs (22 repeats of each stimulus). After undergoing the fMRI scan subjects were removed from the scanner bore and brought to a separate room where they were offered a full bottle of chocolate milkshake (1656.12 mL) to drink while the experimenters broke down the delivery equipment. When the experimenters returned (10 minutes) they debriefed the subject and measured the amount of milkshake consumed.

fMRI Data Acquisition

The images were acquired on a Siemens 3 T Trio magnetom scanner at Yale University Magnetic Resonance Research Center, using parameters similar to previous studies. Susceptibility-weighted single-shot echoplanar imaging was used to measure the blood oxygenation-level dependent (BOLD) signal as an indication of cerebral brain activation with TR, 2000 ms; TE, 20 ms; flip angle, 80°; FOV, 220 mm; matrix, 64 × 64; slice thickness, 3 mm; and acquisition of 40 contiguous slices. Slices were acquired in an interleaved mode. The MR signal was allowed to equilibrate at the beginning of each functional run over six scans for a total of 12 s, which were then excluded from analysis. A high-resolution T1-weighted three-dimensional anatomical scan was acquired with the following parameters: TR/TE, 2530/3.66 ms; flip angle, 20°; matrix, 256 × 256; 1 mm thick slices; FOV, 256; 176 slices.

Analysis of Behavioral and BMI Data

We performed Pearson's correlations in SPSS 16.0.0 for Windows in order to evaluate whether any of the multiple measures that were recorded (BMI, hunger, fullness, intensity, pleasantness, wanting to eat) were associated with ad libitum milkshake intake.

Analysis of FMRI Data

Data were analyzed using SPM5 software (Statistical Parametric Mapping; Wellcome Department of Imaging Neuroscience, London, UK). Functional images were time-acquisition corrected to the slice obtained at 50% of the TR and realigned to the mean image. Anatomical and functional images were normalized and segmented using the VBM toolbox in SPM5. Functional time-series data was detrended, a method that eliminates at each voxel any linear component matching the global signal (Macey et al., 2004). Functional images were smoothed using a 6 mm full-width at half maximum isotropic Gaussian kernel.

Sessions in which head movement during scanning exceeded a predetermined limit of 1 mm in any direction were excluded.

A design matrix was created at the individual subject level, which identified the onset and duration of each event. Events of interest were defined as mini-blocks with a duration of 4 s after onset (when solution entered the subject's mouth). Milkshake and tasteless presentations were modeled as events of interest. According to SPM5 convention, a 128 high-pass filter was applied to the time-series data with the aim to remove low-frequency noise and slow signal drifts. General linear model was used to estimate, at each voxel, condition-specific effects. A canonical hemodynamic response function, including a temporal derivative, was used to model neural response to events.

To assess group effects, the parameter estimate images for the conditions for each subject were entered into an ANOVA contrasting Milkshake - Tasteless. T-map threshold was set at p uncorrected < 0.001 and a 3-voxel cluster size. Unpredicted responses were considered significant at $p < 0.05$ false discovery ratio (FDR) corrected across the entire brain for multiple comparisons. For predicted responses the WFU Pick Atlas tool in SPM5 was used to perform region of interest (ROI) analyses to test for chemosensory activations (e.g. insula, orbitofrontal cortex) to milkshake – tasteless and peaks were considered significant at $p < 0.05$ FDR corrected across the total number of voxels across all ROIs (Maldjian et al., 2003). Small-volume searches with ROIs defined using peaks from Small et al 2001 to create 15 mm diameter spheres were performed to test whether response in the midbrain, insula, striatum and medial and lateral OFC were related to intake.

Next, we regressed *ad libitum* milkshake intake, BMI, hunger, and pleasantness ratings against BOLD response to Milkshake – Tasteless across all of the subjects in a whole-brain regression analysis in SPM5. This analysis was used to test *whether* and *where* we observe significant correlations in neural response to milkshake and any of these variables. The parameter estimate images from the Milkshake - Tasteless contrast were entered into a regression design along with a vector of the variable of interest (milkshake intake, BMI, hunger, or pleasantness rating).

Three additional regression analyses were performed in which *ad libitum* milkshake intake was treated as a regressor of interest, whereas BMI, hunger, or pleasantness respectively were included as nuisance variables. The resulting correlation maps specifically show regions that show a response with ad libitum milkshake intake that is independent of BMI, hunger, or pleasantness.

Results

Behavioral Results

Average hunger and fullness ratings were near neutral before and after scanning ((mean ± SEM) hunger before scan: 8.92 ± 6.83; hunger after scan: 12.24 ± 7.21; fullness before scan: -14.95 ± 7.98; fullness after scan: -19.20 ± 7.14) and did not differ as a function of time (hunger, $F(1,19) = 0.345$, $p = 0.564$; fullness, $F(1,19) = 0.231$, p = 0.636). Before and after scanning, the milkshake was rated as pleasant (before scan: 26.34 ± 3.04; after scan: 25.19 ± 3.05 – note that a score of 17 corresponds to "like moderately" and a score of 44 to "like very much" with reference to all hedonic sensations imaginable), moderately intense (before scan: 20.95 ± 2.30; after scan: 20.05 ± 2.02), and wanted (before scan: 29.68 ± 4.83; after scan: 20.32 ± 6.03). There was no significant effect of time on pleasantness ($F(1,19) = 3.754$, p = 0.068) or intensity ratings ($F(1,19) = 0.803$, p = 0.382) but wanting ratings decreased pre to post scan ($F(1,19) = 7.445$, $p = 0.013$). The tasteless solution was rated as neutral in pleasantness (before scan: -0.47 ± 1.93; after scan: 0.66 ± 2.23), barely detectable

in intensity (before scan: 3.40 ± 0.63; after scan: 3.34 ± 0.70), and moderately unwanted (before scan: -15.26 ± 7.72; after scan: -15.82 ± 7.24), with no significant effects of time (pleasantness, $F_{(1,19)} = 0.298$, $p = 0.592$; intensity, $F_{(1,19)} = 0.015$, $p = 0.903$; wanting, $F_{(1,19)} = 0.015$, $p = 0.904$). The milkshake was rated as significantly more pleasant ($F_{(1,18)}=54.38$, $p<0.001$), more intense ($F_{(1,19)}=83.823$, $p<0.001$), and more wanted ($F_{(1,17)}=31.12$, $p<0.001$) than the tasteless solution.

Following the scan subjects consumed on average 216.91 ± 187.8 milliliters of milkshake, equivalent to 166.51 calories. To address whether quantity of *ad libitum* milkshake consumption was related to BMI, ratings of internal state or milkshake, or eating style, we correlated milliliters drank with each of these variables across subjects. How much milkshake a subject drank was not correlated with BMI ($r = -0.015$, $p = 0.951$), hunger ($r = 0.120$, $p = 0.615$), fullness ($r = 0.203$, $p = 0.390$), pleasantness ($r = 0.284$, $p = 0.225$), intensity ($r = -0.175$, $p = 0.460$), wanting of the milkshake ($r = 0.315$, $p = 0.189$), or eating style (TFEQ restraint, $r = 0.226$, $p = 0.353$; TFEQ disinhibition, $r = -0.199$, $p = 0.415$; TFEQ hunger, $r = -0.152$, $p = 0.534$; PFS, food available, $r = -0.076$, $p = 0.758$; food present, $r = -0.054$, $p = 0.826$; food tasted, $r = -0.078$, $p = 0.750$).

fMRI Results

A main effect of milkshake vs. tasteless was observed in the mid insula bilaterally, indicating that the task activates the expected chemosensory area (Figure 1). A full list of the activations that resulted from the main stimulus contrast can be found in Table 1.

Next, we examined the relationship between brain activity and milkshake consumption by regressing ad libitum milkshake intake against BOLD response to milkshake vs. tasteless. As predicted, a significant positive association was observed between intake and response in the midbrain (Table 1; Figure 1). We also observed a trend towards a similar positive relationship between intake and activity in the mOFC (Table 1; Figure 1). However, no other positive correlations were observed and none of the predicted negative associations were observed. Unexpectedly, the midbrain activation that was observed originates from the peri-aqueductal gray (PAG) region rather than the ventral tegmental area (Mai, Assheuer and Paxinos, 2004). Since there is evidence that spatial normalization procedures aimed at whole brain registration may have shortcomings in the midbrain we re-did the analysis using the Automated Brainstem Coregistration method outlined by Napadow and colleagues (2006). Spatial coregistration was performed in two steps. First, we derived a globally defined affine transformation and then we refined this transformation by using a brainstem-weighted affine transform (MNI-152 brainstem mask). This analysis again produced significant activation centered around the PAG (-3, -31, -20; $k = 15$; $z = .05$; data not shown).

No significant associations were observed between behavioral measures (BMI, hunger, wanting, and pleasantness) and BOLD response. Including BMI, hunger, and wanting as covariates did not markedly change the magnitude of the relationship between the midbrain and mOFC response and ad libitum milkshake intake (Table 1). However, when pleasantness was included as a covariate the association between milkshake intake and the BOLD response in both the midbrain and the mOFC became weaker (non-significant at $p < 0.005$ uncorrected for multiple comparisons). We therefore performed a mediation analysis to test whether pleasantness ratings mediated the relation between BOLD response in midbrain and mOFC and subsequent intake. They did not ($p = .54$ for the midbrain and $p = .16$ for the mOFC).

Finally, to test for non-linear effects we examined brain response to milkshake *minus* tasteless with three additional regressors; quadratic, cubic and log transformations of the

original intake regressor. The medial OFC showed non-significant trends with the quadratic and cubic transformations. No other effects were observed.

Discussion

The primary aim of this study was to test whether response to tasting small quantities of milkshake predicts subsequent milkshake intake in subjects who report being neither hungry nor full. In other words, we wished to isolate regions of the brain that might promote eating in the absence of hunger. Our primary prediction was that enhanced responses in the midbrain, insula, striatum and medial OFC and decreased responses in lateral OFC and lateral prefrontal cortex to the taste of milkshake would be associated with milkshake intake. In keeping with our prediction, response in the midbrain, possibly corresponding to the PAG, was positively associated with immediate subsequent *ad libitum* intake of milkshake. Also consistent with our hypothesis was a trend towards a similar effect in the medial OFC. However, in contrast to our hypotheses no relationships were observed in the insular, striatum, lateral OFC or lateral prefrontal cortex.

In humans, midbrain response to chocolate consumption correlates positively with pleasantness ratings (Small et al, 2001), while response here to food pictures has been shown to correlate with individual differences in sensitivity to reward (Beaver et al., 2006) and with behavioral preferences for foods (O'Doherty et al., 2006). Frank and colleagues found that midbrain response to the taste of sucrose, but not sucralose, correlates with ratings of stimulus pleasantness (Frank et al., 2008), which suggests that the midbrain response to pleasurable foods is also dependent upon metabolic effects. However, an important consideration is that the effects reported in these studies appear to be largely restricted to the ventral tegmental area and substantia nigra. In contrast, in the current study, the midbrain response can be localized primarily to the PAG. This dorsomedial region of the midbrain has a well-known role in pain, but there is only indirect evidence implicating the region in feeding. First, the PAG is rich in opioid receptors and endogenous opioid peptides (Gutstein et al., 1998; Mansour et al., 1995a; Mansour et al., 1995b). Naturally occurring opioids have been repeatedly implicated in the hedonic aspects of feeding (Peciña et al., 2006), enhancing food palatability and contributing to the consummatory experience of "liking" (Kelley et al., 2002; Wassum et al., 2009; Yeomans and Gray, 1996; 2002). Therefore it is possible that the association we observed reflects a relationship between opiod signaling in the region and subsequent food intake. However, arguing against this possibility, while we found that inclusion of subjective pleasantness, but not hunger ratings, impacted the relationship between midbrain response and intake, pleasantness ratings did not mediate the effect of the midbrain on intake. This suggests that midbrain responses might reflect an aspect of reward that was not assessed in the current study, such as incentive motivation or the reinforcement value of the food. Consistent with this possibility, medial prefrontal cortex projections to the PAG (Mota-Ortiz et al., 2009) are thought to be critically involved in opiod activation of the PAG to promote food foraging behavior in rats (Miranda-Paiva et al., 2003).

Another possibility is that the BOLD signal reflects cannabinoid signaling. Intravenous injection of the synthetic cannabinoid agonist HU210 modulates BOLD responses in the mouse PAG (Shah et al., 2004), which is intriguing since cannabinoid agonists produce clear hyperphagia (Abel, 1971; Williams et al., 1998; Koch, 2001 and Higgs et al., 2003).

Finally, there are dopamine neurons in the PAG, also known as the A10 dorsocaudal group (Hokfelt et al., 1984; Hasue and Shammah-Lagnado, 2002). Although the role for these cells in reward has not been well studied, they have been implicated in mediating the rewarding and sensitizing properties of heroin (Flores et al., 2006). Whether PAG dopamine neurons,

like the ventral tegmental dopamine neurons (Abizaid et al., 2006a; Berthoud, 2002) are directly modulated by hormones to promote eating is unknown. However, it is nevertheless possible that the association between PAG response to milkshake and intake reflects dopaminergic activity in this region.

One potentially fruitful avenue for future research will be to include the PAG in region of interest analyses that have been used to evaluate the influence of dopamine, cannabinoid, and opiod antagonism on brain response to food (e.g. Rabiner et al., 2011; Dodds et al., 2012). Additionally, it would be interesting to test whether common polymorphisms that are related to food intake and dopamine or opiod signaling may influence the association between PAG response and intake. For example, Davis and colleagues reported that obese individuals are more likely to possess the G allele of the A118G polymorphism of the mu-opiod receptor gene (Davis et al., 2009) if they carry a diagnosis of binge eating disorder. Testing whether the association between PAG response to palatable food and intake is modulated by this genotype might provide insight into the neurochemistry behind BOLD responses observed here.

Like the midbrain and medial OFC, response in the insula is positively associated with reported pleasure during chocolate consumption (Small et al., 2001). The insula has also been implicated in compulsive eating (Woolly et al., 2007) and in weight gain in youth at risk for obesity by virtue of parental obesity (Stice et al., 2011). Response here remains elevated in post-obese individuals (Del Parigi et al., 2004) and it was recently reported that weight loss is associated with a reduction in insular responses (Cornier et al., 2012). These data strongly suggest that variation in insular response may be associated with susceptibility for weight gain. However, in the current study, although we observed strong insular responses to milkshake – tasteless in the insula, this response was unrelated to subsequent intake. This suggests that the role the insular cortex plays in regulating (or dysregulating) feeding is more likely related to variations in homeostatic or internal state controls rather than in the influence of the hedonic aspects of food to influence consumption. This possibility is consistent with the fact that the insula is sensitive to hunger states (Tataranni et al., 1999) and that response to food cues is modulated by endocrine factors such as ghrelin (Malik et al., 2008). Another possibility is that insular responses reflect an interaction between internal state and the appetitive characteristics of foods to influence intake. In this case we would have failed to observe an association because our subjects were not hungry.

We also failed to observe associations between lateral OFC or lateral prefrontal cortex and intake. These regions have been shown to play an important role in inhibiting hedonic eating (Hare et al., 2008 and 2011) and in consciously regulating desire for food upon exposure to food images (Wang et al., 2009; Hollmann et al., 2012). Both areas are also less responsive to food cues in individuals with high vs. low BMI (Batterlink et al., 2010; Del Parigi et al., 2003; Volkow et a., 2009). There are a variety of possible reasons for this lack of association. Most notably, our paradigm did not measure choice or explicit attempts to exercise self-control, nor did we examine intake of foods other than the milkshake. Therefore it is critical to interpret this negative finding with caution. Our group of subjects also all scored low on self-reported disinhibition towards foods, with all but one subject scoring between a 2 and an 8 on this subscale of the Three Factor Eating questionnaire. Finally, women were scanned in various phases of the menstrual cycle, which might have contributed to increased variance in response. It will be important for future studies to consider these and other factors to have a more comprehensive understanding of brain responses that predict *ad libitum* intake.

In summary, our findings show that response in the PAG to the taste of milkshake positively predicts immediate subsequent *ad libitum* intake of that milkshake, thus linking brain

response in this region with feeding behavior. We suggest that this result provides support for the possibility that PAG response to palatable food is related to the motivational drive behind food consumption in the absence of hunger.

Acknowledgments

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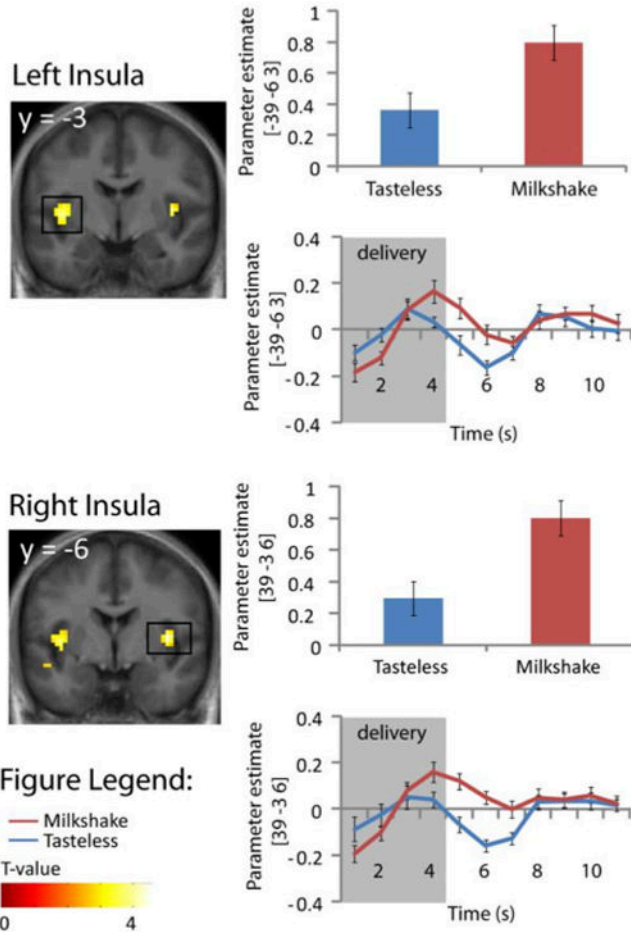
Highlights

1. Periacqueductal gray response to milkshake is correlated with subsequent milkshake intake.

2. This relationship is not influenced by ratings of hunger, BMI, or eating style.

3. Insular response to milkshake is NOT correlated with subsequent milkshake intake.
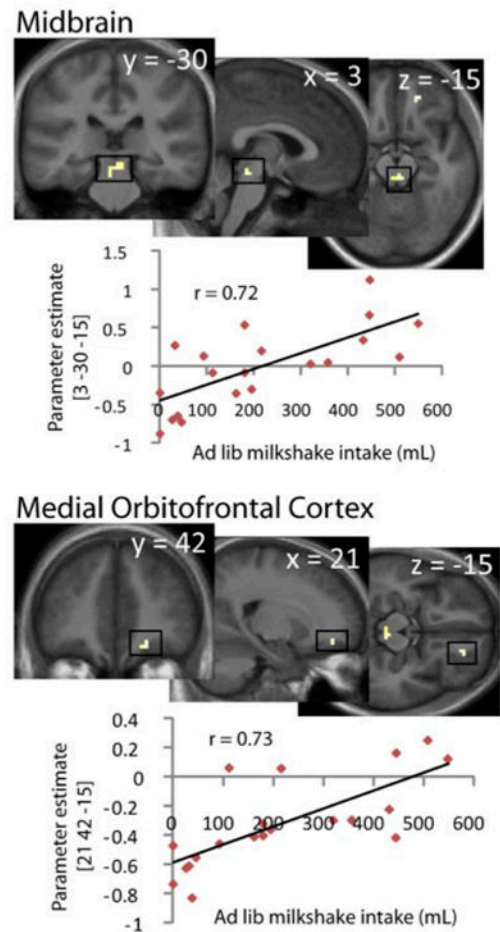
Figure 1. fMRI Results

Images displayed at p < 0.005. Color bar depicts T-values. **A.** Significant response in the left and right mid-insula to Milkshake – Tasteless. Bar graphs for tasteless solution (blue) and milkshake (red) (from the peak voxel). Time courses for tasteless solution (blue) and milkshake (red) (from the peak voxel). **B.** Positive correlation between BOLD response and ad libitum intake. Response in the midbrain is positively related to intake. The scatter plots illustrate the correlation between ad libitum milkshake intake (x-axis), and parameter estimate (PE) (y-axis).

Table 1

	x,y,z MNI[a]	K[b]	Z	P_FDR
Main effect Milkshake – Tasteless				
Right mid-insula	39 −3 6	22	4.86	0.030[c]
Left mid-insula	−39 −6 3	51	4.28	0.042[d]
Left Anterior insula	−33 18 6	5	3.29	0.072[d]
Positive Correlation of Milkshake – Tasteless and Ad Lib Intake				
Right medial OFC	21 42 −15	4	3.67	0.061[d]
Covariate: BMI		4	3.57[d]	
Covariate: Hunger		4	3.86[d]	
Covariate: Wanting		9	3.41[d]	
Covariate: Pleasantness		4	3.40[d]	
Midbrain	3 −30 −15	9	3.59	0.045[d]
	−3 −30 −21		3.38	0.999[d]
Covariate: BMI		10	3.69[d]	
Covariate: Hunger		11	3.56[d]	
Covariate: Wanting		5	3.37[d]	
Covariate: Pleasantness		4	3.31[f]	

[a]Italics indicate that a peak falls under the same cluster as the preceding peak;

[b]Cluster size in voxels;

[c]Significant at P_FDRcorrected = 0.05 across the whole brain

[d]Significant at P_FDRcorrected = 0.05 across SVCs;

[e]Significant at P_FDRcorrected = 0.05 across the ROIs;

[f]Thresholded at p = 0.05