FOOD CUE REACTIVITY UNDER STRESS: A COMPARISON OF OBESE AND HEALTHY WEIGHT INDIVIDUALS USING FMRI

by

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(Under the Direction of SARAH FISCHER)

ABSTRACT

Obese individuals exhibit increased BOLD activation in limbic system regions after exposure to food cues. Few studies have examined neural processing of food cues in states of stress. The current study examined neurobiological responses to food cues in obese and healthy weight individuals prior to and following stress induction utilizing fMRI. Healthy weight individuals exhibited greater activation in anterior cingulate cortex (ACC) and nucleus accumbens (NAc) than obese participants after exposure to food cues and a stress induction. All participants exhibited a decrease in activation in these regions, and the insula, following stress induction; and had increased activation in the orbitofrontal cortex when contrasting post stress to pre stress food cues. Exploratory analyses indicated that regions other than a priori ROIs exhibited changes in activation from pre-stress to post-stress exposure to food cues. Results suggest that stress may cause deactivation in limbic system response to food cues in obese participants.

INDEX WORDS: Obesity, fMRI, Stress, Food, Cue reactivity

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B.S., The University of Georgia, 2008

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2013

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May 2013

DEDICATION

Throughout the course of this project I have learned a great deal about the work I love and my passion for this work. Most importantly, this time in my life has taught me more about myself than anything else. This thesis is dedicated to my family, closest friends, and major professor who have given me constant support and guidance. It is especially dedicated to my mom and dad who have provided unconditional love and support in all my endeavors. I love you both and so deeply cherish our relationship.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my major professor, Dr. Sarah Fischer for her support, patience, and guidance throughout the completion of this project. Many thanks are also extended to the members of my committee, Dr. L. Stephen Miller and Dr. James MacKillop, for their continued support. I would also like to thank collaborators, Carlos Faraco and Doug Terry on this project for their assistance, as well as the members of the Eating, Drinking, and Personality Lab at the University of Georgia.

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CHAPTER 1

INTRODUCTION

Obesity is defined by the World Health Organization (2000) as a body mass index (BMI) of 30 or higher, in addition to a waist circumference of 35 inches or greater in females and 40 inches or greater in males. In the preceding decades obesity (OB) has become a serious health problem within the United States. Approximately 34% of adults in the United States are obese. Furthermore, the CDC reported in 2010 that no state had an OB prevalence rate under 20%. There are a multitude of factors that promote the increasing rate of obesity within the United States (Brownell &Horgen, 2004). The overconsumption of foods high in fat and sugar is one such factor that contributes to the development of obesity, and has also been linked to psychosocial stress (World Health Organization, 2009). Additionally, cues for foods high in fat and/or sugar are omnipresent in the environment in the United States. The availability of high fat/high sugar foods and the presence of food cues are also linked to over consumption, and hence, obesity (Brownell & Horgen, 2004).

Cue Reactivity & Craving

Cue reactivity is the response an individual has to a stimulus with salient meaning. Several investigators conceptualize cue reactivity as a form of classical conditioning (Drobes, Saladin, & Tiffany, 2001; Niaura et al., 1988;Tiffany, 1995). When an individual consumes a substance, initially neutral stimuli become paired with the physiological processes involved in consumption. After many repeated pairings of neutral cues with consumption, the presentation of the cue alone can reliably elicit the same physiological response that occurs prior to or during consumption of that substance. For example, contextual cues in alcohol and drug addiction serve a major

function in alcohol and drug consumption, such that exposure to cues is associated with use (Siegel & Ramos, 2002). Furthermore, a meta-analysis of physiological responses to alcohol and drug cues found that substance cue-exposures produced reactions such as increased heart rate, increased sweat-gland response, and decreased skin conductance (Carter & Tiffany, 1999). The same meta-analysis demonstrated a large effect size for self-reported craving following cue-exposure. Thus, exposure to substance related cues can be understood to influence both physiological responses and craving(Carter & Tiffany, 1999).

It has long been established that some individuals tend to eat in response to environmental cues, as opposed to internal feelings of hunger (Schachter, Goldman, & Gordon, 1968). Healthy weight individuals react to food exposure physiologically with changes in gastric activity, heart rate, blood pressure, and salivation (Jansen, 1998; Nederkoorn, Smulders, & Jansen, 2000;Wardle, 1990). These changes are hypothesized to occur in response to learned associations between cues that signal food and impending consumption, and are thought to prepare the body for consumption and digestion (Jansen, 1998). Additionally, there are positive associations between these physiological changes and craving, and between craving and food intake (Jansen, 1998; Nederkoorn, et al., 2000). For example, presentation of food cues vs. neutral cues elicits craving for food in laboratory paradigms (Sobik, Hutchison, & Craighead, 2005). Cravings specifically for highly palatable foods (foods high in fat or sugar) are associated with increased consumption of those food items compared to other foods in a laboratory setting (Martin, O'Neil, Tollefson, Greenway, & White, 2008). In short, laboratory paradigms elicit craving in response to food cues, and several studies indicate that there is a positive association between craving ratings and subsequent consumption. This data suggests that environmental cue elicited craving

plays a motivational role in the excessive consumption of food, in a parallel fashion to cue elicited craving and consumption of drugs.

Reward System Activation in Response to Food

There are two pathways through which the human body regulates food intake: homeostatic and hedonic (Lutter & Nestler, 2009). The homeostatic pathway regulates eating by signaling the body when energy has been depleted via the release of leptin and ghrelin (Lutter & Nestler, 2009; Zigman & Elmquist, 2003). The hedonic pathway, also referred to as reward-based regulation, regulates the reward salience of food. The hedonic pathway may override the homeostatic pathway when there is a strong desire to eat (Kelley, Baldo, Pratt, & Will, 2005; Lutter & Nestler, 2009), Kelley and colleagues (2005) describe a complex neurobiological animal (rat) model through which the appetitive motivation for food may lead to overconsumption. This model posits that dopaminergic, GABAergic, and opiate peptide systems interact to influence overconsumption (Kelley, et al., 2005). These systems are thought to interact with corticolimbic structures and structures that regulate energy balance and food intake (e.g. the hypothalamus). Results from the investigation of many laboratory feeding paradigms are discussed below, which Kelley and colleagues incorporated into their model of neural mechanisms related to overconsumption.

First, GABA output neurons within the nucleus accumbens (NAc) shell have a direct influence on mechanisms for feeding motor patterns. GABA acts to inhibit activity within the shell of the NAc and increases strategies involved in food-seeking behavior (Kelley et al., 2005). When glutamate (an excitatory neurotransmitter) is stimulated in the shell of the NAc, consumption of palatable substances decreases in food deprived animals (Haberny, Berman, Meller, & Carr, 2004; Kelley & Swanson, 1997). One study examined the effect of lesions in the

shell of NAc (producing a complete lack of activity), and found that excitoxic lesions were associated with weight gain (Maldonado-Irizarry & Kelley, 1995). Of note, the investigators were able to distinguish that GABA activity within the NAc shell was related to increased intake of high-fat and sucrose food, but was not related to water or non-saccharin solutions (Maldonado-Irizarry & Kelley, 1995).

To explain the circuitry regulating GABA activity within the NAc shell, investigators probed the role of the lateral hypothalamus, which is the only region that directly projects to the shell (Kelley et al., 2005; Stratford & Kelley, 1997). The lateral hypothalamus contains mostly GABA substrates. Several studies support the functional link between these two structures in that GABA blockades between the two structures does not lead to food intake; and increased activity of GABA substrates in the lateral hypothalamus is necessary for rapid food intake mediated by the NAc shell (Stratford & Kelley, 1997; Baso & Kelley, 1999; Maldonado-Irizarry, Swanson, & Kelley, 1995). These results can explain how GABA modulation can affect food preference and intake beyond that which is necessary for homeostatic needs, but does not yet address the role of motivation or positive reinforcement for eating behavior (Stratford & Kelley, 1999).

In fact, a separate neurotransmitter system, the dopaminergic system, is involved in conditioned reinforcement of food intake (Berridge & Robinson, 1998; Salamone & Correa, 2002; Wise, 2004). Laboratory studies demonstrate that dopamine released within the NAc are related to the approach phase for food intake (Hajnal, Smith, & Norgren, 2004). Chemical lesions within the dopaminergic system, and depletion of dopamine in the NAc contribute to reductions in approach related behavior for food intake in animals (Baldo, Sadeghian, Basso, & Kelley, 2002; Kelley et al., 2005). Dopamine levels in the NAc are also related to orienting responses to conditioned stimuli (Rotiman, Stuber, Phillips, Wightman, & Carelli, 2004). Thus

far, it is apparent that GABAergic and dopaminergic systems within and surrounding the NAc have distinct effects on eating behavior.

Kelley and colleagues propose that these dopaminergic and GABAergic systems work together to incorporate anticipatory and consummatory effects of eating, and that a special network (striatal opiod) may develop for eating beyond what is allocated by homeostatic needs. This model highlights the above-mentioned proposal that hedonic pathways have a specialized network that provides ability to override homeostatic pathways. Given the support found for neurotransmitter activity within the corticolimbic system in animal models, it is appropriate to use fMRI to investigate the activity of these same regions in response to palatable food in humans.

Limbic System and Food Consumption

There is evidence in both rodent and human populations that drug abuse and consumption of foods high in fat or sugar are both regulated by the limbic system, which is associated with motivated behavior (Nestler, 2001, 2005). It is thought that this motivation is influenced by the release of dopamine within the mesolimbic dopamine pathway. The release of dopamine in the NAc is believed to mediate conditioned learning (the association of food stimuli and food consumption) as well as desire for food rewards (de Araujo et al., 2008). Several studies have documented the release of dopamine, as well as endocannaboids and opioids, within the limbic system in response to palatable food (Adam & Epel, 2007; Cota, Tschop, Horvath, & Levine, 2006), analogous to it's response to drugs (Boutrel, Kenny, Markou, & Koob, 2005; Harris, Wimmer, & Aston-Jones, 2005). Furthermore, the release of dopamine during this process may contribute to learned associations between excessive consumption and specific outcome

expectancies. In short, rodent and human models support the hypothesis that increased activation in the limbic system, mesolimbic dopamine system, and other pathway discussed, is related to excessive consumption of food and drugs (Kelley, et al., 2005; Volkow, Fowler, & Wang, 2002; Volkow & O'Brien, 2007; Volkow & Wise, 2005; Wang et al., 2001).

The Use of fMRI to Examine Limbic System Response to Food Cues

Previous research utilizing fMRI data acquisition has demonstrated that visual cues of palatable foods compared to neutral cues increase activation in the orbitofrontal cortex, the premotor cortex, the ventral striatum (including the NAc), the insula and the anterior cingulate cortex in healthy weight individuals (Beaver et al., 2006; Geliebter et al., 2006; Karhunen et al., 2000; Pelchat, Johnson, Chan, Valdez, & Ragland, 2004; Rolls & McCabe, 2007; Stice, Spoor, Bohon, Veldhuizen, & Small, 2008; Stoeckel et al., 2008). Various types of food stimuli and/or subject conditions have been manipulated in previous studies. One such condition is hunger vs. satiety. Activation in the insula, amygdala, and OFC appears to increase when individuals are exposed to food cues under conditions of hunger compared to satiety (Killgore & Yurgelun-Todd, 2006; LaBar et al., 2001; Siep et al., 2009). An additional manipulation is the presentation of palatable food cues vs. low calorie food cues instead of neutral cues. Typically, palatable or high calorie food cues elicit increased activation in all regions of interest described above compared to low calorie food cues (Rothemund et al., 2007; Stoeckel, et al., 2008). One study compared appetizing foods to bland foods and disgusting foods in individuals with varying levels of motivation for reward. Results revealed a linear trend, such that individuals strongly motivated for reward had greater BOLD signal change in response to appetizing food within the reward system (Beaver, et al., 2006).

Thus, several studies have indicated that limbic system activation increases in response to cues for palatable foods compared to other stimuli under varying conditions. The various empirical studies examining limbic system response to food cues can be integrated in the following manner. Acute increases in dopamine in the accumbens and striatum in response to cues increase motivation to consume, and reinforcement from consumption, while activation in the amygdala may link memories of food to their rewarding properties. Impaired inhibitory control due to increased activation in the prefrontal and anterior cingulate cortex may ultimately lead to the behavioral choice to engage in repeated consumption. These studies have contributed to our understanding of the neurobiological pathways that may lead to overconsumption of food. However, they were all conducted with healthy weight, non-eating disordered individuals.

Response to Food Cues in Obese Samples using fMRI

Several studies have utilized fMRI to examine changes in neural activation in response to food cues in obese individuals compared to health weight individuals. Rothemund and colleagues investigated blood oxygen level dependent (BOLD) signal differences between obese and healthy weight participants while they viewed high calorie foods (Rothemund et al., 2007). Activation within the putamen, caudate, anterior insula, hippocampus, and parietal lobule was greater in the obese group compared to the healthy weight group. However, the same visual food cues were repeatedly presented in this study. Hence, habituation to stimuli may have occurred. In order to address some of these methodological concerns, Stoeckel and colleagues used a block design without repeated images to compare differences in BOLD signal for high and low calorie foods in both healthy weight and obese women (Stoeckel, et al., 2008). In this study, obese women displayed greater BOLD signal activation to high calorie food cues within the orbitofrontal cortex, medial prefrontal cortex, anterior cingulate cortex, insula, amygdala, NAc,

ventral pallidum, and hippocampus than healthy weight women. They also displayed greater activation to high calorie vs. low calorie foods in the orbitofrontal cortex, medial prefrontal cortex, anterior cingulate, insula, NAc, amygdala, hippocampus, caudate, and putamen (Stoeckel, et al. 2008). The investigators found similar results when comparing high calorie foods to neutral stimuli. Viewing low-calorie vs. high calorie foods did not produce significantly greater activation in these regions within the obese group (Stoeckel, et al., 2008).

Other studies have utilized fMRI to investigate BOLD response in obese and healthy weight samples when participants view high calorie foods before a consumption of a meal compared to after consumption of a meal (Martin, et al, 2010; Dismitropoulos, Tkach, Ho, & Kennedy, 2011). In the premeal condition, obese individuals displayed greater activation for food vs. nonfood cues than the control group in the following regions: anterior cingulate cortex, medial prefrontal cortex, medial frontal gyrus, middle frontal gyrus, and the inferior frontal gyrus (Martin, et al., 2009). The control group did not display greater activation in response to these stimuli in any area compared to the obese group before consuming a meal. In the post-meal condition, obese participants displayed greater activation than control participants in medial prefrontal cortex, superior frontal gyrus, caudate, and hippocampus. Similar to the pre-meal condition, control group participants did not display greater activation in any of these regions than obese participants. Self-reported disinhibition and hunger were associated with increased activation in the anterior cingulate cortex and the medial prefrontal cortex in obese participants (Martin, et al., 2009).

The second study to examine the effects of satiety on food cue processing had a standardized waiting period following the meal prior to scanning (Dismitropoulos, et. al., 2011). In this study, obese participants had greater activation in response to both high and low calorie food cues after

eating compared to control participants. Similar to previous findings (Martin, et al., 2009), obese participants exhibited greater activation than control participants in similar limbic and frontal regions when comparing high vs. low calorie cues after eating a meal (Dismitropoulos, et. al., 2011). These results are particularly interesting in that obese participants reported decreases in hunger following the meal, but displayed greater reward system activation than healthy weight individuals. Thus, in several studies of BOLD response to food cues in which hunger and satiety were manipulated, obese participants displayed different neurobiological responses to those cues than healthy weight participants. This may indicate that there is a relationship between weight status and reward system processing of food cues.

Another study manipulated cues indicating the actual delivery of a milkshake (into the mouths of participants via a tube) vs. neutral solution, and demonstrated that obese individuals have differential activation in several regions in response to anticipation of food in comparison with healthy weight controls (Stice, Spoor, Bohon, Veldhuizen, and Small, 2008). The study found that obese participants had increased activation compared to healthy weight participants in the insula and operculum when anticipating and consuming a milkshake. These regions are thought to be involved in sensory and hedonic aspects of food intake. Interestingly, obese (vs. lean) participants had decreased activation within the caudate nucleus in response to milkshake consumption. Investigators hypothesized that this may be a result of decreased dopamine receptor availability (Stice et al., 2008).

In 2011, Stice and colleagues replicated this study in order to examine the decreased striatum response previously observed in obese individuals during food consumption. The study was also extended to investigate how the perception of calorie content affected reward processing during food consumption. The original results were replicated in this study, except that hypo-activation

in the striatum was not observed. Obese (vs. lean) individuals did display differential activation based on the perception of low vs. high fat milkshake. The results speak to how meaningful food cues can be in reward processing from both anticipating and consuming palatable foods of different caloric content.

Since many studies have documented differences between obese and healthy weight participants in reward system processing, a key question is whether functional interactions between key reward network areas may be disrupted. In one such study, a path analysis was used to investigate group differences of network connections between NAc, amygdala, and orbitofrontal cortex for high vs. low calorie foods in obese and normal-weight participants (Stoeckel, et al., 2009). The results supported abnormal connectivity between these regions for the obese vs. normal-weight participants. Specifically, obese participants had a deficiency in the amygdala modulation of activation in NAc and orbitofrontal cortex (Stoeckel et al., 2009). These deficient pathways may be involved in affective and motivational aspects of the reward salience of food. This model indicates that greater activation within a specific region is not the only pathway to disrupted reward system processing.

A multitude of methods and designs have been used to investigate neurobiological responses to food cues in obese individuals. Taken together, these studies indicate that obese individuals display significantly greater activation in several regions (limbic, gustatory, somatosensory) then healthy weight individuals when exposed to palatable food cues. However, food cues are not the only trigger for overconsumption of high fat/high sugar foods. To date, there is a dearth of research being conducted in the fMRI environment in which emotional state is manipulated while viewing food cues. Overconsumption of high fat and high sugar foods has been specifically linked to psychosocial stress (Coccurello, D'Amato, & Moles, 2009). Research on

activation of the limbic system in response to food cues under stressful conditions is an important step towards understanding the chronic nature of overconsumption that is often associated with obesity.

Neurobiology of Stress and Food Consumption

Exposure to Stress Increases Craving in Response to Food Cues

It has long been thought that stress influences diet choices and eating behavior (Wardle & Gibson, 2002; Torres & Nowson, 2007). Stress is generally defined as "the generalized, non-specific response of the body to any factor that overwhelms, or threatens to overwhelm, the body's compensatory abilities to maintain homeostasis," (Sherwood, 2001). Stressors vary in type and effect on physiological response. For example, stressors may be acute or chronic in nature. Stress typically produces a fight or flight response that induces activity within the sympathetic adrenal medullary system. A passive response to a stressor is thought to activate the hypothalamic pituitary adrenal (HPA) axis. Hyperactivity of the HPA contributes to the release of glucocorticoids, specifically cortisol (Lackshweitz et al., 2008). Stress can either increase or decrease likelihood of food consumption via activation of these pathways and subsequent release of hormones.

Animal models of feeding behavior under stress conditions indicate that chronic exposure to stress increases consumption of highly palatable, high fat foods (Dallman, Pecoraro, & la Fleur, 2005; Fachin et al., 2008). In animals, this is likely due to food choice as opposed to chronic excessive caloric consumption. It is important to note that while acute stress in animal models often leads to weight loss, exposure to chronic stress also leads to the preference for high fat foods (Dallman et al., 2003). For example, rats in a chronic stress condition chose foods such as lard or sugar over other types of foods (Dallman et al., 2003, 2005). Ingestion of highly palatable

foods increases activation of opioid and cannabinoid systems (Adam & Epel, 2007), leading some to hypothesize that the ingestion of foods high in fat and sugar reduces the acute effects of stress.

Several laboratory studies have examined the effect of stress on eating behavior in humans. Specific studies of food choice in humans, similar to animal studies, indicate that individuals choose high fat foods under stressful conditions (Grunberg & Straub, 1992; Oliver, Wardle, & Gibson, 2000). For example, daily hassles such as ego threatening stressors, work stressors, and interpersonal stressors are associated with increased consumption of snack foods (O'Connor et al., 2008). The only daily stressors found to be associated with decreased snacking behavior were physical stressors (O'Connor et al., 2008). Attentional bias to food cues also appears to increase in conditions of acute stress (Newman et al., 2007). The relationship between stress and snacking may be stronger in obese individuals than healthy-weight individuals (O'Connor et al., 2008).

Gender is one variable that may affect HPA activity in response to stress. Some research suggests that under stressful conditions, women increase food consumption more than men (Greeno & Wing, 1994). Even within gender there can be variation in how HPA axis activation contributes to changes in eating behavior under stress. For example, in a laboratory study of stress, cortisol response, and eating behavior in pre-menopausal women, the subjective experience of stress did not predict whether participants would eat high vs. low calories foods. However, women who produced high levels of cortisol in response to the stress condition ate more calories and chose high fat foods compared to women who produced low levels of cortisol post stress (Epel et al., 2001). While a subgroup of human individuals consume less and lose weight during exposure to stress, most report increased consumption (Adam & Epel, 2007; Epel et al., 2004).

Several paradigms indicate that it is not simply the emotional experience of stress that leads to increases in food consumption, but also the acute increase of cortisol activity which influences this behavior. Individuals with high cortisol activity tend to consume more food in a laboratory stress paradigm (Epel, Lapidus, McEwen, & Brownell, 2001). High cortisol activity is also associated with increased consumption in a naturalistic setting following a laboratory stressor (Newman, O'Connor, & Conner, 2007). Additionally, individuals in stress manipulation conditions who produce high levels of cortisol chose high fat foods as opposed to low calorie foods (Epel et al., 2001; Newman et al., 2007). Finally, acute administration of glucocortoids in humans causes an increase in food consumption (Tataranni et al., 1996; George, Khan, Briggs, & Abelson, 2010). Thus, it is plausible that stress plays a role in dysregulated eating behavior and food craving.

Stress and the Limbic System

A review by Sinha (2001) identified common pathway between stress and response to rewards (both in illicit drugs and palatable foods) in the mesolimbic dopamine system and the HPA axis. Under conditions of stress, the HPA axis is activated, which releases corticotropin-releasing hormone from the hypothalamus, adrenocorticotropic hormone (ACTH) from the anterior pituitary, and cortisol (in humans) from the adrenal cortex (Pruessner et al., 2008). Studies in rodent populations have indicated that cortisol influences activation in several components of the limbic system, including the hippocampus, amygdala, and prefrontal cortex (Pruessner et al, 2008). These studies have identified that the hippocampus has an inhibitory effect on the HPA axis (Herman et al., 2003), which may cause disruption in the feedback loop of cortisol (Bruder, Jacobson, & Raff, 2005; Herman, Ostrander, Mueller, & Figueiredo, 2005). The prefrontal cortex and specifically the anterior cingulate (a neocortical regulatory structure)

may have both inhibitory and excitatory effects on the HPA axis depending on which specific nuclei are activated, while the amygdala is purely excitatory (Herman et al., 2005). The complex relationship between neuroendocrine stress responses and the limbic system is not fully understood. However, preliminary data suggest that disruption in the HPA axis may have profound effects on stress and reward processing in the limbic system. Given the common neurocircuitry involved in both responses to food cues and acute stress, there is a need to understand the role stress plays in the neurobiological response to food cues, and how this response may influence excessive consumption.

fMRI and Acute Stress Inductions

A meta-analysis (Dedovic, D'Aguiar, & Pruessner, 2009) examined the impact of acute stress inductions on BOLD activation in various brain regions. This meta-analysis highlighted that various methods have been used to induce stress within the fMRI environment and have not always produced consistent effects across methodologies. For example, recall of personally distressing or stressful situations produces BOLD activation in different regions than serial subtraction tasks (Tillfors, et al., 2001; Dedovic et al., 2009). Although there are some inconsistent findings, other regions have been identified that display common patterns of activation in response to acute stress, regardless of method of stress induction. These common patterns include decreased activation in the orbitofrontal cortex, increased activity in frontal lobes (specifically the anterior cingulate cortex), and deactivation within the hippocampus (Dedovic et al., 2009).

Because of the role stress can play in the relapse of addictive behaviors, several recent studies have investigated neural processing of stress during cue-reactivity paradigms. Dagher and colleagues investigated neural activity in response to smoking cues following a stress induction

(Dagher et al., 2009). They found that deactivation within the NAc during the stress induction was positively correlated with activation in the medial prefrontal cortex, anterior cingulate cortex, caudate, amygdala, hippocampus, dorsomedial thalamus, posterior cingulate cortex, and primary and extra-striate visual areas during exposure to smoking cues post-stress induction (Dagher et al., 2009). In sum, this study demonstrated that acute stress induction increased neural response to smoking cues within many limbic regions.

The shared circuitry of stress and cue-reactivity has also been investigated with regard to eating behavior. In one study, investigators presented participants with three types of stimuli (acute stress, cues for favorite foods, or neutral/relaxing cues) in the fMRI environment to examine common underlying neural circuitry and its relationship to subjective ratings of stress and food craving (Jastreboff, et al., 2013). Increased BOLD activation was present in similar regions across acute stress conditions and favorite food cue conditions. Specifically, the thalamus, striatum, hippocampus, anterior cingulate, and medial frontal gyrus (among other regions) were involved in both stress and cue-related processing (Jastreboff, et al., 2013). This study was one of the first studies to directly compare neural circuitry between an acute stress induction and food cue reactivity. However, it did not examine neural response to food cues while participants were in an acute stress state. Thus, this study did not address how acute stress directly impacts neural networks involved in food cue processing.

Cues, Craving, Stress, and Reward Response

Cues for high fat/high sugar food elicit craving for those foods, and craving has been linked to food consumption. Food cues are omnipresent and have been linked to overconsumption and hence obesity (Federoff, Polivy, & Herman, 1997; Halford, Gillespie, Brown, Pontin, & Dovey,

2004; Jansen, et al., 2003; Laibson, 2001; Martin, O'Neil, Tollefson, Greenway, & White, 2008; McKennah 1972; Rogers & Hill, 1989; Tom & Rucker, 1975; Wansink, 2001, 2004; Wansink, Painter, & North, 2005). Food cues tend to produce BOLD activation in several regions of the brain, especially regions that have also been implicated in drug and alcohol addiction. Thus, in parallel, increased limbic system activation to food cues is also hypothesized to influence craving, motivation for food, and hence consumption. Additionally, obese individuals tend to have increased BOLD activation in response to food cues in comparison to healthy weight individuals. This suggests that neurobiological responses to food cues may be dysregulated in obese individuals. A large body of research has also linked acute psychosocial stress to the consumption of high fat/high sugar foods. Disruptions in the limbic system have also been noted under conditions of acute psychosocial stress. However, psychosocial stress tends to produce decreases in activation in several of the same regions that exhibit increases in activation in response to palatable food cues. Very few studies to date have examined both acute psychosocial stress and processing of food cues in an fMRI environment. Thus, limbic system response to food cues under conditions of psychosocial stress is unknown.

Current Study

The purpose of this study was to examine BOLD activation in obese individuals compared to healthy weight controls in response to exposure to food cues prior to and following an acute stress induction. This study examined brain responses in an fMRI laboratory paradigm designed to model two conditions, which influence excessive consumption; environmental cues and stress exposure. The study used a two-group mixed design, with the group independent variable being obese weight status vs. healthy weight status, and the within-subjects independent variable being pre-stress and post-stress response to food cues. In addition, the study investigated obese vs.

healthy weight participants' responses to food vs. neutral cues. It was hypothesized that (1) compared to neutral cues, all participants would exhibit increases in BOLD activation in the following regions of interest: orbitofrontal cortex (OFC), the premotor cortex (PMC), the nucleus accumbens (NAc), amygdala, the insula, and the anterior cingulate cortex (ACC) in response to palatable food cues. (2) Compared to healthy weight individuals, obese individuals would exhibit significantly greater BOLD activation when contrasting food cues to neutral cues in a priori hypothesized regions of interest: OFC, PMC, NAc, amygdala, insula, and ACC. (3) Compared to pre-stress conditions, participants would demonstrate within group increases in BOLD activation following a stress induction in response to visual presentation of food cues in the hypothesized regions of interest: OFC, PMC, NAc, amygdala, insula, and ACC. (4) Compared to healthy weight individuals, obese individuals would exhibit significantly greater BOLD activation in response food cues following the stress induction in the following regions of interest: OFC, PMC, NAc, amygdala, insula, and ACC.

CHAPTER 2

METHODS

The current study utilized a mixed design. The study utilized fMRI to compare BOLD activation changes in response to food cues vs. neutral cues in non -eating disordered obese participants vs. non-eating disordered healthy controls, and within group changes in response to food cues following a stress induction. (Obesity in this study is defined as having a body mass index (BMI) of 30 or higher, and a waist circumference of 35 inches or more for women and 40 inches or more for men). The between groups variable is obesity vs. normal weight, and the within groups variable is pre vs. post stress induction. The primary outcomes of interest are differences in BOLD activation changes in obese vs. normal weight individuals in the insula, NAc, orbitofrontal cortex, premotor cortex, amygdala, and anterior cingulate cortex in response to visual presentation of food cues prior to and following a stress induction.

Participants

A total of 10 obese participants and 10 healthy weight controls were recruited from the northeast Georgia region. All participants were screened via the telephone for eligibility. Participants who were affected by any of the following were excluded from the study: ferrous-based tattoos, contraindicated surgical or metal implants, women who were pregnant or possibly pregnant, women who were nursing, individuals with Type I or Type II Diabetes, history of traumatic brain injury (TBI), Anorexia Nervosa (AN), Binge Eating Disorder (BED), Bulimia Nervosa (BN) (including subclinical binge eating and purging behavior), women taking oral contraceptives, individuals with substance dependence, history of psychotic symptoms, a diagnosis of Major Depression in the last 12 months, and anyone under the age of 18 or over the age of 45. The study was approved by the Institutional Review Board.

Procedure

Assessment Session

After meeting preliminary criteria via phone screen, participants were asked to come in for an assessment session. During the assessment, trained clinical interviewers obtained informed consent and administered the Structured Clinical Interview of DSM Disorders-I (SCID-I) modules for substance dependence, depression, and psychotic symptoms; the Eating Disorder Examination (EDE) Interview; the assessment of conditions prohibiting scanning and affecting cortisol reactivity (claustrophobia, metal implants or fragments in the body, pacemakers, internal stimulators, shunts, surgical staples, screws, plates, respiratory conditions, and intrauteran devices); Dutch Eating Behavior Questionnaire (DEB-Q); Eating Expectancies Inventory (EEI); Purging Motives Questionnaire (PMQ); Eating Motives Questionnaire (EMQ); Food Craving Inventory (FCI); and a demographics questionnaire. Participant's height, weight, waist circumference, and shoulder width measurements were collected to ensure eligibility for scanning. Following assessment, participants were given compensation for their participation in the study.

Scan Session

Following the assessment session participants returned for a second session to acquire fMRI data. Each session began at approximately 1:30 pm, as this time was optimal for examining hunger due to natural variations in hunger-related hormone fluctuation. At the beginning of this session, participants provided a baseline rating of subjective craving and stress. Each participant was provided a standardized meal consisting of approximately 20% fat, 20% protein, and 60% carbohydrates. Participants waited approximately one hour in order to digest the meal. During this time, trained interviewers administered a timeline follow-back eating

assessment for the 48 hours leading up to the scan session. Participants completed a stressors questionnaire during this time. Participants rated their subjective levels of craving and hunger for a second time after the one-hour waiting period. Participants were trained how to complete three different tasks in the scanner. Specifically, each participant was trained how to use button response pads to answer subjective rating for craving and stress, to choose picture stimulus orientation (horizontal vs. vertical), and lastly to choose correct answers to arithmetic problems. Participants were lead to the scanning room where conditions prohibiting scanning were re-reviewed by an MRI technician. Scanning began at approximately 3:00 pm. After fMRI data acquisition participants were debriefed and compensated for their participation in the second session.

Materials

Assessments and Measures

Structured Clinical Interview of DSM Disorders-I (SCID-I) for substance dependence, major depression, and psychotic symptoms (First, Spitzer, Gibbon, & Williams, 1996). The SCID-I is a semi-structured interview used for diagnosing DSM-IV Axis I disorders. A clinician or trained mental health professional typically conducts this interview. The SCID-I consists of separate modules for each axis I diagnosis. The modules used in the current study include assessment for substance dependence, major depression, and psychotic symptoms.

Eating Disorder Examination (EDE; Cooper & Fairburn, 2006**)** interview is a semi-structured interview designed to assess eating disorder symptoms and to assign eating disorder diagnoses. The EDE has four subscales including weight concern, shape concern, eating concern, and restraint. The composite score of theses four subscales creates a global EDE score, which

indicated levels of cognitive symptomatology and eating pathology. The EDE also yields a frequency count of binge eating and purging behaviors within the past 28 days, 60, and 90 days.

Dutch Eating Behavior Questionnaire (DEB-Q; van Strein, 1986) is an 33-item self-report measure of eating behavior. Each item is scored on a 5-point likert scale ranging from "1 - never" engaging in the behavior, to "5" engaging in the behavior "very often." Scores on the DEB-Q are divided into seven categories: very high, high, above mean, mean, below the mean, low, and very low. The DEB-Q has been was normed on 1170 individuals including men and women, and individuals of an obese status. The DEB-Q has emotional, external, and restrained eating subscales. Items on the external eating subscale assess individual differences in the tendency to eat in response to external cues. Cronbach's alpha for the external eating subscale in this sample was .91.

Eating Expectancies Inventory (EEI) (Hohlstein, Smith, & Atlas, 1998), is a 34 item self-report measure of what outcomes individuals expect from eating food. These are collectively referred to as eating expectancies, which are thought to have developed over the course of an individual's life through various learning experiences. An example of an eating expectancy is "eating fills some emotional need" or "eating helps me deal with feelings about inadequacy about myself." Five factor structures were identified in this measure and have been confirmed with independent samples (Hohlstein, et al., 1998). These factors include expectations that eating will reduce negative affect, induces positive moods, result in loss of control, enhance cognition, and relieve boredom. The five factors comprised within the EEI have also shown discriminant and convergent validity various clinical groups with eating disorders (Hohlstein, et al., 1998). Cronbach's alpha in the current sample was adequate (alpha = .80).

Eating Motives Questionnaire (EMQ) (Jackson, Cooper, Mintz, & Albino, 2003) is a 20 item self-report measure that was adapted from the Drinking Motives Questionnaire. The EMQ has four subscales to assess different motivations for eating including, social motivation, coping with negative affect, compliance, and enhancement. Cronbach's alpha for each subscale in this sample was >.80.

The Food-Craving inventory (FCI: White, Whisenhunt, Williamson, Greenway, & Netemeyer, 2002) was developed as a self-report measure of general and specific food cravings. Twenty-eight food items representative of four food types are rated for frequency of craving using a 5-point Liker scale ranging from "Never" to "Almost every day." Exploratory and confirmatory factor analyses reveal a four- factor structure representing four subscales: high fats, sweets, carbohydrates/starches, and fast-food fats. Each factor is also highly correlated, suggesting that each is a unitary dimension of a higher order factor of food-craving. Psychometrics in the development sample revealed moderate to high internal consistency (range of α coefficients. 0.76 - 0.93) and test retest reliability (range, 0.79 - 0.91) for all subscales and the total score. Self-reported craving, as measured by the FCI, has been associated with subsequent specific food consumption within a laboratory setting, suggesting that specific cravings are related to corresponding food intake (Martin, O'Neil, Tollefson, Greenway, & White, 2008). Cronbach's alpha in the current sample was .98.

Other questionnaires included were a demographics questionnaire, a questionnaire assessing conditions prohibiting scanning and affecting cortisol reactivity, a stressors questionnaire, and subjective ratings of stress and craving. The demographics questionnaire assesses demographic information from participants including: age, gender, social economic status, education level, and ethnicity. The stressors questionnaire was a measure of the total

number of stressful life events that occurred within the last year. Examples of life stressors include, but are not limited to, death in the family, separation or dissolution of family, and serious illness. The stressors questionnaire was included to account for factors that may exacerbate participant's subjective stress levels during the study. Subjective ratings of stress and craving are self-report measures of stress and craving utilized within and out of the MRI system. Participants are asked to rate their levels of stress/craving on a seven point likert scale ranging from "0 - no stress at all/no craving to eat at all," to "6 – the most stress I can handle/strong urge to eat food if it is available."

Stimuli

Pilot study.

Visual food cues and neutral cues were purchased from Shutter Stock Photos (www.shutterstock.com) based on reference IAPS (International Affective Picture Set; Lang, Bradley, & Cuthbert, 2005) images. Pilot data was collected to ensure that food and neutral cues purchased from Shutter Stock Photos were comparable to IAPS cues. The criteria for comparison of food cues and neutral cues between purchased photos and IAPS cues are discussed below. 15 participants (ages ranged from 22-30 years of age) were asked to rate 60 food cues on a Likert scale of 1 to 5. A rating of "5" represented that each cue was clear, easy to interpret, and appetizing. A rating of "1" represented that the cue had poor resolution, may be confused for other food, or was not appetizing. Participants were encouraged to rate food cues based on their preferences, not on the preferences of the general public (e.g. if a participant did not like apple pie, they were encouraged to rate it a "1", even though another person may enjoy apple pie).A rating of "5" for food cues included that the cue had good resolution, was easily perceived, and was appetizing to the point of craving for the food item. Participants were additionally asked to

rate 60 neutral cues on a Likert scale of "1" to "5".A rating of "5" for neutral cues included that the cue be clear and easily perceived. Additionally, the cue should not have elicited an emotional response or resemble food or drinks in any way. Neutral cues that failed to meet these criteria (where cues that were difficult to interpret, were reminiscent of food/drink, or aroused any emotion) were rated a "1". The mean of each cue was calculated. Only cues that had a mean of 3.00 or higher were used in the current paradigm.

IAPS stimuli.

Visual food cues and neutral cues were selected from the IAPS stimulus set (Lang, Bradley, & Cuthbert, 2005) and the pilot study described above. High palatability foods were used for several reasons. First, the theoretical model used to generate the hypotheses tested is based on a model of reward-motivated behavior. Consumption of food beyond what is required for energy balance/maintenance is one factor that contributes to overconsumption. This excessive consumption is hypothesized to be driven by several hedonic or reward based mechanisms. Foods that are viewed as highly rewarding/palatable are more likely to be those that are consumed in excess(Yeomans, Lee, Gray, & French, 2001). Additionally, previous research demonstrates that individuals exhibit differences in activation in response to photographs of high calorie foods vs. low calorie foods (Arana et al., 2003; Killgore et al., 2003). The neutral cues utilized in the current study have similar levels of visual complexity as the palatable food images, and so are hypothesized to require similar levels of visual processing.

Stress Induction

Stress was induced using a paradigm based on the Trier Social Stressor Task (TSST; Kirschbaum, Pirke, &Hellhammer, 1993). During the TSST, participants are asked to complete difficult math problems while being socially evaluated on their performance, in addition to being

asked to prepare and deliver a public speech on an unfamiliar topic, also while being socially evaluated on their performance. The current study utilized the arithmetic paradigm portion of the TSST. Several studies have documented that solving difficult arithmetic problems resulting in failure reliably induces stress (Kirschbaum, et al., 1993; Pruessner, Hellhammer, & Kirschbaum, 1999; Dickerson & Kemeny, 2004). While the mental arithmetic itself may produce stress, the social component is critical to the stress induction because it evokes social evaluative threat (Dickerson & Kemeny, 2004). The individual is expected to necessarily experience frustration during mental arithmetic, be informed of their failure as it occurs, and be aware that the failure is communicated to an audience. All of these components are conducive to the collection of fMRI data, whereas other parts of the original TSST are not. For example, giving a speech during a scanning session is an inefficient use of time and may introduce error secondary to head movement. The Montreal Imaging Stress Task (MIST) is another stress induction paradigm similar to the TSST and the paradigm used in the current study (Dedovic et al., 2005). It has also produced consistent data reflecting induced stress in fMRI data acquisition (Pruessner et al, 2008). The paradigm designed for the current study is a simpler version of the MIST and the TSST, but includes all elements necessary to induce stress.

In order to examine the effects of stress in fMRI data, a congruent behavioral task must be used as a comparison for the stress induction. Before inducing stress, participants completed a series of subtraction problems that require minimal mental effort under timed constraints (e.g. 9999 – 0 = 9999). Both the TSST and the MIST have demonstrated that continually subtracting 17 from 4-digit numbers under time constraints results in enough failure to induce stress (Kirschbaum, Pirke, & Hellhammer, 1993; Dedovic et al., 2005). Therefore the paradigm in this study requires all participants to perform serial 17 subtractions under timed conditions. In both

the control and stress inducing problems, the participants chose their answer from four options using a 4-button serial response pad and were then informed of their accuracy. The participants were told that the accuracy of their performance was relayed to an audience.

fMRI Data Acquisition

Structural scan.

Participants were placed in the scanner ,which uses an 8-channel phased-array head coil for data acquisition. Participants were fitted with Resonance Technology Inc. stereo headphones and LCD goggles. An initial 2D gradient echo fast sequence scout (localizer) scan was acquired for setting landmarks. Participants then completed a high-resolution 3D spoiled gradient anatomical scan sequence covering the full brain and a 24 cm FOV, a TE of 7 ms, a TI of 450 ms, and a 1.2mm slice thickness with 0.0 slice gap. Following these scans, participants were asked to rate their subjective stress level and their subjective level of food craving with a 4-button serial response pad which links to a set of visual analog scales presented on their LCD goggles screen. The visual analog scales have the same seven point anchors describing subjective stress and craving as the ratings collected earlier during the scan session.

Functional scan.

The functional scan protocol consisted of a T2*-weighted gradient EPI pulse sequence in an oblique plane, with 22 cm FOV, a Flip angle of 90, a TE of 25ms, a TR of 2000 ms, 1 echo with interleaved slices of 4 mm thickness and 0.0 slice gap.

Participants underwent four functional runs. In the first run participants viewed eight blocks of three neutral cues, taken from or interpolated from the IAPS. The second run consisted of eight blocks of three highly palatable foods cues (pre-stress food cues) taken from or interpolated from the IAPS. In the third run, participants underwent the stress induction

paradigm while remaining in the scanner. In the final run, participants were exposed to eight blocks of highly palatable food cues (post-stress food cues). Refer to Figure 1 for ordering of the full paradigm and each run. Interspersed between all blocks of visual stimuli were baseline blocks of a centered crosshair for initial contrasts. Likewise, a centered crosshair was interspersed between each block of arithmetic problems. Photographs of all visual stimuli were presented as either in "landscape" mode or "portrait" mode in a fixed random manner by stimulus type. In the first, second, and fourth run participants were instructed to respond to each visual cue by indicating stimulus orientation with the button response pad. This was done to ensure participants were alert and attending to stimuli. No image was used more than once in order to minimize habituation.

Subjective ratings of stress and craving were assessed following the structural scan and immediately following each run. Figure 1 also depicts the time points at which behavior measures were collected.

fMRI Analyses

Individual-level analysis.

In the first level, functional analysis was carried out using FEAT (FMRI Expert Analysis Tool), part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Each subject's functional runs were analyzed separately and include quality assurance steps, intensity normalization, slice-timing correction, motion correction, and spatial smoothing. After motion correction, images were temporally high-pass filtered with a cutoff period of 48 seconds and smoothed using an 6 mm Gaussian FWHM algorithm. For each condition, the design was convolved with a gamma function to produce an expected BOLD response. Data were fitted to the model using FSL's implementation of the general linear model (GLM), with motion

components included as confound EVs. Each participant's statistical data was then warped into a standard space based on the MNI-152 atlas. FLIRT (FMRIB's Linear Image Registration Tool) was implemented to register the functional data to the atlas space. Functional images were aligned with the high-resolution co-planar T2-weighted image. The co-planar volume was registered to the standard MNI atlas. Because a block-design was used for stimulus presentation, statistical activation maps from each run were used to compare BOLD signal changes in response to food cues and neutral cues for obese and healthy-weight controls in the next level of group analysis.

Group-level analysis.

In the group-level pipeline, the statistical maps from the first level were resampled to a standard space and combined into a group analysis using the GLM model implemented in FSL. Analyses were conducted with FSL using FEAT, ANOVA fixed-effects models to compute group and condition differences. Z (Gaussianized T/F) statistic images were thresholded using $Z>2.3$ and a (corrected) cluster significance threshold of $p = 0.05$ (Worsley, Evans, Marrett, Neelin, 1992; Forman, et al., 1995; Friston, Worsley, Frackowiak, Mazziotta, Evans, 1994). For each group (OB or healthy-weight controls) statistical changes for 1. pre-stress food vs. neutral cues and 2. post-stress vs. pre-stress food cues were analyzed. Statistical map differences prior to and following the stress induction were also analyzed for between group comparisons (OB and healthy-weight controls).

ROI-based analyses.

Based on our a priori hypotheses, as described in the introduction, we used a region of interest (ROI) analysis of variance (ANOVA) block analysis. The predetermined regions of

interest were OFC, PMC, VS, insula, and ACC. ROIs were generated using automated parcellation with the Harvard-Oxford Cortical and Subcortical Structural Atlas provided by FSL (Desikan, et al., 2006).

Tests of hypotheses.

Analyses were carried out using the data generated in the preceding analyses. Initial comparisons were made between the two groups on demographic and descriptive variables to determine any potential group differences. We then conducted two 2 x 2 ANOVAs on BOLD activation for each of the following ROIs: OFC, PMC, NAc, ACC, insula, and amygdala. In order to get insight into ANOVA results, mean BOLD percent signal change (a measure of neural activity) was calculated for each group and condition.

The first ANOVA consisted of condition (neutral cue/pre-stress food cue) x group (OB/healthy-weight control) comparisons; and the second consisted of cue-type (pre-stress food cue/post-stress food cue) x group (OB/healthy-weight control). For the first ANOVA, we hypothesized that there would significantly greater activation during pre-stress food cues compared to neutral cues in each region of interest (a main effect of condition), and differentially greater reactivity to pre-stress food cues in OB individuals compared to controls (a main effect of group). Similarly, in the second ANOVA, hypotheses include significantly greater activation during post-stress food cues compared to pre-stress food cues in each region of interest (a main effect of condition), and differentially greater reactivity to post-stress food cues in OB individuals compared to controls (a main effect of group). Lastly, we hypothesized an interaction of group x condition in which each group would have greater activation in each region of interest for pre- stress food cues compared to neutral cues, and post-stress food cues compared to pre-stress food cues, but in which the OB group will have differentially greater activation than the

healthy-weight controls at each time point. Thus, we hypothesized the nature of the change in each condition would vary by weight status.

CHAPTER 3

RESULTS

Participants

106 participants were screened for the study. 50 qualified for the study following the phone screen, 49 completed an in-person assessment, and 26 completed the fMRI portion of the study. Due to technological complications and error within the fMRI data, six participants' data were not included in the final analyses. The final sample consisted of six obese females, four obese males, (mean BMI = 37.22, sd = 6.60) and six control females and four control males (mean BMI = 21.85, sd = 1.47). None of the participants in either group exhibited symptoms of disordered eating.

The total sample was70% Caucasian, 20% African American/Black, 5% Hispanic, and 5% biracial. In the obese group, 40% of the participants identified themselves as African-American, and 60% identified as Caucasian. In the healthy weight group, 10% identified themselves as Hispanic, 80% as Caucasian, and 10% as biracial. Participants ranged in age from 19 to 40 years old. There were no significant differences between groups in age. Finally, there were no significant differences between the groups in the number of stressful events experienced over the past year ($t = 1.17$, $p<.26$).

Behavioral Results

Bivariate correlations of craving and stress on the scan day with BMI and self-report data.

Table 1 displays correlations for BMI, weight, participants' subjective ratings of pre-meal craving, post meal craving, pre-stress induction craving, post stress induction craving, and all eating/craving measures. There was a significant correlation between BMI and self-report

craving in the fMRI paradigm following neutral cues, indicating that obese participants reported higher levels of craving following the neutral cue presentation. However, none of the other correlations between self-report data and behavioral data were significantly different in obese compared to healthy weight participants. Therefore the correlational data for the total sample was combined in Table 1. Measures of subjective craving levels (e.g. pre-meal craving, post-meal craving, baseline craving, neutral cue craving, pre-stress food craving, TSST craving, post-stress craving) across different time points during the scan day were all positively associated with each other. Additionally pre-stress food cue craving was positively associated with a measure of external eating behavior (DEBQ External Eating), as were craving levels following the stress induction (TSST craving), and craving levels after viewing post-stress food cues. There were also several positive associations between measures of eating behavior, eating expectancies, and eating motivations. However, none of the other self-report measures of eating motives, expectancies, or eating behavior was significantly correlated with craving on the scan day. Finally, there were no significant correlations between the number of life stressors in the last year and subjective ratings of stress in the fMRI environment.

Subjective ratings of craving and stress during fMRI paradigm.

Independent-samples t-tests were used to examine between groups differences on subjective levels of stress and craving at each sampling point on the scan day. There were no significant differences between obese and healthy weight participants on subjective ratings of stress or craving at any point throughout the paradigm (*t* values ranged from -1.34 to .76). As there were no significant differences between the two groups on any subjective rating at any time point, behavioral data for the groups was combined for analysis of within participant changes in craving and stress across conditions.

Craving significantly decreased from pre-meal (3.00) to post-meal (.70) (t = 8.64, p<.001). Craving did not significantly change from baseline ratings in the scanner to the neutral cue condition. However, as expected, craving significantly increased following the neutral condition (.80) to following the pre-stress food cue condition (1.35) (t = -2.77, p<.01). Craving significantly increased again from the stress condition (1.10) to the post-stress food cue condition (1.65) (t = -3.58, p<.01).

Subjective ratings of stress appeared to decrease pre-meal (1.35) to post-meal (1.00) (t = 2.93, p<.01). There were no significant changes in stress level following the post meal period until the stress induction was introduced in the fMRI environment. Subjective levels of stress significantly increased from the pre-stress food condition (1.40) to the stress condition (2.45) (t = -7.76, p<.001). After viewing food cues again following the stress induction, subjective ratings of stress significantly decreased (t = 6.19, p<.001). Thus, subjective levels of craving significantly increased following exposure to food cues for both groups, and subjective levels of stress significantly increased following the stress induction in both groups.

A Priori ROI Analysis Results

Results from 2x2 ANOVA for six a priori regions of interest are discussed below and summarized in Tables 2 and 3.

Obese vs. healthy weight by neutral cue vs. pre-stress food cue comparisons.

The first ANOVA (See Table 2 and Figure 2) examined effects across two groups (obese and healthy weight) and two conditions (neutral and pre-stress food cues). Within the ACC there was a main effect of group, in which the healthy weight group exhibited greater activation than the obese group. There were no significant main effects of condition on activation within the ACC. There was a main effect of group, but not condition, bilaterally within the NAc. In the

neutral condition, both healthy weight and obese participants exhibited decreased percent signal change relative to baseline in the NAc. In the pre-stress food cue condition healthy weight participants exhibited increased percent signal change above baseline, while obese participants exhibited a decrease in percent signal change from the neutral condition. This differential response in the pre-stress food cue condition is likely driving the main effect of group.

There was a main effect of condition on activation in the PMC. Within the PMC, both groups exhibited a similar positive percent signal change in response to the neutral cue condition. However, in the pre-stress food cue condition the obese group exhibited a large decrease in percent signal change. The data appear to exemplify a group x condition interaction (see Figure 4), but this interaction was not found to exceed thresholds of Z =2.3. Lastly, there was a main effect of condition in both the Amygdala and OFC, in which pre-stress food cues elicited greater BOLD response than neutral food cues.

Obese vs. healthy weight by pre-stress food cue vs. post-stress food cue comparisons.

The second ANOVA (See Table 3 and Figure 3) included the same groups (obese and healthy weight), but different conditions (pre-stress food cues and post-stress food cues). Results revealed significant main effects of group and condition on activation in the ACC, but no interaction. The healthy weight group exhibited a greater BOLD response to post stress food cues than the obese group in this region. The main effect of condition indicated that there was a greater BOLD response for pre-stress food cues than post-stress food cues. As the response to condition was similar for both groups, the interaction effect was not significant. Healthy weight individuals also exhibited a greater BOLD response to food cues post stress compared to pre-stress than OB individuals in the NAc.

There was a main effect of condition on activation in the insula, in which pre-stress food cues elicited a greater BOLD signal than post-stress food cues. A different pattern was observed for the main effect of condition within the OFC, in which post-stress food cues evoked a stronger BOLD signal than pre-stress food cues. Lastly, there was a main effect of group within the PMC. Activation of each group varied, by condition, which indicates that the main of effect of group may be trending towards an interaction. Figure 5 displays a graph of percent signal change for each group and condition. The obese group had increased activation from pre-stress to post-stress food cues, but the healthy weight group had decreased activation from pre-stress to post-stress food cue conditions. The percent signal change for both groups remained positive across conditions and was similar after exposure to post-stress food cues.

Whole Brain Analyses and Post-Hoc ROI Analyses

In addition to examination of a priori ROIs, whole brain analyses were conducted to examine diffuse areas of activation for each group in response to each condition. None of the originally hypothesized regions were found to be significantly activated (above a threshold of Z=2.3) in whole brain maps. While conducting post-hoc analyses on regions found to be active in whole brains maps can be problematic due to circularity, these analyses are warranted given the novelty of the paradigm in the current study. Additionally, the regions identified as post-hoc ROIs are consistent with several other studies reports of BOLD response to various food cue reactivity conditions (Beaver, et al., 2006; Killgore, et al. 2003; Schienle, Schäfer, Hermann, & Vaitl, 2009; Siep, et al, 2009; Schur, et al., 2009). Any region with activation that surpassed threshold across groups or conditions was considered for post hoc analyses. Whole brain activation maps revealed eight regions that surpassed significance threshold levels for either group or condition comparisons, including the occipital cortex, fusiform gyrus, thalamus,

caudate, precuneus, frontal cortex, inferior frontal gyrus, and prefrontal medial cortex. We utilized the same two 2 x 2 ANOVA designs implemented in a priori analyses to probe main effects and interaction in post-hoc ROIs. Tables 4 and 5 and Figures 6 and 7 summarize the findings that are discussed below.

Obese vs. healthy weight by neutral cue vs. pre-stress food cue comparisons in post hoc regions.

In the first ANOVA that considered both groups (obese and healthy weight) and conditions (neutral cues and pre-stress food cues) there were several significant main effects and interactions. The occipital cortex displayed the greatest level of activation in whole brain analyses. There were significant main effects of group, condition, and an interaction within this region and in the fusiform gyrus. Probing of the results revealed that the healthy weight group had greater activation than the obese group across conditions in both the occipital cortex and fusiform gyrus. BOLD responses to pre-stress food cues in both of these regions were elevated in comparison to neutral cues. The interaction effect indicated that both groups had similar BOLD responses to neutral cues in the occipital cortex, but that the obese group had a greater increase in percent signal change following pre-stress food cues in this region than the healthy weight group. The second significant interaction of group x condition indicated that both groups had a similar response to neutral cues in the fusiform gyrus, and an increase in BOLD signal following pre-stress food cues, but the percent signal change was greatest for the healthy weight group in the pre-stress food cue condition.

Similar to main effects of group in the occipital lobe and fusiform gyrus, main effects of group in thalamus and frontal cortex indicated that healthy weight participants had a greater BOLD response than obese participants across conditions. The main effects of condition also

follow the same pattern. Pre-stress food cues evoked a greater BOLD response than neutral cues across groups in the thalamus and frontal lobe. There were no significant interactions for group x condition in the thalamus or frontal lobe.

There were main effects of both group and condition in the inferior frontal gyrus. Within this region, obese participants exhibited a greater BOLD response than healthy weight participants across conditions, and pre-stress food cues evoked a greater BOLD signal than neutral cues across groups. There was a main effect of condition within the caudate, in which pre-stress food cues produced a greater BOLD signal than response to neutral cues. There was no main effect of group.

Main effects of group and condition were found in the precuneus and medial prefrontal cortex (mPFC) in which the obese group exhibited less BOLD activation than the healthy weight group. For both regions, both groups' percent signal change was negative relative to baseline in the neutral cue condition. The percent signal change remained negative relative to baseline in the obese group following pre-stress food cues, but was positive relative to baseline for the obese group in both regions. In the main effect of conditions within these regions, there was greater negative BOLD signal response relative to baseline for neutral cues compared to pre-stress food cues across groups. Lastly, there was a significant interaction of group x condition in activation in the prefrontal medial cortex, but not in the precuneus.

Obese vs. healthy weight by pre-stress vs. post-stress food cue comparisons in post hoc regions.

The second set of ANOVAs also revealed several main effects and interactions of group (obese and healthy weight) and condition (pre-stress food cues and post-stress food cues). There were main effects of group and condition in both the posterior occipital cortex and the fusiform

gyrus, as well as interactions. Obese participants exhibited greater response to cues than healthy weight participants in both regions, and there was a greater response to post-stress food than pre-stress food cues in both regions. The interaction of group x condition in the posterior occipital cortex indicated that the healthy weight group had a small increase in BOLD percent signal change from pre-stress to post-stress food cues and that the obese group had a very large increase in BOLD percent signal change from pre-stress to post-stress food cues. The same pattern of findings was observed for the interaction of group x condition in the fusiform gyrus.

In the frontal lobe, the healthy weight group exhibited greater percent signal change across conditions than the obese group. Furthermore, there was a greater percent signal change for pre-stress food cues compared to post-stress food cues across groups. The main effect of condition was similar within the inferior frontal gyrus. However, the significant main effect of group indicated that the obese group exhibited a greater percent signal change across conditions than the healthy weight group. As with results from the first set of ANOVAs, there were reductions in BOLD signal to below baseline in both the precuneus. There were also significant main effects of group in both regions, in which the obese group exhibited a more negative BOLD percent signal change than the healthy weight group across conditions. There was a main effect of condition within the precuneus, but not the prefrontal cortex. Within the precuneus, post-stress food cues created a more negative BOLD percent signal change than the pre-stress food cues.

CHAPTER 4

DISCUSSION

The purpose of this study was to examine BOLD activation in obese individuals compared to normal weight controls in response to exposure to food cues prior to and following an acute stress induction. In addition, this study sought to replicate previous findings of corticolimbic activity in response to palatable food cues compared to neutral cues in both groups. Self-report measures of eating behavior, experiences of stress, and behavioral data regarding changes in craving and stress throughout the paradigm were also collected.

The relationship of self-report variables to each other did not differ by obese vs. healthy weight status. The external eating subscale of the Dutch Eating behavior questionnaire was positively associated with craving levels after pre-stress food cues, craving following the stress-induction, and craving after post-stress food cues. Questions from this subscale assess an individual's likelihood of eating food after being exposed to cues in the environment (e.g. being more likely to eat when preparing a meal). Thus, the positive relationship between this scale and self-report craving following exposure to food cues is not unexpected. There were no significant relationships between number of life stressors in the last year (e.g. death, family discord, job change) and stress ratings on the day of the scan.

Several manipulation checks were conducted with the self-report data gathered while participants were in the fMRI environment. Both healthy weight and obese individuals had decreases in craving following the standard meal. This was an important step to ensure that all participants' levels of hunger and craving were minimal before exposure to food cues. Additionally, levels of craving significantly increased following exposure to pre-stress food cues as well as post-stress food cues. Regarding the amount of stress experienced on the day of the

scan, both groups experienced an increase in stress after the stress induction and a decrease in stress after the post-stress food cues were viewed. Qualitatively, all participants in the study commented during debriefing that the math problems made them feel uncomfortable and stressed.

Summary of fMRI findings for neutral cues and pre-stress food cues

The current study replicated findings of increased neural activity in the OFC, posterior occipital cortex, fusiform gyrus, thalamus, caudate, frontal lobe, inferior frontal gyrus, and amygdala for both obese and healthy weight groups when contrasting pre-stress food cues to neutral cues (Rothemund et al, 2007; Stoeckel et al., 2008; Beaver, Beaver, et al., 2006; Killgore, et al., 2003; Schienle, Schäfer, Hermann, &Vaitl, 2009; Siep, et al., 2009; Schur, et al., 2009).

Contrary to our hypotheses, the healthy weight group had more neural activity than obese participants in the ACC and NAc across both neutral and pre-stress food cue conditions. Furthermore, the percent signal change relative to baseline (e.g. attending to a fixation cross) in these regions was negative for the obese group, but not the healthy weight group. While this finding was inconsistent with our original hypotheses of hyperactivation, a recent review of neuroimaging studies supports a theory of hypoactivation in areas related to cognitive control/attention (e.g. ACC) in obese populations (Carnell, Gibson, Benson, Ochner, & Geliebter, 2011). The ACC is involved in monitoring error and cognitive control. In our task, participants were asked to indicate if each cue was presented in landscape or portrait format. These instructions were given to ensure that participants were awake and attending to stimuli. However, it may be that obese individuals' cognitive control processes during this task was disrupted by the presentation of pre-stress food cues, where this was not the case for healthy weight individuals (Carnell, Gibson, Benson, Ochner, & Geliebter, 2011). Bohon, Stice, and

Spoor (2009) investigated the neural response of anticipation of food receipt and food consumption in neutral and negative mood conditions. Their results revealed that emotional eaters had increased activity in the ACC under negative mood state (compared to neutral) when receiving a milkshake, but this effect was not found for non-emotional eaters who had decreases in ACC activity under negative mood states. It is possible that since obese and healthy weight individuals in the current study did not have disordered eating symptoms that they may have had similar responses to 'non-emotional eaters' under negative mood states.

Obese participants also exhibited reduced or negative activation in the NAc, while healthy weight participants exhibited increased or positive activation in the pre-stress food cue condition. This difference may have been affected by the state of satiety of participants in the current study. Previous studies investigating corticolimbic activation in obese groups for food cues (vs. neutral cues) under varying hunger conditions have found increased NAc activation in fasted conditions; but a lack of significant NAc activation in fed states(Martin, et al, 2010). All participants in our study were fed a meal prior to scanning and reported decreases in craving levels before scanning. Additionally, one study has demonstrated disruption in neural circuitry of NAc for adaptive processing of food cues in obese participants compared to controls. This study of effective connectivity within the limbic system demonstrated deficiencies in amygdala modulation of NAc, and a disproportional influence of OFC on NAc activity. A lack of sufficient influence from the amygdala on NAc is thought to be related to interpretation of reward and motivational properties of food cues, while the undue influence of OFC on NAc activity could contribute to increased motivation for eating (Stoeckel, et al., 2009). Evidence of disruption in this network may explain decreased activity in the NAc for the obese group but not the healthy weight group in the current study.

Our sample also exhibited greater activity in the PMC for neutral cues than pre-stress food cues across groups. This finding contrasts with previous studies that report increases inactivation in the PMC in lean/control groups when contrasting palatable food cues with other cues (Cornier, Von Kaenel, Besseman, &Tregellas, 2007; Cornier, 2009; Siep et al., 2008; Passamonti et al., 2009). We hypothesized that the obese group would display hyperactivity in this region for food cues given the role of the premotor cortex in preparation for eating behavior (Miyai, Suzuki, Kang, Kubota, & Volpe, 1999; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001). Given that several of our neutral stimuli are objects that can be manually manipulated (e.g. blocks, screws) it is possible the premotor activity was greater in both groups due to properties of these neutral cues.

There were several regions identified in exploratory whole-brain and ROI analyses in which healthy weight participants exhibited greater neural activity than OB participants. These regions include posterior occipital cortex, fusiform gyrus, thalamus, and the frontal lobe. Both the occipital cortex and fusiform gyrus have previously been identified as regions involved in food cue processing because of their role in attention (Murdaugh & Cook, 2011; McCaffery, et al., 2009; Malik, McGlone, &Dagher, 2011; LaBar et al., 2001; Killgore et al., 2003; Killgore, et al., 2007). Initially activation found within the fusiform gyrus was not well understood. Yokum and colleagues (2012) hypothesized a correlation between BMI and fusiform activity in response to food cues because of the fusiform's role in attention orienting. It was originally thought that increased activity within the fusiform would indicate attentional biases towards food cues in individuals with compromised food cue processing. This hypothesis was not supported. A meta-analysis of food cue reactivity revealed that 41% of studies demonstrated fusiform gyrus activity when contrasting food to non-food cues (Van der Laan, De Ridder, Viergever, &Smeets, 2011).

Amygdala and ACC are thought to be top down regulators of visual activity (Lang, et al., 1998). Given the there is evidence of disruption in amygdala functioning of obese individuals; visual processing could be impacted in obese groups, but not healthy weight groups (Stoeckel, et al., 2009).

In the current study, there were significant interactions of group x condition for activation in both the occipital cortex and the fusiform gyrus. Both groups exhibited activation increases from neutral cue exposure to pre-stress food cue exposure. The healthy weight group had greater activation in both conditions compared to the obese group. However, the slope of percent signal change across both conditions was steeper for the obese group, potentially indicating a greater change in BOLD response to pre-stress food cues that the healthy weight group had, even though the healthy weight group had greater overall activation. It may be that the obese group showed greater sensitivity to these cues than the healthy weight group. However the degree of change across conditions within each group is different than the pattern of activation exhibited in the occipital cortex. The healthy weight and obese group had almost identical responses (in regard to percent signal change) during the neutral cue condition, but the healthy weight group had a greater slope or degree of percent signal change across conditions. This change across conditions in the fusiform gyrus was different than the change observed within the occipital cortex.

Similar main effects were obtained in the thalamus, which is known for relaying sensory information to areas within the neocortex. The healthy weight group had greater activation than the obese group across conditions. Similar activation in the thalamus has been observed in response to food cues in healthy weight participants and under conditions of anticipation of food (Small, Veldhuizen, Felsted, Mak, &McGlone, 2008; Stice, Spoor, Ng, & Zald, 2009). No

published studies to date have implicated the thalamus's role in food cue processing in obese groups.

In the present study, healthy weight participants exhibited increased activity in the frontal lobe than the obese group across conditions. Depending upon which area of the frontal lobe exhibits increased activation, there are several hypotheses that could explain this effect. In general, craving or an "irresistible urge to consume" and abnormalities within the frontostriatal circuit are related to overeating (Wang, Volkow, Thanos, & Fowler, 2004). Specifically, food craving is related to increased or hyperactivity in the OFC and ACC with insufficient regulation from later prefrontal inputs, specifically the dorsolateral prefrontal cortex (Uher, et al., 2004). An investigation of repetitive transcranial magnetic stimulation over prefrontal regions in women who frequently crave food was found to attenuate food cravings (Uher, et al., 2005), which further describes the role of frontal and prefrontal circuitry in craving. Our results demonstrated hyperactivity of OFC in both groups and increased activity in the ACC for healthy weight compared to obese participants. The evidence previously cited for "crosstalk" and dysregulation of the ACC circuitry in obese individuals (Carnell et al., 2011) may explain why the healthy weight group had increased frontal lobe activity compared to the obese group across cue conditions. It is likely that appropriately functioning frontal networks display hyperactivity compared to dysregulated frontal networks.

Inferior frontal gyrus (IFG) is often coupled with findings of OFC activation (van der Laan, et al., 2011), as these structures have adjacent borders. The OFC is not fully understood, but is thought to be involved in sensory integration, processing affective salience of reinforcers, cues for rewards and punishments, decision-making, and expectation. The right IFG (rIFG) is thought to be related specifically to response inhibition (Aron, Robbins, & Poldrack, 2004). TMS

and lesion studies support the role of IFG in response inhibition, demonstrating that damage to the IFG increases the likelihood of risky decision-making (Knoch, et al., 2006). An fMRI study using a Stop Signal Task demonstrated that rIFG also is recruited when salient cues are detected regardless of response inhibition demands (Hampshire, Chamberlain, Monti, Duncan, &Owen, 2010). Our finding of increased IFG activation in obese compared to healthy weight participants is consistent with previous studies (Martin, et al., 2009). IFG activation has also been found in response to binge and non-binge foods for a sample of obese binge eaters; and was activated in lean individuals in the same cue conditions (Geliebter, et al., 2006). Our obese sample did not engage in binge eating or dieting behavior, similar to the sample from Martin et al. (2009). It appears that regardless of these behaviors, something about the obese weight status contributed to increased activity within the IFG compared to controls participants.

Precuneus is a multipurpose area of the brain, serving roles in sensorimotor, cognitive association, and visual related tasks. Imaging studies have shown than have functional activation decreases in the precuneus for both obese and healthy weight groups following a meal (Gautier, et al., 2000; Gautier, et al., 2012). Another study demonstrated group differences across multiple food cue conditions between normal weight and obese participants (McCaffery, et al., 2009). This study did not indicate if the groups had increased or decreased precuneus activity, but supports the role of differences between groups. On the other hand, Martin et al. (2009) found that after a meal obese groups had greater reactivity to food cues (vs. non-food cues) than a normal weight group after a meal. The authors did not indicate whether precuneus activity in the obese group increased or decreased relative to baseline. Data from the current study indicate a main effect of group and condition, but no interaction. Both groups initially displayed decreased activity to neutral cues, which is not surprising given the role of the precuneus in resting states

processes (García-García, et al., 2012). In the pre-stress food cue condition, both group had increases in activity from neutral food cues, but the obese group remained below baseline and the healthy weight group exhibited positive activity relative to baseline. Our exploratory data are consistent with data that show healthy weight groups having greater reactivity to food cues than obese groups in this region.

The medial prefrontal cortex is involved in processing reward value from reinforcers (Francis, et al., 1999; Gallagher, McMahan, & Schoenbaum,1999; O'Doherty, Critchley, Deichmann, & Dolan, 2003;O'Doherty, Rolls, Francis, Bowtell, &McGlone, 2001; Tremblay & Schultz, 1999). mPFC cortex activity has been reported in many neuroimaging studies of food cue reactivity, but its role is somewhat difficult to discern. One reason for this difficulty is the inconsistent naming of ROIs across studies and poorly defined ROIs across studies. For example, ventromedial PFC encompasses orbitofrontal and medial prefrontal regions (O'Doherty, 2012). Some imaging studies in this field report results for the ventromedial PFC, when the OFC and mPFC may have very distinct, but related, roles for food cues processing. This is only one variation of PFC labeling, and there are many more. In our study, there were main effects of group, condition, and an interaction in the mPFC (separate from the OFC). A trend similar to that observed in the precuneus indicated activation in response to neutral cues in the mPFC in which both groups had decrease BOLD activation relative to baseline and increases in BOLD signal during the pre-food stress induction. Again, similar to the effects observed in the precuneus, activation for the obese group within the mPFC stayed below baseline and increased above baseline for the healthy weight group in the pre-stress food cue condition.

One study using single photon emission computed tomography (SPECT) has documented that overweight healthy individuals (compared to lean healthy individuals) had decreased

regional cerebral blood flow in the prefrontal cortex (Willeumier, Taylor, & Amen, 2012). Another group replicated findings of decreased metabolism within the prefrontal cortex during baseline conditions using positron emission tomography (Volkow, et al., 2009). Martin and colleagues (2009) study of satiation effects on neural food cue processing showed that obese groups (vs. healthy weight) exhibited increased mPFC activity during both fasted and fed states when viewing food cues. However, food vs. baseline (instead of food vs. non-food) contrasts indicated that the healthy weight group exhibited greater activation in the mPFC than the obese group. Some studies that documented increased activity in mPFC (Del Parigi, et al., 2002; Gautier, et al., 2001; Tataranni et al., 1999) have been reanalyzed and now indicate decreases in this region (Le., et al., 2007; 2006; Martin, et al., 2009). Still, there is evidence of greater activity within mPFC for obese vs. healthy weight groups in response to food cues in multiple conditions. Our data show increases across groups for pre-stress food cues vs. neutral cues, and that healthy weight participants exhibited greater activity than obese participants (an interaction of group x condition). Synthesizing these results is difficult due to mixed findings, methodological flaws in previous studies, and in consistent naming of ROIs. A general finding replicated by our data is that there are differences in mPFC activity between obese and healthy weight samples in response to food cues compared to neutral cues.

While that current literature generally demonstrates "hyperactivity" for obese compared to healthy weight individuals in NAc, caudate, OFC, insula, amygdala, mPFC, ACC, and other corticolimbic regions, these findings may be hard to replicate. There have been many different and nuanced methods for investigating abnormal neural processing in obese individuals including, but not limited to: fasted/fed conditions, various cue conditions (visual: high calorie, low calorie, neutral, eating utensils; olfactory; gustatory), various and sometimes poorly defined

exclusionary criteria; use of only one gender. One example how variation of these methods affects in neural processing is that several ROIs no longer exhibit significant effects for food cue processing when comparing fed to fasted states (Martin et al., 2010; Farooqi, et al., 2007;Malik, McGlone, Bedrossian, &Dagher, 2008). This implicates that being hungry vs. sated influences neurobiological responses within the limbic system in general, but also in obese groups. This is one variation in methodology that has contributed to difficultly in synthesizing the state of the current literature for reward system processing of food cues in obese samples. Our findings were partially consistent with a "hyperactivity" model of corticolimbic reactivity to food cues compared to neutral cues. However, our results also support "hypoactivation" models. Lastly, our results extend literature that food cue processing in obese individuals is simply different from that of healthy weight individuals.

Summary of fMRI findings for pre-stress and post-stress food cues

No study to date has directly examined the effect of an acute stress induction on food cue processing in a fed state with fMRI methodology. Thus our original hypotheses regarding food cue processing under negative mood (stressful) conditions were theory driven. Results from behavioral studies demonstrate that stress affects "wanting" for food and food intake post-stress induction. Stress had been found to increase intake of sweet foods in healthy and overweight participants (Rutters, Nieuwenhuizen, Lemmens, Born, & Westerterp-Plantenga, 2008). In a separate study, under conditions of stress overweight participants report increased "wanting" of food and consumed more calories than healthy weight participants (Lemmens, Rutters, Born, &Westerterp-Plantenga, 2011). Recent fMRI studies have sought to understand how acute stress affects limbic activity. Two studies have found decreased activity within the limbic system in response to stress inductions. Specifically, healthy participants demonstrated decreased activity

in the hypothalamus, medio-orbitofrontal cortex, and anterior cingulate cortex (Pruessner, et al., 2008b). This study employed a psychosocial stressor very similar to the stress induction used in the current study, but only included healthy participants. A second study produced results of limbic deactivation under stressful states, and also investigated effects of neural processing on food "liking" and "wanting" during fMRI data acquisition. Activity was decreased in putamen, amygdala, hippocampus, and cingulate cortex when participants were choosing food to eat in a satiated state vs. fasted state. Investigators reported that after experiencing psychosocial stress participants chose foods with higher energy density (Born et al., 2009).

Given that stress may contribute to overconsumption of highly palatable foods and hence weight gain, and the role of corticolimbic activity in the processing of rewarding properties of food, we hypothesized hyperactivation for the obese group in our six primary ROIs. Additionally, we hypothesized that post-stress food cues vs. pre-stress food cues would also produce increased activity within the limbic system. There were no a priori hypotheses regarding activity in the eight post-hoc ROIs. We found some support for our hypotheses regarding differences in food cue reactivity in pre- and post-stress cue exposure conditions. Differential effects of post-stress food cue reactivity was observed between groups, but not in a priori ROIs. Consistent with our hypotheses, increased activity was observed in the OFC for post-stress food cues across groups when contrasted with pre-stress food cues. The same effect was observed in the occipital cortex and fusiform gyrus. In post-hoc regions, the obese group exhibited greater activity in occipital cortex, fusiform gyrus, and IFG across conditions compared to healthy weight participants. It appears that visual and specific prefrontal regions may potentiate reactivity to food cues under stress. Evidence currently exists that supports the hypothesis that there is increased attentional biases towards food in obese samples (Castellanos, et al., 2009;

Nijs, Muris, Euser, & Franken, 2010). Activation in the OFC, occipital cortex, and fusiform gyrus all play critical roles in visual attention. Hyperactivation in these regions in obese participants compared to healthy weight participants and post-stress compared to pre-stress may suggest a neural basis for increased attentional bias towards food under acutely stressful conditions.

Several findings suggest an overall pattern of deactivation in stress conditions in the obese group. During the pre-stress food cue condition there was greater BOLD response than to post-stress food cues in the ACC, insula, frontal lobe, and IFG. Healthy weight groups exhibited a greater response to food cues across conditions in the ACC, NAc, and frontal lobe. There was also differential activity within the PMC across conditions. BOLD signals in the healthy weight group decreased from pre to post stress food cue conditions whereas obese participants exhibited increased activity. Neural activity in the PMC during post-stress food cue conditions was similar in both groups. ROIs that exhibited deactivation during post-stress food cues are primarily in the limbic/reward system, consistent with results from other imaging studies of neural correlates of stress. It appears that decreased neural activity during concurrent acute stress and food cue processing is specific to limbic regions.

The healthy weight group also exhibited greater activation in the precuneus and mPFC than the obese group. As stated previously, healthy weight participants exhibited neural activity above baseline in both the precuneus and mPFC during pre-stress food cues; and the obese group exhibited regional BOLD signals below baseline. Following the stress induction, both the healthy weight and obese groups' regional neural activity decreased and was below baseline, similar to the neutral cue condition. Both groups showed decreases in BOLD percent signal change across food cue conditions. There was also a main effect of condition within the precuneus where

reactivity to pre-stress food cues was greater than post-stress food cues. The precuneus and mPFC are both involved in default mode network (DMN) processing, and evidence supports altered DMN connectivity in obese compared to lean samples (García-García et al., 2012). During pre-stress and post-stress food cue processing healthy weight individuals exhibit activation in these regions as they may be useful for eating behavior. Obese individuals do not appear to modulate activity in these regions in response to food cues. However both groups show deactivation following the experience of acute stress. Stress may be responsible for regional suppression in the DMN. Further investigation of suppression in DMN is needed to understand its role in eating behavior.

A similar study was recently published that investigated acute stress- in food cue-induced craving. Jastreboff and colleagues (2013) designed personalized stress and favorite-food cue scripts for obese and lean participants. Consistent with literature supporting the similar role of corticolimbic activation for food cues and stress (Sinha, 2001; Lemmens, et al., 2011), investigators reported that obese and not lean individuals had greater BOLD response in striatal, insular, and hypothalamic regions in response to favorite-food cue imagery and stress scripts (Jastreboff, et al., 2013). ROIs specific to food cue reactivity in obese individuals included the putamen, insula, thalamus, hypothalamus, parahippacampus, and IFG. Similarly, there was increased activity in the putamen, insula, and IFG during readings of the stress script for obese, but not lean individuals (Jastreboff, et al., 2013). These results of hyperactivity are consistent with our original hyperactivity hypotheses and subsequent findings (e.g. OFC, IFG) for the obese vs. healthy weight sample. It is important to note that the Jastreboff group did not investigate simultaneous stress and food cue reactivity, but instead examined neural responses to these two processes independently. This methodological difference may account for the discrepant findings

in the current study. For example, the study discussed above did not report any regions where lean individuals had greater activity than obese individuals.

Another recent fMRI study also used personalized neutral and stressful guided imagery scripts in overweight and obese women with chronic stress, and fed participants a milkshake during delivery of each script (Rudenga, Sinha, & Small, 2013). Investigators found that there was activity in the insula, somatosensory cortex, ventral striatum (which includes the NAc), and thalamus during receipt of a milkshake, but that these regions were not impacted by acute stress. However, right amygdala hyperactivity was found to be specific to the experience of acute stress during food receipt. Additionally BMI and stress reactivity during milkshake delivery was positively correlated with BOLD signal response in the OFC. Taken together, results from this study suggest that both body weight and chronic stress can potentiate BOLD signal responses in limbic regions upon food receipt. Data from the current study extend that stress potentiates OFC activation during food cue processing. Results from Rudenga et al. (2013) are specific to stress and reward circuitry in a specific population during a specific stage in food consumption. There were also several differences in the stress construct examined in the current study compared to Rudenga et al. (2013). For example, chronic stress and acute stress may differentially affect reward processing during food receipt. Additionally, personalized stress inductions vs. modified TSST stress inductions appear to provoke activation in different regions (Dedovic et al., 2009).

This study design was novel for its inclusion of an acute stress induction and investigation of food cue processing in a state of stress in an fMRI environment. Our participants' self-report of subjective stress levels during scanning, and qualitative reports after scanning, demonstrated that our stress induction was successful across groups. Our recruitment methods were stringent, in order not to confound disordered eating symptoms with weight. And,

in fact, comparison of self-report measures in our sample confirmed few differences in eating and craving behavior between groups. Therefore, differential activation by group in our sample can be considered a function of weight status, not a third variable such as a form of psychopathology that might influence both weight gain and response to stress.

While the current study had many strengths, there are some limitations. Menstrual cycle has been shown to affect reward circuitry (Van Vugt, 2010; Dreher, et al., 2007) and food cue reactivity (Frank, Kim, Krzemien, & Van Vugt, 2010). In this study we did not match women on the stage of their cycle. We did not have study participants rate pleasantness of food items utilized in the study. However, in efforts to optimize palatability of food cue images we assessed pleasantness of food cues in a separate pilot study. Additionally, craving increased after presentation of pre-stress food cues for both groups. Regarding statistical ROI analyses, we did not use hand drawn ROI masks for each participant, which may have decreased our ability to detect within group and between group differences. Automated parcellation masks created available in the Fslview atlas toolbox cannot account for individual variation in brain structures. Another issues related to the use of automated ROI masks is that all functional data was masked by and co-registered to MNI space, whereas automated anatomical labeling ROI masks can be applied the individual level and not averaged across groups and conditions (Poldrack, 2007). Despite the fact that hand-drawn ROI masks offer optimal methods for examining ROI effects, it is acceptable to use probabilistic atlases of cortical and subcortical structures, such as FSL's Harvard Cortical and Subcortical Atlases (Poldrack, 2007; Desikan, et al., 2006). Lastly, while a sample of 10 participants per group is sufficient for investigating hypotheses in this study, a larger sample size would be optimal for detecting effects of group, condition, and interactions of

group x condition. It is possible that with a larger sample size more statistically significant interactions would have been observed in the current paradigm.

The investigation of neural responses in obese individuals under varying conditions has dramatically increased in the last 10 years. There are still many unanswered questions regarding the interaction of multiple neural circuits involved in stress and food cue processing. Future directions in this area of research include examining neurobiological response to food cues under acute stress in other populations (e.g. samples of individuals with bulimia nervosa or binge eating disorder). Additionally, it would be interesting to utilize a psychosocial stress induction during fasted states to examine neural activity of concurrent stress and reward response. Evidence from behavioral studies suggests increased high-energy food intake in obese groups following stress inductions. The addition of a feeding condition at the end of this study would provide behavioral data to examine in conjunction with neural activity and group variables.

Future studies should attempt to induce acute psychosocial stress for periods long enough to provide sufficient fMRI data for further analysis. Understanding the temporal nature of BOLD signal response across stressful and food cue conditions would shed important light on alterations in corticolimbic activity in various populations and conditions. Demographic variables should be considered as moderators in the future, as was done in other studies in which effects of gender were compared in fasted and fed states for obese groups (Geliebter et al, 2012). Some work is already being conducted investigating differences in differences in obesity in minority groups (Luo, et al., 2013). These additions to the current paradigm are several directions, which would provide valuable information regarding the processing of food cues under stress and how that in turn influences eating behavior.

In conclusion, our study provided continued support for altered corticolimbic processing in obese groups compared to healthy weight groups. Furthermore, our data can be incorporated into models of hyper- and hypoactivation. Participant responses to pre-stress food vs. neutral food cues replicates many previous findings of reward system circuitry. Comparisons of post-stress food to pre-stress food cues shows that visual-attention systems may be heightened towards food related stimuli in obese individuals compared to healthy weight individuals. However, the reward system appears to be deactivated under stress. There are also implications for DMN activity during and after acute stress contributing to altered appetitive processing in obese vs. healthy weight groups. More investigation is needed to replicate finding of how acute stress disrupts reward system processing of food cues.

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Table 1.

Bivariate correlations of BMI, weight, craving and related measures on scan day.

	BMI	Weight	Pre-Meal Craving	Post-Meal Craving	Baseline Craving	Neutral Craving	Pre-Stress Craving	TSST Craving	Post-Stress Craving	Eating Concern	Restraint	FCI a	FCI b	DEBQ External	EEI Negative Affect	EMQ
BMI	1	**.93****	-.07	.30	**.45***	**.50***	.36	.27	.35	.07	.35	.31	.30	.06	.25	.39
Weight		1	.00	.34	**.54***	**.59****	**.46***	.33	**.50***	.10	.18	.34	.38	.11	.26	.32
Pre-Meal Craving			1	**.59****	**.57****	**.51***	**.59****	**.67****	**.59****	.24	-.07	-.32	-.15	.14	-.07	.00
Post-Meal Craving				1	**.90****	**.90****	**.75****	**.84****	**.71****	-.10	-.20	-.19	.09	.26	.26	.30
Baseline Craving					1	**.97****	**.82****	**.86****	**.82****	-.10	-.26	-.21	.06	.15	.20	.22
Neutral Craving						1	**.82****	**.87****	**.82****	-.12	-.17	-.05	.22	.24	.36	.37
Pre-Stress Craving							1	**.89****	**.95****	.12	.06	-.01	.19	**.45***	.33	.34
TSST Craving								1	**.90****	-.16	-.06	-.10	.13	**.45***	.32	.34
Post-Stress Craving									1	.07	.01	.05	.24	**.48***	.36	.36
Eating Concern										1	**.50***	.12	.08	-.15	-.13	-.07
Restraint											1	**.56***	.37	.28	.18	.34
FCI a												1	**.92****	.44	**.46***	**.57****
FCI b													1	**.49***	**.51****	**.66****
DEBQ External														1	**.66****	**.62****
EEI Negative Affect															1	**.83****
EMQ																1

Note: Pre-Meal Craving, Post-Meal Craving = subjective craving measures before and after meal provision; Baseline Craving, Neutral Cue Craving, Pre-Stress Food Cue Craving, Pre-stress Craving, TSST Craving, Post-Stress Craving = craving measure during functional scans; TSST = Trier Social Stressor Task; Eating Concern = Eating Disorder Examination, Eating Concern subscale; Restraint = Eating Disorder Examination, Restraint Subscale; FCI a = Food Craving Inventory, craving subscale; FCI b = Food Craving Inventory, "giving in" subscale; DEBQ External = Dutch Eating Behavior Questionnaire, External Eating subscale; EEI Negative Affect = Eating Expectancies Inventory, Negative Affect subscale; EMQ = Eating Motive Questionnaire total score; * = p >.05; ** = p >.0

Table 2.

Results of 2x2 ANOVA for Group (OB, HW) x Condition (Neutral, Pre-Stress Food)

Region	Main Effect	*k*	Cluster	Z-max	*x*	*y*	*z*
ACC	*Group*	296	1	5.15	6	42	-2
	HW > OB				2	48	4
					2	42	16
					-8	44	0
					8	36	14
					8	44	16
NAc	*Group*	26	1	4.59	-12	6	-12
	HW > OB	11	2	2.87	12	10	-8
Amygdala	*Condition*	190	1	4.72	-20	-6	-16
	F1 > N				-14	-6	-16
		119	2	4.00	22	-4	-14
OFC	*Condition*	419	1	6.98	26	28	-16
	F1 > N				36	32	-12
		395	2	7.67	-28	34	-16
					-38	18	-22
					-36	26	-8
					-40	22	-2
					-48	20	-12
PMC	*Condition*	292	1	3.54	0	-6	50
	N > F1				0	-10	50
					6	-8	48
					0	0	52
					2	-14	62
					-6	0	52

Note: k = the number of voxels that surpassed a threshold of Z = 2.3; Cluster will indicate it either 1 or 2 clusters in the region surpassed threshold; Z-max = the peak Z threshold statistic of an individual voxel within ROIs; x, y, and z = coordinates of peak activation within ROIs; " > " indicates that percent signal change is greater than baseline; ACC = anterior cingulate cortex; NAc = nucleus accumbens; OFC = orbitofrontal cortex; PMC = premotor cortex; HW = healthy weight control group; OB = obese group; N = neutral cue condition; F1 = pre-stress food cue condition.

Table 3.

Results of 2x2 ANOVA for Group (OB, HW) x Condition (Pre-Stress Food, Post-Stress Food)

Region	Main Effect	*k*	Cluster	Z-max	*x*	*y*	*z*
ACC	*Group*	1007	1	6.99	6	42	-2
	OB < HW				2	48	4
					-4	46	0
					2	44	14
					4	36	10
					10	36	16
	Condition	427	1	4.38	2	48	6
	F1 > F2				0	32	30
					2	28	34
					8	42	20
					10	22	34
					-2	42	22
NAc	*Group*	47	1	3.74	-10	6	-12
	HW > OB	19	2	3.24	12	8	-8
Insula	*Condition*	226	1	5.13	32	18	-12
	F1 > F2						
OFC	*Condition*	289	1	5.13	32	18	-12
	F2 > F1				42	34	-10
					50	28	-10
					48	20	-8
PMC	*Group*	138	1	3.94	-2	8	56
	HW > OB				8	0	66
					-2	0	64
					-4	-4	68

Note: k = the number of voxels that surpassed a threshold of Z = 2.3; Cluster will indicate it either 1 or 2 clusters in the region surpassed threshold; Z-max = the peak Z threshold statistic of an individual voxel within ROIs; x, y, and z = coordinates of peak activation within ROIs; " > " indicates that percent signal change is greater than baseline; ACC = anterior cingulate cortex; NAc = nucleus accumbens; OFC = orbitofrontal cortex; PMC = premotor cortex; HW = healthy weight control group; OB = obese group; F1 = pre-stress food cue condition; F2 = post-stress food cue condition.

Table 4.

Results of 2x2 ANOVA for Group (OB, HW) x Condition (Neutral, Pre-Stress Food)

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*z*
Occipital	*Group*	3213	1	8.21	6	-94	30
	HW > OB				-12	-96	30
					-34	-94	20
					24	-100	18
					34	-96	14
					14	-92	12
	Condition	4062	1	8.21	-16	-96	30
	F1 > N				24	-96	24
					16	-98	24
					20	-98	24
					-14	100	22
					26	-96	14
	Group x Condition	724	1	8.21	26	-94	26
					30	-94	24
					-6	-102	18
					-12	-102	8
					-4	-100	2
					12	-104	0
		584	2	8.21	-14	-100	0
					-32	-98	-4
					-12	-96	30
					-14	-92	34
					-26	-94	26
					-8	-94	30
Fusiform	*Group*	849	1	8.21	-18	-88	-12
	HW > OB				-22	-90	-12
					-14	-90	-20
					-18	-88	-22
					-26	-64	-8
					-30	-80	-10
		682	2	8.21	26	-72	-4
					32	-82	-18
					28	-84	-18
					24	-86	-18
					20	-88	-18
					36	-72	-20
	Condition	1034	1	8.21	-14	-90	-12
	F1 > N				-18	-90	-14
					-12	-84	-16
					-20	-80	-18
					-30	-78	-20
					-22	-90	-20
		982	2	8.21	32	-84	-14
					28	-86	-14
					24	-88	-14
					20	-90	-14
					16	-88	-16
					30	-78	-18

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*z*
Fusiform	*Group x Condition*	393	1	7.06	-24	-88	-20
(cont.)					-30	-82	-20
					-26	-88	-16
					-18	-90	-16
					-14	-90	-16
					-42	-74	-18
		204	2	3.95	24	-72	-12
					38	-68	-14
					28	-70	-2
					28	-64	-6
					32	-62	-12
					22	-64	-10
Thalamus	*Group*	175	1	3.73	24	-32	-2
	HW > OB				20	-34	2
					6	-30	2
					20	-32	8
					14	-30	6
	Condition	312	1	3.65	-6	-6	0
	F1 > N				6	-2	2
					6	-6	-4
					-8	-6	4
					-6	-12	4
					-8	-2	10
Caudate	*Condition*	113	1	3.7	-10	4	6
	F1 > N						
Precuneus	*Group*	1098	1	6.7	-4	-54	32
	OB < HW				-4	-44	38
					10	-44	36
					-10	-82	40
					12	-42	42
					2	-50	16
		427	2	7.13	-2	-66	62
					4	-54	68
					4	-58	68
					4	-68	60
					8	-68	62
					-2	-80	48
	Condition	2527	1	6.47	-6	-54	18
	N < F1				-2	-56	18
					-10	-54	32
					-2	-50	34
					4	-58	32
					4	-66	32
Frontal	*Group*	1045	1	5.47	-16	62	14
	HW > OB				0	56	-4
					2	58	24
					-22	44	26
					-24	40	26
					0	56	8

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*Z*
Frontal	*Group*	525	2	7.04	44	52	-12
(cont.)	HW > OB				30	58	-16
					50	50	-10
					52	46	8
					48	42	14
					20	56	-18
	Condition	2651	1	6.05	-10	62	20
	F1 > N				-14	60	14
					-8	64	8
					-10	60	16
					-8	64	12
					-46	40	14
IFG	*Group*	153	1	4.37	58	28	0
	OB > HW				56	32	0
					54	36	10
		87	2	3.43	-54	34	4
					-52	32	8
					-50	28	-6
	Condition	399	1	5.77	-46	38	12
	F1 > N				-42	36	10
					-46	24	10
					-52	22	10
		194	2	4.14	56	30	10
					50	30	10
					46	24	10
					42	28	6
mPFC	*Group*	569	1	5.74	0	50	-6
	OB < HW				6	40	-18
					-2	54	-4
					10	34	-20
					10	40	-20
					-6	52	-14
	Condition	307	1	5.09	-6	52	-10
	N < F1				-4	56	-2
					-10	40	-10
					-4	46	-20
					12	50	-10
	Group x Condition	99	1	3.3	6	34	-20
					-10	36	-20
					0	36	-18
					-2	30	-24
					-4	30	-20
					-6	36	-20

Note: k = the number of voxels that surpassed a threshold of Z = 2.3; Cluster will indicate it either 1 or 2 clusters in the region surpassed threshold; Z-max = the peak Z threshold statistic of an individual voxel within ROIs; x, y, and z = coordinates of peak activation within ROIs; > = indicates percent signal change is greater than baseline; < = indicates percent signal change is

less than baseless; Occipital = posterior occipital cortex; Fusiform = fusiform gyrus; frontal = frontal lobe; IFG = inferior frontal gyrus; mPFC = prefrontal medial cortex; HW = healthy weight control group; OB = obese group; N = neutral cue condition; F1 = pre-stress food cue condition.

Table 5.

Results of 2x2 ANOVA for Group (OB, HW) x Condition (Pre-Stress Food, Post-Stress Food)

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*z*
Occipital	*Group*	3251	1	8.21	12	-92	38
	OB > HW				-22	-92	34
					-18	-92	32
					16	-96	32
					-24	-96	28
					-32	-94	22
	Condition	2273	1	8.21	18	-98	26
	F2 > F1				30	-94	24
					16	-104	14
					-12	-102	8
					18	-104	4
					-4	-100	2
	Group x Condition	724	1	8.21	26	-94	26
					30	-94	24
					-6	-102	18
					-12	-102	8
					-4	-100	2
					12	-104	0
		584	2	8.21	-14	-100	0
					-32	-98	-4
					-12	-96	30
					-14	-92	34
					-26	-94	26
					-8	-94	30
Fusiform	*Group*	802	1	8.21	-18	-88	-12
	HW > OB				-14	-90	-20
					-18	-88	-22
					-30	-80	-10
					-46	-66	-20
					-26	-64	-8
		766	2	8.21	34	-80	-18
					30	-82	-18
					22	-90	-18
					36	-72	-20
					24	-90	-12
					28	-64	-4
	Condition	962	1	8.21	-34	-82	-18
	F2 > F1				-16	-88	-18
					-22	-90	-18
					-38	-76	-20
					-26	-84	-20
					-46	-66	-20
		912	2	8.21	28	-78	-18
					22	-84	-18
					28	-64	-10
					30	-80	-6
					20	-90	-14

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*z*
Fusiform	*Group x Condition*	388	1	8.21	-38	-76	-20
(cont.)					-22	-88	-18
					-18	-90	-16
					-26	-80	-20
					-12	-82	-10
					-22	-72	-14
		136	2	6.17	36	-74	-10
					28	-80	-8
					26	-74	-2
					40	-64	-12
					44	-64	-16
					38	-64	-18
Precuneus	*Group*	2284	1	8.04	0	-52	32
	OB < HW				-10	-82	40
					2	-46	36
					8	-44	36
					-12	-72	28
					8	-80	48
		398	2	6.03	8	-68	62
					4	-48	64
					6	-62	66
					-2	-66	62
					4	-56	62
					10	-54	62
	Condition	1183	1	4.89	-6	-56	34
	F2 < F1				0	-50	38
					6	-56	36
					-2	-36	44
					4	-40	38
					8	-60	46
Frontal	*Group*	1863	1	6.33	-2	56	-2
	HW > OB				-22	40	24
					2	56	6
					34	42	20
					-12	60	10
					-12	60	14
	Condition	1696	1	5.36	42	44	-8
	F1 > F2				20	58	18
					36	46	0
					16	52	24
					36	48	12
					34	36	32
IFG	*Group*	149	1	4.67	56	30	14
	OB > HW				54	28	22
					54	36	12
	Condition	138	1	3.48	50	24	6
	F1 > F2				48	24	12
					50	22	2
					48	30	16
					48	28	12
					44	30	16

Region	Main Effect/Interaction	*k*	Cluster	Z-max	*x*	*y*	*z*
mPFC	*Group*	505	1	6.39	0	50	-6
(cont.)	OB < HW				10	34	-22
					-6	40	-20
					0	30	-22
					4	34	-20
					2	34	-14

Note: k = the number of voxels that surpassed a threshold of Z = 2.3; Cluster will indicate it either 1 or 2 clusters in the region surpassed threshold; Z-max = the peak Z threshold statistic of an individual voxel within ROIs; x, y, and z = coordinates of peak activation within ROIs; > = indicates percent signal change is greater than baseline; < = indicates percent signal change is less than baseline; Occipital = posterior occipital cortex; Fusiform = fusiform gyrus; frontal = frontal lobe; IFG = inferior frontal gyrus; PFMC = prefrontal medial cortex; HW = healthy weight control group; OB = obese group; F1 = pre-stress food cue condition; F2 = post-stress food cue condition.

“BASELINE” CRAVING & STRESS SUBJECTIVE RATINGS

Run 1.

\+ (3) N + (3) N + (3) N + (3) N + (3) N + (3) N + (3) N + (3) N +

“NEUTRAL” CRAVING & STRESS SUBJECTIVE RATINGS

Run 2.

\+ (3) F1 + (3) F1 + (3) F1 + (3) F1 + (3) F1 + (3) F1 + (3) F1 + (3) F1 +

“PRE” CRAVING & STRESS SUBJECTIVE RATINGS

Run 3.

\+ (4) MC + (4) MC + (4) MC + (4) MC + (4) MS + (4) MS + (4) MS + (4) MS +

“STRESS” CRAVING & STRESS SUBJECTIVE RATINGS

Run 4.

\+ (3) F2 + (3) F2 + (3) F2 + (3) F2 + (3) F2 + (3) F2 + (3) F2 + (3) F2 +

“POST” CRAVING & STRESS SUBJECTIVE RATINGS

Figure 1. Ordering of cue and stress conditions during functional fMRI scans. (3), (4) = the number of stimuli presented during on trials; N = neutral cues; F1 = pre-stress food cues; MC = math control/easy problems; MS = math stress problems (serial 17 subtractions); F2 = post-stress food cues.

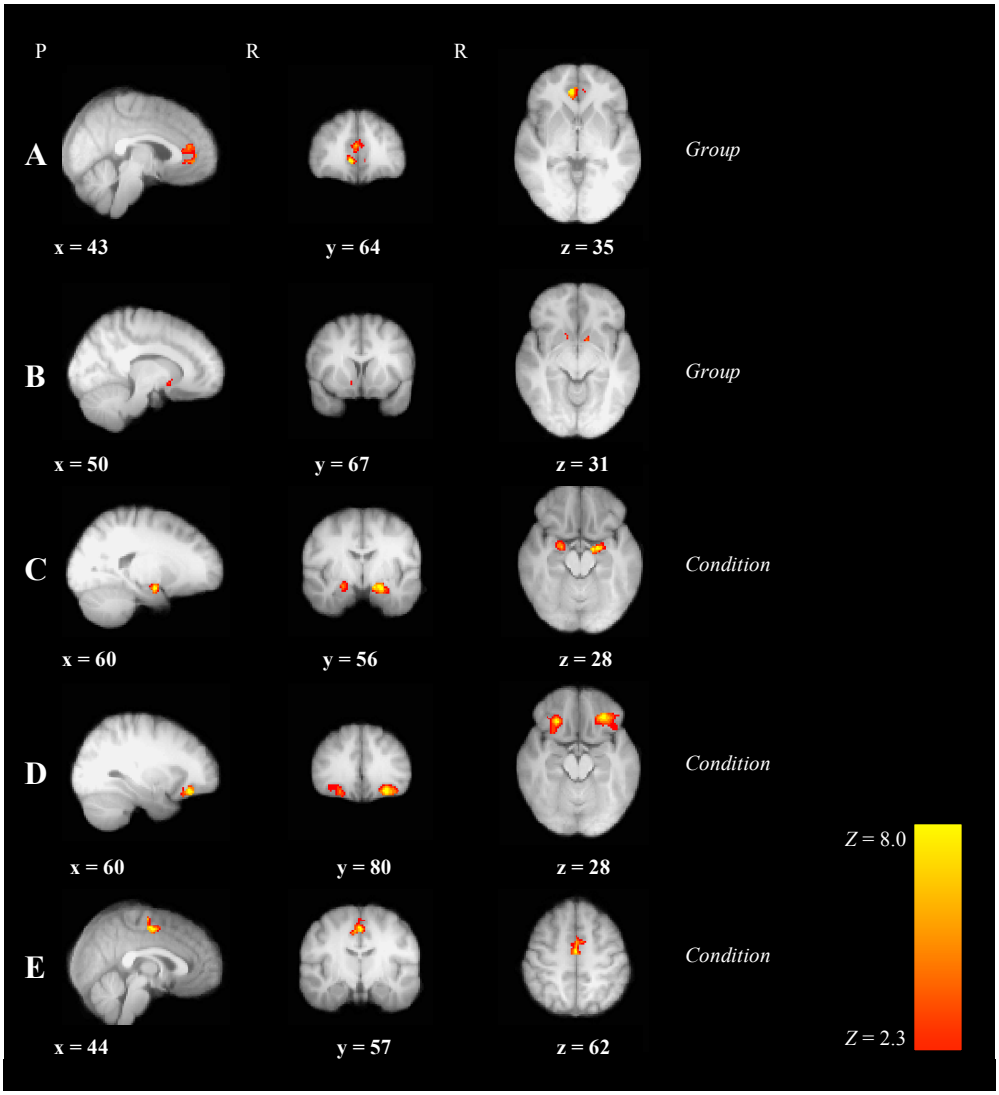

Figure 2. A priori ROI *F*-test results for neutral and pre-stress food cue conditions. The color scale indicates the extent to which regions of interest surpassed a threshold of Z =2.3. A = anterior cingulate cortex; B = nucleus accumbens; C = amygdala; D = orbitofrontal cortex; E = premotor cortex; R = right; P = posterior; *Group* = main effect of group in the region; *Condition* = main effect of condition in this region; z, y, z = MNI coordinates of slice location.

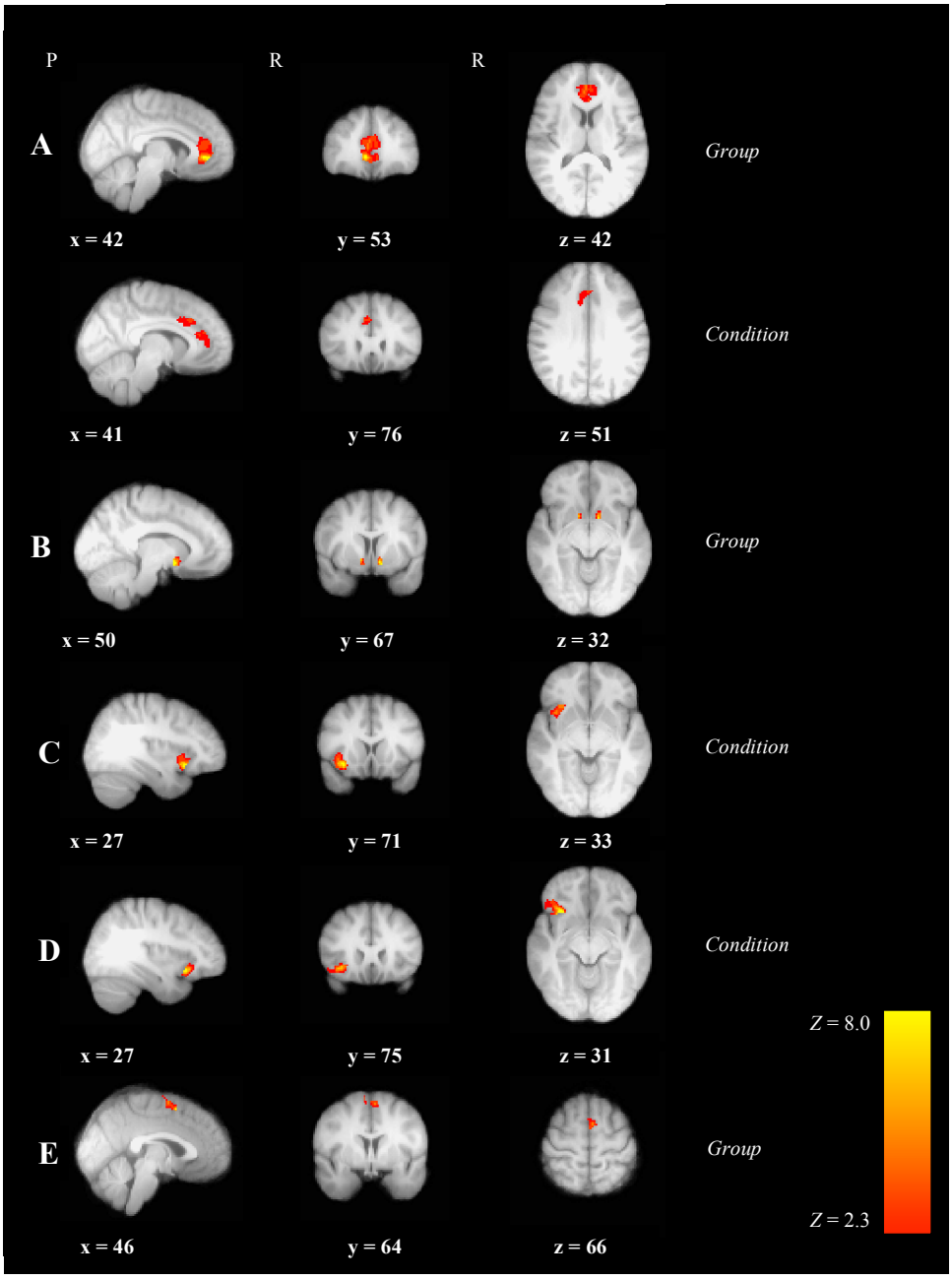

Figure 3. A priori ROI *F*-test results for pre-stress and post-stress food cue conditions. The color scale indicates the extent to which regions of interest surpassed a threshold of Z =2.3. A = anterior cingulate cortex; B = nucleus accumbens; C = insula; D = orbitofrontal cortex; E = premotor cortex; R = right; P = posterior; *Group* = main effect of group in the region; *Condition* = main effect of condition in this region; z, y, z = MNI coordinates of slice location.

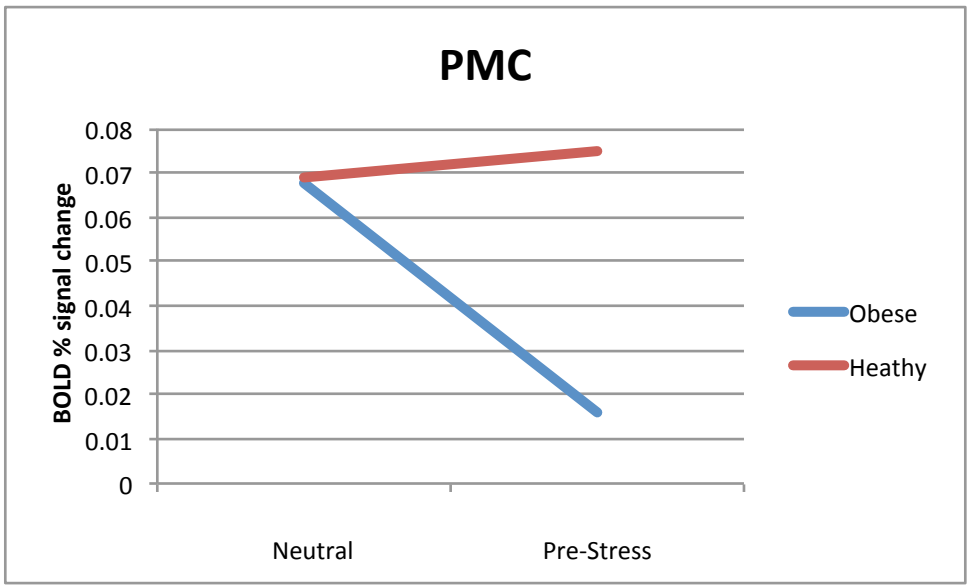

Figure 4. Percent signal change within the premotor cortex in neutral and pre-stress food cue conditions. Neutral = neutral cue condition; Pre-Stress = pre-stress food cue condition.

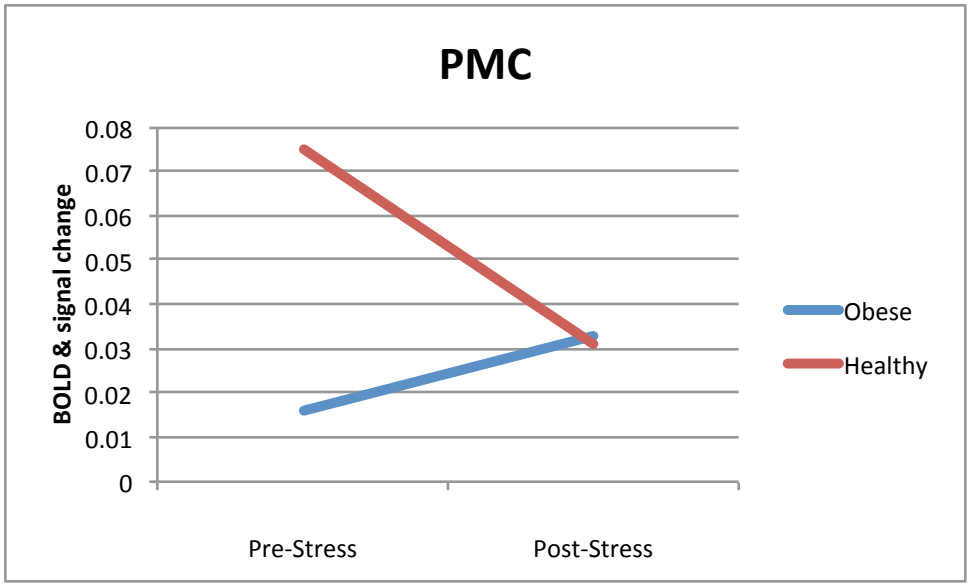

Figure 5. Percent signal change within the premotor cortex in pre-stress and post-stress food cue conditions. Pre-Stress = pre-stress food cue condition; Post-Stress = post-stress food cue condition.

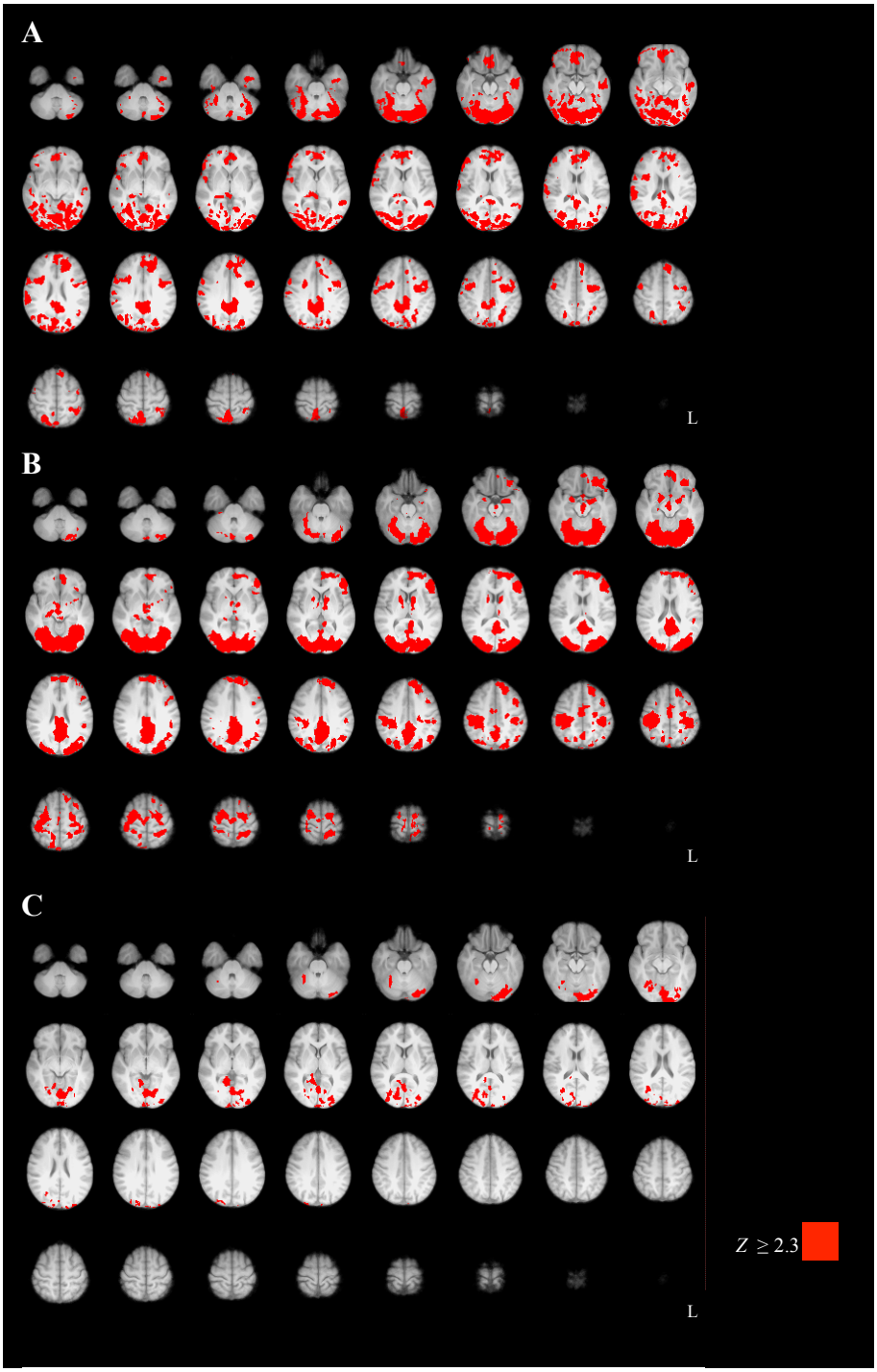

Figure 6. Post-hoc whole-brain activation maps of binary activation for all participants in neutral and pre-stress food cue conditions. A = *F*-test results displaying main effects of group; B = *F*-test results displaying main effects of condition; C = *F*-test results displaying interaction of group x condition.

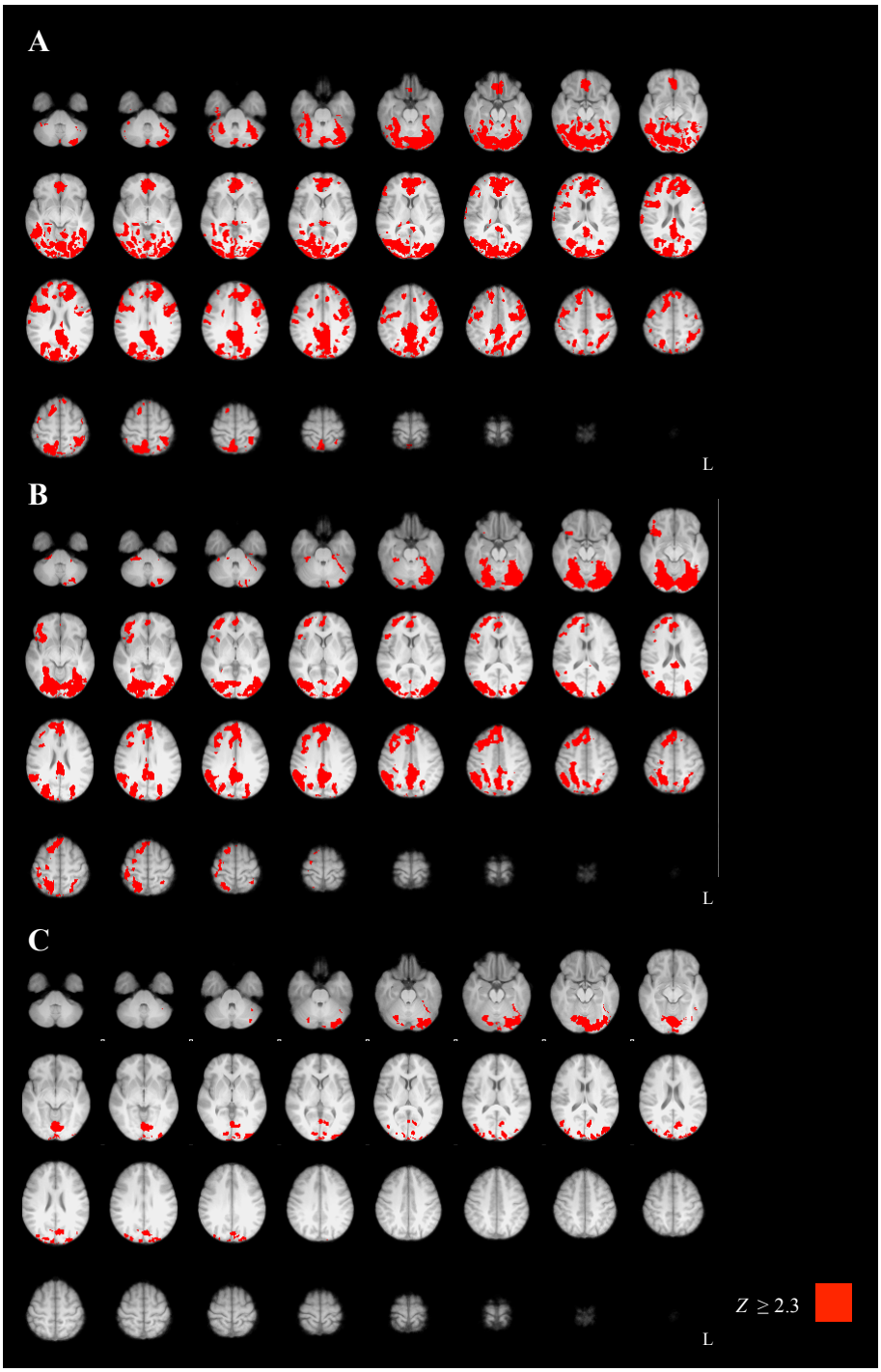

Figure 7. Post-hoc whole-brain activation maps of binary activation for all participants in pre-stress and post-stress food cue conditions. A = *F*-test results displaying main effects of group; B = *F*-test results displaying main effects of condition; C = *F*-test results displaying interaction of group x condition.