SELF-REGULATORY BRAIN NETWORKS OF EATING BEHAVIOR

BY

BRIELLE M. PAOLINI

A Dissertation Submitted to the Graduate Faculty of

WAKE FOREST UNIVERSITY GRADUATE SCHOOL OF ARTS AND SCIENCES

in Partial Fulfillment of the Requirements

for the Degree of

DOCTOR OF PHILOSOPHY

Neuroscience

May, 2015

Winston Salem, North Carolina

Approved By:

W. Jack Rejeski, Ph.D., Co-Advisor

Paul J. Laurienti, M.D. Ph.D., Co-Advisor

Sean L. Simpson, Ph.D., Chair

Jonathan H. Burdette, M.D.

Barbara Nicklas, Ph.D.
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my co-mentors, Paul Laurienti, MD, PhD and W. Jack Rejeski, PhD. They have both been tremendous sources of guidance for me inspiring me to expand my scientific thinking, to work harder, and to enjoy the pursuit of scholarship. For this, I am extraordinarily grateful. I also would like to thank my committee members, Sean L. Simpson, PhD, Barbara Nicklas, PhD, and Jonathan H. Burdette, MD for their advice and support over the past few years. None of this data could not have been collected or analyzed without following grant support: (a) National Heart, Lung, and Blood Institute grant R18 HL076441, (b) National Institute for Aging grant P30 AG021332 (c) internal grants from the Translational Science Center of Wake Forest University and the Sticht Center on Aging at Wake Forest University School of Medicine, (d) Wake Forest University School of Medicine, and (d) the generous contribution of Charlotte and Roy Smith.

It is also important to mention that none of this work was completed in isolation; it is the result of multiple collaborations amongst lab members. Thus, I would like to thank all of the members of the Laboratory for Complex Brain Networks (LCBN) team; I would never have been able to complete this work without them! Not only am I grateful for their intellectual assistance but also for making it a pleasure to come to work each day. I would like to give a special thanks to Ashley Morgan for training me to run the CLIP imaging study and to Robert Lyday for his persistent aid with data analysis. I am also appreciative of Debra Hege’s cheerful helpfulness and of Rhiannon Mayhugh’s reflections during our tea-time chats. Finally, I would like to thank my family for being a source of infinite encouragement and support during this journey.
# TABLE OF CONTENTS

LIST OF ABBREVIATIONS ................................................................................................. v

ABSTRACT .............................................................................................................................. viii

CHAPTER 1 ............................................................................................................................. 1
  INTRODUCTION ................................................................................................................... 1

CHAPTER II ........................................................................................................................... 31
  MEAL REPLACEMENT: CALMING THE HOT-STATE BRAIN NETWORK OF APPETITE 31
  Published in Frontiers Psychology, 2014, volume 5, issue 249 .............................................. 31

CHAPTER III ......................................................................................................................... 70
  NETWORK ANALYSIS: METRICS MATTER! ........................................................................ 70
  In Review at Frontiers in Aging Neuroscience, 2015 ............................................................. 70

CHAPTER IV .......................................................................................................................... 84
  BRAIN NETWORK INTEGRATION AND INCREASING SELF-EFFICACY PREDICT
  SUCCESSFUL WEIGHT LOSS IN OLDER ADULTS ........................................................ 84
  In Review at Frontiers Aging in Neuroscience, 2015 .......................................................... 84

CHAPTER V ............................................................................................................................ 106
  DISCUSSION ....................................................................................................................... 106

CURRICULUM VITAE ............................................................................................................. Error! Bookmark not defined.
LIST OF FIGURES AND TABLES

CHAPTER 1
FIGURE 1.1 Network Highlighting Degree and Global Efficiency Differences………………16
FIGURE 1.2 Direct and Indirect Connections………………………………………………18

CHAPTER 2
FIGURE 2.1 Cartoon Demonstrating Direct and Indirect Connections……………………………39
TABLE 2.1 Summary of hubs of the HBN-A………………………………………………42
FIGURE 2.2 Degree maps demonstrating the hubs of the HBN-A…………………………..43
TABLE 2.2 Summary of direct connections between nodes of the HBN-A…………………45
TABLE 2.3 Summary of indirect connections between nodes of the HBN-A…………………45
FIGURE 2.3 Direct and Indirect Connections from the Insula………………………………47
FIGURE 2.4 Direct and Indirect Connections from the STP…………………………………49
FIGURE 2.5 Direct and Indirect Connections from the ACC…………………………….51
FIGURE 2.6 Direct and Indirect Connections from the Amygdala……………………….53
FIGURE 2.7 Direct and Indirect Connections from the Hippocampus……………………55
FIGURE 2.8- A & 2.8- B Cartoon of the Direct and Indirect Connections of the HBN-A………………57

CHAPTER 3
FIGURE 3.1 Network Highlighting Degree and Global Efficiency Differences………………75
FIGURE 3.2 Modularity Maps for the Bilateral Insula…………………………………….78

CHAPTER 4
FIGURE 4.1 The Hot-State Brain Network of Appetite……………………………………94
TABLE 4.1 Results from Linear Regression………………………………………………96
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>Anterior cingulate cortex</td>
</tr>
<tr>
<td>AAL</td>
<td>Automated Anatomic Labeling atlas</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>AT</td>
<td>Aerobic exercise training</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood oxygenation level dependent</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CLIP-II</td>
<td>Cooperative Lifestyle Intervention Program- II</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DLPFC</td>
<td>Dorsolateral prefrontal cortex</td>
</tr>
<tr>
<td>DMN</td>
<td>Default mode network</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion tensor imaging</td>
</tr>
<tr>
<td>FC</td>
<td>Functional connectivity</td>
</tr>
<tr>
<td>FCQstate</td>
<td>Food Craving Questionnaire</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
</tr>
<tr>
<td>FSL</td>
<td>FMRIB Software Library – a collection of functional and structural brain analysis tools</td>
</tr>
<tr>
<td>GE</td>
<td>Global efficiency</td>
</tr>
<tr>
<td>HBN-A</td>
<td>Hot-state brain network of appetite</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass correlation coefficient</td>
</tr>
<tr>
<td>IDED-IV</td>
<td>Interview for the diagnosis of eating disorders</td>
</tr>
<tr>
<td>K</td>
<td>Degree</td>
</tr>
<tr>
<td>L</td>
<td>Shortest path length</td>
</tr>
</tbody>
</table>
MBI: Mindfulness-based intervention
MEG: Magnetoencephalography
MetS: Metabolic syndrome
MI: Myocardial infarction
MNI: Montreal Neurological Institute (in reference to MNI space/coordinates)
MR: Meal replacement
MRI: Magnetic resonance imaging
N: Number of nodes in a network
OPFC: Orbitofrontal cortex
PH: Parahippocampal gyrus
PCA: Principal component analysis
PCTA: Percutaneous transluminal coronary angioplasty
PFS: Power of food scale
PBN: Parabrachial nucleus
RCD: Reduced calorie diets
ROI: Regions of interest
RT: Resistance exercise training
SE: Standard error
SD: Standard deviation
SI: Scaled inclusivity
SPSS: Statistical Package for the Social Sciences (SPSS), a software package for statistical analysis
SPM: Statistical Parametric Mapping, a software package for analysis of brain imaging data
STP: Superior temporal pole
TFEG: Three-Factor Eating Questionnaire
TP: Temporal pole
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIF</td>
<td>Variance inflation factor</td>
</tr>
<tr>
<td>VMPF</td>
<td>Ventromedial prefrontal cortex</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
</tr>
<tr>
<td>WEL</td>
<td>Weight Efficacy Lifestyle questionnaire</td>
</tr>
<tr>
<td>WFU</td>
<td>Wake Forest University</td>
</tr>
<tr>
<td>WL</td>
<td>Weight loss treatment</td>
</tr>
</tbody>
</table>
ABSTRACT

Obesity is currently a public health crisis in North America not sparing the rapidly expanding population of older adults. While lifestyle interventions remain the most popular treatment for obesity, many individuals fail to monitor their eating behavior and lose weight. The over-reaching purpose of this thesis was to elucidate a brain network of self-regulatory failure among older adults predictive of real-world weight loss.

The first objective was to verify a Hot-state Brain Network of Appetite (HBN-A) that exists after a brief period of food restraint. Fourteen overweight/obese adults came to our laboratory on two occasions. On both visits they ate a controlled breakfast and then fasted for 2.5 hours. Depending on the randomization schedule, they either consumed a meal replacement or water prior to completing a resting-state fMRI scan. Using a data-driven approach, we identified five hubs relevant to the HBN-A: the insula, anterior cingulate cortex (ACC), the superior temporal pole (STP), the amygdala and the hippocampus. The consumption of a liquid MR dampened interconnectivity between the nodes of the HBN-A as compared to water. A secondary analysis of this dataset found that the HBN-A’s level of integration with the rest of the brain, as measured by global efficiency (GE), was inversely related to subjective experiences of hunger and craving.

Since greater HBN-A integration was associated with less hunger and craving, we hypothesized that greater baseline HBN-A integration would be predictive of weight-loss after 6-months of treatment. To test this hypothesis, we obtained baseline resting-state brain network analyses on 56 individuals prior to a randomized weight-loss trial. Higher levels of GE were associated with greater weight loss even after controlling for baseline weight, age, sex and self-regulatory efficacy. The HBN-A is comprised of limbic regions important in the processing of emotions and visceral sensations. Improved integration of these regions may provide superior awareness and processing of sensory cues leading to successful self-regulation and thus greater weight loss. Further work is
warranted to test whether individuals can volitionally improve their HBN integration and weight-loss success through awareness training (i.e. mindfulness-based-therapies) or whether intentional weight loss itself can change HBN-A integration.
CHAPTER 1

INTRODUCTION

Brielle M. Paolini, W. Jack Rejeski, and Paul J. Laurienti
In the United States and other developed nations, obesity has emerged as a public health crisis due to its rapid increase in prevalence and its wide range of associated morbidities. This crisis has not spared older adults, which represent the fastest growing portion of the U.S. population (Ogden, 2000; Ogden et al., 2014). From a practical standpoint, the problem of obesity is most often addressed through lifestyle interventions involving weight loss, with considerable variability in actual weight lost; moreover, weight regain is the norm. The over-arching goal of this thesis is to evaluate whether there is a brain signature that becomes activated during periods of food restraint and whether this signature predicts weight loss during the intensive phase of a weight loss intervention.

Three primary aims were delineated to address this goal: 1) to characterize the brain network associated with short-term food restriction; 2) to tie this circuitry to real-world behavioral consequences; and 3) to test if this resulting brain network is predictive of weight loss during the 6-month, intensive phase of a weight loss intervention among older adults. A link will be provided from brain-network signatures to behavioral experiences during food restraint (i.e. hunger and craving) and to 6-month weight loss. These data will be used to better understand self-regulatory failure and to propose novel therapeutics and intervention modalities to treat those at risk.

The perils of obesity in an aging population will be reviewed along with exiting literature related to self-regulation and eating behavior. The concept of hot versus cold visceral and emotional states will be introduced, emphasizing how they might contribute to self-regulatory failure. The neuroscience of eating behavior, especially in relation to weight-loss interventions will be examined. Finally, background information will be covered as it relates to the creation of complex brain networks and their analysis.

1.0 Obesity and Aging

Obesity among adults has doubled in the past 25 years, with the most recent national survey showing that around 35% of adult Americans are obese (Body Mass Index (BMI) > 30 kg/m2) and
69% are overweight (Ogden et al., 2014). This problem is even greater in persons aged 65 and older as 37% of older adults are obese (Ogden, 2000; Ogden et al., 2014). Obesity has particular public health importance in an aging population as it is a major risk factor for physical disability (Rejeski et al., 2009) and for many chronic health conditions, including cardiovascular disease (CVD), type 2 diabetes mellitus, arthritis, and other orthopedic complications, several types of cancers, urinary incontinence, and depression (Han et al., 2011; Samper-Ternent and Al Snih, 2012). Obesity is also associated with all-cause mortality and poorer quality of life (Samper-Ternent and Al Snih, 2012). Because these chronic diseases are costly, the obesity crisis will place an increasing financial and social burden on the U.S. health care system in the coming years with the graying of the baby boomer generation (Yang and Hall, 2008). For this reason, research topics addressing the etiology of eating behavior and weight loss among older adults are extremely timely and important.

2.0 Self-regulation during Weight Loss

Lifestyle interventions are the most common treatment prescribed and followed for individuals with obesity; however, there is significant variability in weight-loss success among individuals during the intensive phase of treatment and weight regain is common (Kramer et al., 1989; Wing and Hill, 2001; Rejeski et al., 2011). In fact, during weight loss interventions, it has been estimated that study populations realize about 30-40% of their intended goals (Godin and Kok, 1996; Conner and Armitage, 1998). Thus, there is a need for more weight-loss studies to better understand why older adults fail at attempts to self-regulate their diets and physical activity behavior (Samper-Ternent and Al Snih, 2012). Such knowledge could help revolutionize treatment and improve the success of weight-loss interventions among older adults.

2.1 Self-Regulatory Failure

Individuals’ differential responses to weight-loss treatment cannot be fully explained by genetic predispositions or other biological explanations (Weyrich et al., 2007; Haupt et al., 2010). Although
metabolic factors are likely responsible for some variability associated with weight loss and weight regain (Wadden et al., 2001; Maclean et al., 2011), self-regulatory failure to adhere to prescribed dietary and physical activity appears to be a significant barrier to weight loss and maintenance of lost weight (Wing, 2002; Heatherton and Wagner, 2011; Byrne et al., 2012). Weight regain is characterized by self-regulatory failure including behaviors such as increases in total caloric intake, increased frequency of snacking, consuming high levels of dietary fat, selection of poorer quality foods and low levels of physical activity (Katahn, 1982; Jeffery, 1984; Schoeller et al., 1997; McGuire, 1999; Ogden, 2000; Wing and Hill, 2001). Interestingly, those who regain weight have been found to be less conscious about their dietary intake (Byrne et al., 2003), have poorer cognitive restraint (Westerterp-Plantenga et al., 1998; Lejeune et al., 2003), and are not skillful at self-monitoring weight management behaviors (McGuire, 1999; Wing and Hill, 2001).

What is the etiology of this self-regulatory failure? For the past thirty years, the eating behavior literature has been focused on restraint, the intentional efforts to achieve or maintain a desired weight through caloric restriction. Recently, however, more attention has been given to the factors of disinhibition and hunger with the development of the Three-Factor Eating Questionnaire (TFEQ) (Herman and Mack, 1975; Stunkard and Messick, 1985; Laessle et al., 1989; Brownell and Rodin, 1994). Disinhibition is reflective of the tendency to eat and over-eat opportunistically in an obesogenic environment (Bryant et al., 2008). Importantly, disinhibition has been shown to be predictive of less success at weight loss; whereas, decreased disinhibition and hunger coupled with an increase in restraint during a weight loss intervention has been shown to be associated with greater success (Bryant et al., 2008).

The current food environment has begun to be labeled “obesogenic” by researchers since there is currently an ever-increasing overabundance of easily accessible, high-calorie, and highly flavorful foods (Carnell and Wardle, 2008; Murdaugh et al., 2012). This begs the question: how does this obesogenic environment contribute to disinhibition and self-regulatory failure among dieters? One
theory is that individuals differ in susceptibility to food-cues present in their immediate environment. This variability is captured by a new measure, the Power of Food Scale (PFS), which assesses the drive for an individual to consume highly palatable food in an environment that offers an abundance of options to purchase and consume these foods (Lowe et al., 2009). Our laboratory demonstrated that individuals who scored high on the PFS had increased cravings and reduced self-regulatory efficacy after a short term fast (Rejeski et al., 2012). Importantly, after a period of active imagining of food cues, these individuals also had shifts in brain networks paralleling the changes seen in other addictive behaviors (Rejeski et al., 2012).

2.2 Self-Efficacy Beliefs

Self-efficacy is a construct drawn from social cognitive theory as the belief that one can maintain a set of behaviors despite the presence of challenging obstacles (i.e. an obesogenic environment or a tempting social situation) (Bandura, 1977; 1982). Thus, self-efficacy is a measure of one’s self-regulatory beliefs or self-confidence. Clark and colleagues have created a five factor model of self-efficacy specific to the challenge of weight self-regulation called the Weight Efficacy Lifestyle questionnaire (WEL) (1991). The successful completion of a weight-loss intervention has been shown to improve weight-related self-efficacy (WEL scores), and baseline levels of self-efficacy can predict greater weight loss during weight-loss interventions (Jeffery et al., 1984; Bernier and Avard, 1986; Richman et al., 2001; Linde et al., 2006; Warziski et al., 2008; Rejeski et al., 2011; McCarroll et al., 2014). As expected, self-regulatory failure leads to decreased self-efficacy for coping with the challenges common to weight management (Rodin et al., 1988; Clark et al., 1991). Moreover, in a recent, randomized controlled weight loss trial with older adults, our laboratory demonstrated that changes in self-regulatory efficacy beliefs mediated 6-month weight loss. These findings suggest that building cognitive resources to promote effective self-regulation is important to the success of weight loss interventions (Byrne et al., 2012).
2.3 Visceral and Emotional Hot-States

Self-efficacy beliefs provide a cognitive basis for self-regulatory success or failure by providing a “top-down” mechanism for self-regulation; however, it is equally important to study “bottom-up” process that set an individual up for self-regulatory success or failure. Recent reviews on the topic of obesity have noted that the primary cause of obesity—the overconsumption of food—is complex. Overconsumption is due to both homeostatic dysregulation (bottom-up) and to dysfunctional central processing (top-down), the latter being referred to as the hedonic etiology of obesity (Lowe and Butryn, 2007). Along this reasoning, Loewenstein (2005) has proposed that individuals move into and out of “hot states” dynamically over the course of a day as a function of changes in affect or visceral cues (i.e. homeostatic dysregulation). Thus, it follows that the desire for, or preoccupation with, food would be disproportionately higher in hot than cold states (Kavanaugh, 2005). Loewenstein (2005) has shown that when people are in “cold states” they overestimate their self-regulatory capacities. For example, after a noon meal, people are confident that they can control their eating, yet fail miserably at doing so just a few hours later.

Therefore, it would make sense for dieters to avoid entering physiologic or emotional “hot-states” as these states may make them vulnerable to self-regulatory failure. State-of-the-art weight loss interventions have incorporated successful strategies tempering the power of viscerally driven hot-states on self-regulation. Two such strategies are incorporating meal replacements (Heymsfield et al., 2003) and encouraging small frequent meals (Kulovitz et al., 2014).

Liquid meal replacements (MRs) have been successfully used in calorie-restricted diets for both initial weight loss (Rothacker et al., 2001; Annunziato et al., 2009; Frestedt et al., 2012) and weight maintenance (Rothacker et al., 2001; Vazquez et al., 2009). A recent meta-analysis of 6 randomized, placebo-controlled trials of MR found that, with the same calorie goal, weight loss was greater in the MR groups compared to conventional reduced calorie diet (RCD) groups (Heymsfield et al., 2003).
The success of meal replacements could be due to a myriad of factors including ease of use, reduction in meal-planning and preparation, addition of a portion-controlled meal, or the reduction of a high calorie meal (Frestedt et al., 2012). Additionally, meal replacements may provide a satisfying, low-calorie option to relieve the hot-state created by short-term food restriction. Since meal replacements are state-of-the-art in weight loss interventions, we used a MR after a short-term fast in a randomized, cross-over design to alleviate a hot-state (see Chapter 2). This manipulation allowed us to investigate the complex brain networks associated with viscerally driven hot and cold states (see chapter 2).

3.0 The Neuroscience of Eating Behavior

In response to the obesity crisis, the scientific community has become increasingly interested in studying eating behavior and the neuroscience of self-regulation. The reward brain regions important to food consumption, including both the homeostatic and hedonic regions, have been well summarized in a recent review by Stice and colleagues (2013). These regions included the insula, hippocampus, amygdala, anterior cingulate cortex (ACC), parabrachial nucleus (PBN), the ventral tegmental area (VTA), and the orbitofrontal cortex (OPFC) (Stice et al., 2013). This review provides a summary of reward regions important in eating behavior; however, the majority of this work was based on the animal literature limiting its translation to humans.

The majority of human, functional magnetic resonance imaging (fMRI) studies have examined regions of the brain that become active during food-cue visualization tasks (Del Parigi et al., 2002; Rothemund et al., 2007; Stoeckel et al., 2008; Bruce et al., 2010; Martin et al., 2010; Paolini et al., 2012; Rejeski et al., 2012; Bullins et al., 2013). To understand how these brain responses are related to obesity, researchers have conducted cross-sectional studies contrasting the brain activity of obese individuals to the activity of lean, non-dieting controls (Del Parigi et al., 2007; Le et al., 2007; Cornier et al., 2009; McCaffery et al., 2009; Batterink et al., 2010). Overall, these studies have found increased activation in obese individuals within the reward system and associated brain regions, similar to the
proposed reward circuitry for drugs of abuse and include many of the regions reviewed by Stice et al. (i.e. insula, hippocampus, amygdala, ACC, and OPFC) (Volkow et al., 2008;Stice et al., 2013).

These fMRI activation studies have provided a lot of information concerning differential brain responses to food cues in these distinctly different populations; however, they do not explain if these brain activations/regions are related to real-world weight regulation.

3.1 fMRI predicting weight loss from baseline

A few studies have found that certain brain activation patterns are predictive of long-term weight status (Batterink et al., 2010;Yokum et al., 2011;Kishinevsky et al., 2012). In other words, baseline fMRI brain signatures were predictive of natural weight loss or weight gain over a one-to-three year period. To our knowledge, only four studies have demonstrated that baseline brain activity was predictive of long-term weight loss success or failure during an active weight-loss intervention program (Kishinevsky et al., 2012;Murdaugh et al., 2012;Hege et al., 2013;Weygandt et al., 2013).

3.1.1. Executive Control and Weight Loss

Several studies of long-term weight status have demonstrated that high levels of activation in the prefrontal cortex was predictive of weight loss (Yokum et al., 2011;Kishinevsky et al., 2012;Weygandt et al., 2013). Greater prefrontal activation during executive tasks was predicted of weight loss during an active intervention (Weygandt et al., 2013); whereas, decreased activation was predictive of greater natural weight gain over the next couple of years (Kishinevsky et al., 2012).

Specifically, Kishinevsky and colleagues (2012) found that increased activation of the inferior, middle and superior frontal gyri, regions important for executive function, were associated with weight loss over a 1-3 year period. Weygandt and colleagues (2013) demonstrated that greater activity in the dorsolateral prefrontal cortex (DLPFC), a region important for self-control, and the ventromedial prefrontal cortex (VMPF), a region important for signaling the reward of food, was associated with weight loss during the 12 week intervention (Hare et al., 2009;Hare et al., 2011).
Summarizing these results, it appears that greater baseline activation in lateral frontal regions associated with executive function is beneficial for weight-loss.

In contrast to these studies, Yokum et al. (2011) found greater activation of the lateral orbital prefrontal cortex (OPFC) during food cues predicted increases in weight over one year. While the OPFC is anatomically part of the frontal cortex, it is actually a reward region important for valence attribution, as noted by Stice and colleagues (2013). One would expect greater reward activity to be associated with self-regulatory failure and resulting weight gain, and greater executive function and decision-making to be associated with improved self-regulation and weight loss. Consequently, it makes sense that the greater activation of the OPFC, a reward region, was associated with weight gain; whereas, greater activation in the lateral prefrontal cortex, a region known to be important to executive function, was related to weight loss (Yokum et al., 2011; Kishinevsky et al., 2012; Weygandt et al., 2013).

3.1.2 Reward Pathways and Weight Loss

The remaining four studies that relate baseline brain signatures to weight have found activation in other reward centers to be important for weight-loss (Batterink et al., 2010; Stice et al., 2010; Murdaugh et al., 2012; Hege et al., 2013). The majority of cross-sectional studies have indicated that greater food cue reactivity (that is, activation of brain regions) to high-calorie food cues contributes to the development of obesity (Stice et al., 2013); therefore, one would hypothesize that greater baseline activation in reward regions would impair weight-loss during an intervention or be related to natural weight-gain. Murdaugh and colleagues (2012) provide some evidence in support of this hypothesis. They reported that those obese individuals who were least successful at losing weight during a dietary weight-loss intervention had greater pre-treatment activation to food stimuli versus control pictures in brain regions implicated in reward (nucleus accumbens, anterior cingulate and insula), as well as in visual and attention regions.
Using a different food reactivity task, Stice and colleagues (2010) found that activation in different reward regions (frontal operculum, lateral orbito-frontal cortex (OPFC) and the striatum) predicted future increases in body weight in participants during the subsequent year; however, there was a negative correlation between activation in these regions and weight gain in those individuals with genotypes suggestive of impaired dopamine (DA) signaling (Stice et al., 2010). While the Stice study also found that greater reactivity to food-cues was associated with an increase in weight in those with normal DA signaling, different reward regions were identified from the Murdaugh study. The disparity may be due partially to the fact that an alternative food-reactivity task was used; specifically, the task compared the imagined intake of palatable foods with the imagined intake of unpalatable foods or water (Stice et al., 2010). Murdaugh et al. used a task that compared visualization of food versus control pictures. The Stice study was most likely capturing the brain activity of valence attribution; whereas, the Murdaugh study was most likely capturing a food versus no-food effect.

The final two studies predicting weight loss involved more cognitively demanding food-related-tasks requiring either working memory (one-back task) or response inhibition (go-nogo task). Hege and colleagues (2013) used baseline brain activity during a one-back visual memory task with food and nonfood pictures measured by magnetoencephalography (MEG) to study the differences between those individuals who were successful (responders) and unsuccessful at losing weight (non-responders) during a lifestyle intervention. Also, successful individuals (responders) showed greater baseline activation during this working memory task in the right temporal areas, the hippocampus and the fusiform gyrus.

Batterink et al. (2010) using a go-nogo task found that decreased activation in the temporal operculum, a region important for food reward, was associated with future weight gain. Specifically, they reported a negative correlation between brain activation in the temporal operculum on nogo trials with high calorie food pictures of a go-nogo task and weight gain at 1 year.
All four of these fMRI studies predicting weight-change from baseline found different regions of brain activation to be predictive of weight loss. This variability in results may be due to the fact that each study used a different fMRI task. When separate laboratories employ distinct study designs and methods, it makes integration of study findings challenging, if not, impossible.

3.1.3 Limitations of Existing Research

It has recently been proposed by Pessoa (2014) that fixed structure-function relationships (i.e. one-to-one mapping) should give way to a perspective of the brain where regions are dynamically recruited into ‘multi-region assemblies.’ Such assemblies are highly context-dependent; and therefore a given brain region participates in multiple different assemblies across different contexts or tasks (many-to-one mapping) and similar functions can be carried out by diverse regions or assemblies (one-to-many mapping) (Pessoa, 2014).

To date, the neuroscience literature has focused almost exclusively on one-to-one mapping of brain activity during specific tasks. These studies identify specific brain regions that become differentially active between tasks. In other words, traditional neuroimaging methodologies are designed to answer the question, “which area of the brain is most active during a particular task?” Such research questions make the grave assumption that isolated, individual brain regions are uniquely responsible for specific functions. The complexity model dismisses this idea and instead argues that it is the interactions among “multiple-region assemblies” that are most important for understanding brain function and behavior—at rest and during the performance of tasks.

To study the dynamic interactions among “multiple-region assemblies,” one cannot use traditional methods of the past. While such methods have helped us understand the areas important in self-regulation, they do not tell us anything about the complex, interactive pattern of activation during various states. To answer some of these complexity-driven questions, researchers have started applying graph-theory-based network methods to brain imaging data. The upside of such networks is
that they provide opportunity to study the entire brain at once (i.e. multiple regions recruited) for a particular task and quantification of the connectivity patterns or interactions among important regions.

The last few years have seen an explosion of network-related studies in brain imaging; however, until the present thesis, brain network analyses had yet to be incorporated into the study of self-regulation and weight loss. Weygandt et al. (2012) did incorporate functional connectivity (FC) between two frontal ROIs; however, FC is methodologically dissimilar to graph-theory based network analyses (described in detail below).

As mentioned earlier, to date brain imaging research has focused either on brain responses to food-cues or to food-related executive function/memory tasks. It is challenging to summarize this prior body of work since distinct tasks differentially recruit distinct brain circuits or “multiple-region assemblies.” One can imagine that the brain circuitry required for imagining the consumption of a piece of pizza (food visualization task) would be unlike the circuitry needed to remember if a picture of pizza was shown on a previous slide (one back food challenge). Since these tasks and their subsequent brain patterns are often dissimilar, comparison across studies is not possible. Resting-state studies improve the ability to make comparison across studies as compared to cognitive behavioral task studies (Cao et al., 2014).

While food-related visualization, memory and executive function tasks are great experimental paradigms to simulate real-world dietary temptation and the executive function required to harness cognitive control, it is also important to study the brain at rest. The resting brain offers the opportunity to examine underlying integration patterns that may be governing behavior at both conscious and unconscious levels. While resting-state studies have been used in graph-theory brain analysis for several years with great success, prior to the presented work, resting brain networks had not yet been studied in relation to eating behavior or weight-loss interventions. Moreover, all previous research on eating behavior and the brain has focused on young to middle-aged adults. Considering the imminent financial and social burden of an aging overweight and obese population, it is important
to study how these brain networks are working in metabolically compromised older adults as well. Such knowledge will provide insight into the neuroscience of self-regulation in this vulnerable population and could help both target individuals at risk for self-regulatory failure as well as the design of new therapies such as mindfulness-based weight loss interventions.

4.0 Using brain networks to study eating behavior

Graph theory was developed as a branch of mathematics well over two hundred years ago with Leonhard Euler’s thesis on the ‘bridges of Konigsberg (Euler, 1736).’ In this thesis, Euler introduced the idea of representing a connected system with nodes (locations) and edges (bridges) on a graph. The graph structure allowed Euler to demonstrate how the inherent organization and interconnection of a system limits potential movement throughout the system (i.e. efficient travel across the bridges) (Fornito et al., 2013).

The field of network science was reignited when Watts and Strogatz (1998) introduced the small-world network model. A small-world network is characterized by the simultaneous presence of both regional specification (i.e. locally clustered connectivity) and efficient global transfer (i.e. short path lengths) throughout the entire network (Watts and Strogatz, 1998; Telesford et al., 2011; Fornito et al., 2013). For years the brain was thought to possess these features; however, no hard evidence existed for this dual-processing paradigm. When network science was applied to the brain, the brain demonstrated properties of a small-world network (i.e. the simultaneous presence of regional specificity and distributed processing) (Watts and Strogatz, 1998; Stephan et al., 2000). This finding ignited interest in network science. In fact, over the past decade, the field has matured so much that network science has become the method of choice for analyzing functional brain imaging data.

In functional brain networks, a node represents some predefined region of brain tissue, and an edge is defined as a functional connection between a pairs of nodes (Stanley et al., 2013). Researchers define their network nodes in many different ways. Some use anatomical methods (i.e. atlases) to
define nodes based on brain structures while others use functional activation methods relying on prior data from activation studies to determine specific network nodes of interest. We chose to build our networks at the voxel-wise level. Voxels are small cubes (volumetric pixels) representing a region of brain tissue in three dimensional space. The fMRI time-series recorded from these voxels (nodes) are then used to create the functional network.

Links between nodes in a network are measured by a variety of different methods depending on the type of network being analyzed (Friston, 2011). For example, structural networks can be derived from histological samples or diffusion tensor imaging (DTI) data (Sporns et al., 2002; Sporns et al., 2007; Hagmann et al., 2008). Although structural networks provide a way to study the actual hard-wiring of the brain (i.e. map direct connections in the brain), it is well-known that the brain has functional coherence between distantly related brain regions. While the structural connectivity of the brain does predict some functional connectivity (Honey et al., 2007), it does not fully explain it as two brain regions can be functionally connected without a direct anatomical connection between them (Honey et al., 2009).

To study the differences in functional connections (i.e. coherence) among nodes or brain regions, one can build a network of functional connections. This is most commonly done with temporal correlations of the fMRI time-series (i.e. by correlating the time-series of every node (voxel) with every other node in the network). A threshold is applied to the correlation matrix to ensure each network has a similar connection density allowing comparison across individuals (Hayasaka and Laurienti, 2010). We choose to threshold our networks as we believe it provides many advantages; however, other network researchers advocate the use of fully-connected (i.e. no threshold), weighted networks (Rubinov and Sporns, 2011). Without thresholding, weaker links are left in the network. These weaker links are often the result of noise or spurious connections; thus, they can create misleading results (Telesford et al., 2011). Moreover, without a threshold, each node in the weighted network is connected to some degree with every other node in the network. Overall, this creates a
network that loses some of its small-world properties as information is no longer shared locally but is immediately shared globally.

4.1 Common brain network metrics (k, first and second connections, and GE)

With the explosion of the network science in neuroimaging in the past decade, there has been a rapidly expanding choice of metrics for analysis (Rubinov and Sporns, 2010; Telesford et al., 2011). While not a comprehensive list, popular metrics include degree, clustering coefficient, modularity, characteristic path length, global efficiency, local efficiency, small-worldness, k-core and network motifs (Rubinov and Sporns, 2010). Each of these metrics captures a different feature of a complex brain network; thus, it is important not to rely on any single metric when studying a network/system. It should be stressed that consideration of multiple metrics is required to fully capture the complexity inherent in a dynamic system. The current thesis focuses on the following metrics: degree, global efficiency, and step connections. Each metric is described in detail below:

4.1.1. Degree

Degree (K) is the most basic network metric and is a measure of how many direct connections (i.e. functional connections) a particular node has in a given network. Degree answers the question, “how far is a node’s immediate reach?” Therefore, calculation of the degree metric is dependent on its immediate neighbors. This can be illustrated by looking at node A in Figure 1.1. Node A has a degree of eight as it has eight nodal connections (contained within the black box); thus, only these immediate neighbors of node A affect the score for degree.
Above is a sample network illustrating the differences between degree and global efficiency (GE). Degree is the number of connections of a particular node. In the box on the left, node A’s degree is eight as it has eight connections. GE is calculated as the inverse of the average path length. Degree and GE are calculated for every node in the network and are summarized in the table on the bottom right.
4.1.2. Global Efficiency

Global efficiency is a measure of the integrative capacity a node has with the network (Latora and Marchiori, 2001). In other words, GE answers the question, “How far is an individual node’s reach throughout the entire network?”

Specifically, global efficiency (GE) is the inverse of the average shortest path-length of the node and is defined as 

\[ GE = \frac{N}{\sum_{i=1}^{N} L_i} \]

where \( L_i \) is the shortest path length from node \( i \) to every other network node and \( N \) is the number of nodes in the network. Thus, this metric ranges from 0 to 1. A node that is directly connected to all nodes in the network would have a global efficiency of 1 whereas a disconnected node would have a global efficiency of 0. Figure 1.1 demonstrates that the nodes with the highest GE (nodes B and C) are centrally located and thus have the shortest path lengths to every other node in the network. The GE calculation is highly dependent on the overall topological organization of the network. Thus, the GE metric is highly multivariate in nature.

4.1.3. Step Connections

Connectivity among regions of interest (ROIs) or nodes can be quantified by computing the first order (direct) and second order (indirect) connections. The first order connections are the immediate network neighbors of a particular seed node or ROI; whereas, the second order connections are the brain regions that have direct connections to the immediate neighbors of the seed node/ROI. In terms of social networks, your direct connections are your friends and your indirect connections are your friends’ friends (see Figure 2.1).
Figure 2.1 Direct and Indirect Connections

A sample network was created to illustrate the concept of direct and indirect connections. The full network is illustrated at the top of the figure and includes two regions-of-interest (ROI): ROI-A and ROI-B. On the bottom left, the direct connections between these two regions are isolated for visualization. As shown, ROI-A and ROI-B share four direct connections. On the bottom right, the indirect connections between the two ROIs are isolated. Indirect connections between ROI-A and B are those that pass through shared neighbors. Thus, direct connections are one step from the ROI of interest, and indirect connections are 2 or more steps from the ROI of interest. In this example and in the studies presenting in this dissertation, indirect connectivity was limited to 2 steps from the ROI of interest. ROI-A and ROI-B have one shared neighbor (green node). ROI-A has one direct connection with the shared neighbor while ROI-B has four. This example shows that when going from ROI-A to ROI-B there are four indirect (or second order) connections. However, when going from ROI-B to ROI-A there is only one indirect connection.
Direct connections are symmetrical. As shown in Figure 2.1 (bottom left), region A and region B have four direct connections. Indirect connections between two ROIs can, however, be asymmetric because the ROIs may or may not share the same number of connections to those neighbors. This is depicted in Figure 2.1 (bottom right) as region A has four indirect connections to region B; whereas, region B has only 1 indirect connection to region A. This asymmetry for indirect connections between two hubs does not reflect a difference in directionality of information flow; instead, it reflects a difference in the complexity of the connections between the two ROIs. For instance, a large number of indirect connections from ROI-A to ROI-B indicates a large number of connections from ROI-A's neighbors to ROI-B; whereas, a small number of indirect connections from ROI-A to ROI-B indicates that there are a small number of connections from ROI-A's neighbors to ROI-B.

4.1.4. Step Connections versus Functional Connectivity

Functional Connectivity (FC) is a popular technique used in neuroscience imaging literature to assess brain connectivity. While FC is often confused with direct connection analysis in network science, it is actually a very different and simplified technique. In FC, the connection strength from one ROI is mapped throughout the entire brain. The time-series of all voxels within the ROI is averaged to create a single time-series. This resulting time-series is then correlated with every other series in the data-set. The researcher is left with R² values comparing the strength of the ROI’s signal to every other time-series in the brain.

When calculating the number of connections from one ROI to another ROI from a network analysis, each voxel within an ROI is still treated as an individual node in the network. Two ROIs can have multiple connections to each other. For instance, in direct connection analysis, we count the number of these connections (as described in detail above) to gauge the complexity of connectivity between two ROIs. In FC, however, only one connection is possible between two ROIs making connection strength (R²) the only analysis possible.
A distinct advantage of using a whole-brain network is that it also allows investigation of indirect connections between two ROIs. Such information helps paint a more complete picture of the brain network. Indirect connections allow for visualization beyond the reach of a node’s immediate neighbors. Mapping of indirect connections is not a feature available to researchers using traditional FC; consequently, the use of FC exclusively may limit data interpretations. For example, imagine a scenario where the amygdala has a number of direct connections to the insula but not to the anterior cingulate cortex (ACC). The insula, however, does have connections to the ACC. Using traditional methods, one would assume that the amygdala (a rudimentary brain region) would be unable to influence the ACC (a more cognitive brain region) since it lacks a connection to the ACC. Using a connection analysis from graph-theory, it would be possible to know that the amygdala is indirectly connected to the ACC through a shared neighbor (i.e. the insula).

4.2 Validity of Network Imaging Approach

Over the past decade, graph-theory-based network studies have become the method of choice for analyzing functional brain imaging data. To validate this approach, many researchers have studied the test-retest reliability of this technique (Telesford et al., 2010; Whitlow et al., 2011; Braun et al., 2012; Liao et al., 2013; Telesford et al., 2013; Cao et al., 2014; Zuo and Xing, 2014). In summary, these studies have shown reproducibility is maximized with the use of resting-state scans (Cao et al., 2014), of global regression (Braun et al., 2012), of second order metrics (Braun et al., 2012), and of scan durations of around 5 to 6 minutes or longer (Whitlow et al., 2011; Liao et al., 2013; Zuo and Xing, 2014). Telesford’s 2010 investigation of the reliability of resting-state fMRI data using graph theory on voxel-based network in older adults is the most relevant to the current dissertation work. Specifically this work demonstrated that resting brain network measures are reproducible in 45 healthy older adults (mean age=69) scanned twice during the same testing session. Intraclass correlation coefficients were high for the clustering coefficient (ICC= 0.86), global efficiency (ICC= 0.83), and path length (ICC=0.79) (Telesford et al., 2010). Taken together, all of this evidence
demonstrates that graph metrics have robust test-retest reliability and validity and thus, are suitable for clinical studies.

4.3 The Value of Using Networks to Study Eating Behavior

In the new holistic view advanced by Pessoa (2014), brain regions are differentially recruited into ‘mutli-region assemblies’ based on various contexts facilitating understanding of both brain function and behavior. It is the complex pattern of interactions among these regions that is most paramount. Understanding real-world behaviors, such as eating, will require examination of how brain regions interact to form circuits or multiple region assemblies and how these circuits change dynamically from one context to the next. Graph-theory-based network science allows neuroscientists the unique opportunity to analyze these differential activation patterns of brain recruitment during various contexts. These methods also paint a more holistic and detailed picture of the brain network, permitting visualization and analysis beyond the reach of a particular node’s immediate neighbors. Graph-theory-based network science has the potential to revolutionize our understanding of brain functions and of their relationship to complex behaviors, such as the self-regulation of eating.

5.0 Dissertation Outline

The over-reaching purpose of this thesis is to identify a brain network of self-regulatory failure among older adults predictive of weight loss. The first objective is to characterize the resting-state brain network circuitry associated with a vulnerable hot-state (short-term food restriction) among older adults. The second objective is to tie this brain circuitry to real-world behavioral consequences such as hunger and craving. The third objective is to test whether this brain network is predictive of real-world weight loss in a community based intensive weight-loss intervention. This will provide the first evidence on whether individual differences in complex brain networks at baseline are associated with weight loss, and it will be one of the first studies to demonstrate a neural basis of self-regulatory failure within the context of active attempts at weight management. Such knowledge could
revolutionize how researchers address the obesity epidemic. It could allow for targeted treatment of individuals with a brain signature placing them at-risk for failure in efforts to lose and sustain weight loss. Such personalized treatments may enhance weight-loss success and secondary mortality in this vulnerable population; consequently, the research questions presented are extremely important and timely in addressing the climate of increasing economic cost and social issues secondary to the obesity epidemic.
References


theoretical properties during working memory, emotion processing, and resting state. 


Euler, L. (1736). Solutio probematisa d geometriams itus pertinentis, Commentari Academiae Scientiarum Imperialis. _Petropolitanae_, 128-140.


Kavanaugh, J.F. (2005). Brainism: 'we are not our brains; we are embodied persons'. *America (NY)* 193, 8.


CHAPTER II
MEAL REPLACEMENT: CALMING THE HOT-STATE BRAIN NETWORK OF APPETITE

Brielle M. Paolini, Paul J. Laurienti, James Norris and W. Jack. Rejeski

Published in Frontiers Psychology, 2014, volume 5, issue 249
Doi: 10.3389/fpsyg.2014.00249
Introduction

There is a growing awareness in the field of neuroscience that the self-regulation of eating behavior is driven by complex networks within the brain that control “liking” and “wanting” of food. In extensive research, Berridge and colleagues (2010) have shown that ‘liking” represents the hedonic facet of this process whereas “wanting” refers to incentive salience motivation. The authors emphasize that “wanting” can be motivational even when hedonic “liking” does not arise. Although it is not possible to discern “liking” from “wanting” in most experimental paradigms, there is evidence that the desire to consume food is based upon activity in brain networks that vary as a function of individual differences (Rejeski et al., 2012), environmental stimuli (Stoeckel et al., 2009; Bullins et al., 2013; Kullmann et al., 2013), and homeostatic drive (Berthoud, 2012). In line with Loewenstein (2005), people can move in and out of “hot states” dynamically throughout the course of a day as a function of changes in affect or visceral cues; thus, the desire for food or the preoccupation with it is disproportionately higher in hot than cold states (see also (Kavanaugh, 2005)). The goal of the current study is to identify and determine differences in the hot-state brain network of appetite (HBN-A) that exists in a resting state after a brief period of food restraint followed either by the consumption of a meal replacement (MR) or water.

The study design we employed involved having participants (a) come to our laboratory on two occasions so that we could feed them a controlled morning meal, (b) insure that they did not eat for 2.5h, (c) deliver on a randomized schedule either water or a MR, and (d) then have them participate in resting fMRI scans. Thus, we were interested in whether there was an identifiable HBN-A in a resting state following a brief period of food restraint and, whether MR calmed this network as compared to water. In identifying the HBN-A, we were guided by both existing research and an empirically-driven process. Specifically, a recent review by Stice and colleagues has summarized the integrative signaling of the brain reward system, including both the homeostatic and hedonic feeding systems (Lowe and Butryn, 2007; Stice et al., 2013). Much work has been done in this field by other
investigators as well (Berridge et al., 2010; Kringelbach et al., 2012); however, the review by Stice and colleagues (2013) is one of the most comprehensive in identifying areas at all levels of the brain, from the subcortical to the neocortex, that are involved in eating behavior. Based upon this review and other published work (Bechara et al., 2000; Tracy et al., 2001; Kringelbach, 2004; Olson et al., 2007; Stoeckel et al., 2008; Pessoa, 2010; Carnell et al., 2012; Kringelbach et al., 2012; Paolini et al., 2012; Rejeski et al., 2012), we anticipated that the HBN-A would consist of at least 4 primary regions: the insula, hippocampus, amygdala, and anterior cingulate cortex. Due to limitations of fMRI technology, we did not anticipate detecting small, deep brain structures such as the parabrachial nucleus or the ventral tegmental area identified by previous investigators (Kringelbach et al., 2012; Stice et al., 2013). Similarly, because our study involved resting state without active processing of food cues, we did not expect involvement of the orbitofrontal cortex (OPF) (Kringelbach et al., 2012).

Because we are unaware of existing fMRI research on this topic using graph-theory-based methodology, the first phase of our analysis was designed to qualitatively evaluate and empirically confirm the network hubs of relevance to the HBN-A. To ensure high sensitivity in this phase, we used a p value of 0.10 to identify network hubs. Once the structure of the HBN-A was established, we then examined whether the consumption of a MR altered connectivity in this network as compared to water. This second phase, which was the primary aim of the study, employed a per comparison error rate of p=0.05. Based upon clinical research which has shown that MR products are effective in curbing appetite and in promoting weight loss (Rothacker et al., 2001; Heymsfield et al., 2003; Annunziato et al., 2009; Frestedt et al., 2012), we hypothesized that MR would decrease connectivity within the HBN-A when compared to water and that this effect would be evident for both direct and indirect connections within the HBN-A. We also evaluated state craving and hunger and expected both to be higher in the water than MR condition.
Methods

Participants

A sample (n = 14) of older, overweight and obese (BMI ≥ 28 kg/m\(^2\) but ≤ 40 kg/m\(^2\)) adults was recruited from Forsyth County, NC. All participants were between the ages of 50 and 79 and lived independently. The sample included an equal number of men and women. Each participant completed a phone screen, an in-person screening visit, and two 5-hour experimental sessions at Wake Forest School of Medicine, receiving a maximum of $225 for completing all three visits to compensate for their time.

Prescreening and Lost to Follow-up

A telephone screen was administered to interested individuals to determine their qualifications as potential participants. Exclusion criteria included (1) having a BMI outside our established range, (2) the presence of a systemic uncontrolled disease or psychiatric illness, (3) a binge eating disorder, (4) high alcoholic intake (more than 3 drinks per day), (5) the inability to safely undergo magnetic resonance imaging due to claustrophobia or to the presence of implanted magnetic objects/devices, (6) currently undergoing treatment for cancer, (7) active participation in another research study that might interfere with either the study's procedures or objectives, (8) need for assistance while walking, (9) being unable to read or speak English, or (10) the inability to correct eyesight to at least 20/40 in the scanner to complete the required tasks. Over 125 people were contacted by phone about the study, of these 22 were brought in for in-person screening visits, 7 participants did not complete the study due to various reasons: withdrawal due to claustrophobia and poor vision in the scanner. One participant was excluded from the data analyses due to poor quality of brain images during one functional scan that could not be corrected using computer software.

Measures
Food Craving Questionnaire (the FCQstate): The state version of the FCQ was used to measure food cravings and to obtain a measure of hunger as a manipulation check (Cepeda-Benito et al., 2000). The craving measure consists of 15 items that target preferred foods using a 5-point scale (1 = strongly disagree; 5 = strongly agree) with the mid-point being anchored by the label neutral; thus, total scores can range from 15 to 75. In our own work, we have found the FCQ state to be very sensitive to food restraint (Rejeski et al., 2010). The 15-items are averaged for a total score. In addition, three items from the FCQ can be used to derive an index of hunger. The Cronbach alpha internal consistency reliabilities in this study for both scales were excellent, ≥0.90.

The Interview for the Diagnosis of Eating Disorders (IDED-IV): The semi-structured interview described by Kutlesic and colleagues (1998) was employed to exclude any potential participants that might have a binge-eating disorder as defined by the DSM-IV criteria. Dr. Williamson, an investigator involved in the development of the IDED-IV, provided the training on how to screen for Binge Eating.

In-person screening & assessments

An in-person screening visit was completed to obtain an informed consent, collect biometric data, assess current states of physical activity and possible dieting, and to screen for binge eating disorders. The IDED-IV was used to identify and exclude people with possible eating disorders. Eligible individuals were scheduled for two imaging visits 7 to 10 days apart. If necessary, participants were fitted for MRI-safe corrective lenses to be used in the scanner during computer tasks.

Experimental Protocol for the scanning visits

Participants completed two 5-hour visits beginning in the early morning around 8:00 a.m. Participants were asked to arrive in a fasting state, having not eaten breakfast or consumed anything other than water. During each visit, participants ate a prepared breakfast containing 350 calories for females and 450 calories for males. The meals were designed by a staff nutritionist to provide a heart
healthy balance of macronutrients containing approximately 25% fat, 15% protein, and 60% carbohydrates. Participants were allowed to choose macronutrients from a menu. Following the consumption of at least 75% of their breakfast, participants completed a baseline FCQ_{state}. The participants then fasted for 2.5 hours under the supervision of research center nursing staff.

Approximately 45 minutes before the imaging procedure, the research staff then administered the MRI safety form and led each participant in a practice session of the tasks to be completed during the fMRI. About 30 minutes before the scan time, participants either consumed a can of the Nestle MR beverage BOOST® (short term energy surfeit containing 240 calories, vanilla flavor) or an equivalent volume of water. They then completed a second round of the FCQ_{state}. The assignment of the MR and water condition was randomized.

**Resting State Scanning Tasks**

Participants wore goggles (Resonance Technology, [www.mrivideo.com](http://www.mrivideo.com)) in the scanner that were directly interfaced with a computer screen. The MRI consisted of a resting-state session where individuals viewed a cross on the computer screen interfaced with their goggles for a period of 5 minutes.

**Scanning Protocol**

All scans were performed on a 1.5 GTE scanner using an 8 channel neurovascular head coil (GE Medical Systems, Milwaukee, WI, USA) and included anatomic imaging, perfusion, and one resting state fMRI. All fMRI data was used to evaluate differences in brain networks between each individual’s MR and water treatment condition.

Functional images for the network analyzes measured changes in the T2*-relaxation rate that accompany changes in blood oxygenation. The T2* signal is sensitive to changes in blood oxygen content. As brain activity changes, the oxygen content of the blood in the same area also changes.
Thus, the T2* signal is an indirect measure of changes in neural activity (Ogawa et al., 1990). Functional imaging was performed using multi-slice gradient EPI (TR = 2000 ms; TE = 40 ms; field of view = 24 cm (frequency) x 15 cm (phase); matrix size = 3.75 mm x 3.75 mm x 5 mm).

**Imaging Processing and Network Analyses**

In preparation for generating brain networks, all scanning images were realigned and normalized to standard space using FSL (Smith et al., 2004). The time courses were extracted for each voxel in gray matter based on the Automated Anatomical Labeling atlas (Tzourio-Mazoyer et al., 2002) and band-pass filtered to remove signals outside the 0.009-0.08Hz range (Biswal et al., 1995). To account for physiological noise, mean white matter, CSF, and motion correction parameters were regressed from the filtered time series. This regression procedure removes signal fluctuations that are unlikely to be from neuronal activity (Fox et al., 2005). A correlation matrix was then created by computing Pearson correlations between all possible pairs of voxels (~21,000 voxels). This produced a 21,000 x 21,000 matrix which each cell (ij) representing the correlation coefficient between nodes i and j. A threshold was then applied to the correlation matrix and all cells that surpassed this threshold were assigned a value of 1 while remaining cells were assigned a value of zero. The threshold was defined so that the relationship between the number of nodes and average number of connections at each node was consistent across subjects to produce an adjacency matrix. Specifically, the relationship \( S = \log(N)/\log(K) \) was the same across subjects as described above (Hayasaka and Laurienti, 2010). The threshold \( S = 2.5 \) was used for this paper. This resulted in networks with connection densities meeting expected values based on the size of the networks (Laurienti et al., 2011). All remaining analyses were completed using the binary 21,000 x 21,000 adjacency matrix.

To define hubs-of-interest, we generated degree (K) maps for each individual. Degree is the number of functional connections linked to a node. For each individual, we generate a degree (K) map which gives a degree value (i.e. number of connections) for each voxel in the network. In order
to compare regions-of-interest (ROIs) between the two conditions, we generated the average number of connections for each ROI for each individual. This allowed us to run a paired t-test to test the difference in degree or number of connections for each ROI between the MR and water conditions. The same ROIs defined below were used to evaluate average degree for each individual.

To assess network organization, first order (direct) and second order (indirect) connection analyses were performed for ROIs that qualified as hubs-of-interest. First order connections are the immediate network neighbors of the hubs identified because they share direct connections. In this paper, we calculate the direct connections as the number of direct connections between two ROIs (i.e. the insula and the amygdala). Second order connections are areas that have direct connections to the immediate neighbors of the hubs (Figure 2.1). Indirect connections between two ROIs can be asymmetric because the ROIs may or may not share the same number of connections to those neighbors. This asymmetry for indirect connections between two hubs does not reflect a difference in directionality of information flow; instead, it reflects a difference in the complexity of the connections between the two ROIs. For instance, a large number of indirect connections from ROI-A to ROI-B indicates a large number of connections from ROI-A’s neighbors to ROI-B; whereas, a small number of indirect connections from ROI-A to ROI-B indicates that there are a small number of connections from ROI-B’s neighbors to ROI-A.
Figure 2.1 Cartoon Demonstrating Direct and Indirect Connections

Direct connections or first order connections are nodes that are immediate network neighbors (i.e. have a direct connections) with the hub region. Indirect connections or second order connections are areas that have direct connections to the immediate neighbors of the hub.

For our analysis, the following ROIs were used: insula, defined by the Automated Anatomic Labeling (AAL) atlas; amygdalae, defined by the AAL atlas; the superior temporal pole, defined by the AAL atlas; anterior cingulate cortex, defined as a sphere with a radius of 10 mm with the MNI coordinates (x=4, y=38, z=0); the right hippocampus, defined as a sphere with a radius of 6 mm and the MNI coordinates (x=21, y=-7, z=-18) based on the region with the highest degree in the degree map. The ACC and the right hippocampus ROIs used were determined by drawing spheres over the region using the WFU pick-atlas software (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003). For each ROI created with the exception of the hippocampus, both the left and right corresponding regions were included. The ROI used for the hippocampus was unilateral because the right side was the only side that differed between MR and water conditions.

The ROIs generated in pick-atlas software are at a 2mmX2mmX2mm resolution. All ROIs were resliced to 4mmX4mmX5mm, the resolution of the imaging data, for this analysis. Standard reslicing
practices combine a subset of original voxels into a new larger sized voxel. This process causes adjacent ROIs to overlap and to share common voxels. To correct for this issue, every resliced voxel is assigned to a single ROI. This assignment is based on the most frequent assignment of the original 2mmx2mmx2mm voxels in the AAL atlas.

**Analytical Strategy and Statistical Analyses**

In this paper, resting brain network data collected during the water condition was used to provide empirical support for the existence of a hot-state brain network for appetite (HBN-A). We relied on both extant research and an empirical approach to locate ‘hubs of interest’ in the HBN-A during our first-stage, exploratory analysis. Thus, we used a data-driven approach to the identification of our hubs. The hubs were then used as seeds for further analysis of connectivity within the network, which was our primary outcome. It is important to note that we used node degree to inform our connectivity analyses, and the connectivity analyses to inform our identification of hubs. We defined hubs-of-interest as regions-of-interest (ROIs) that had a larger degree (i.e. greater number of connections) in the water than MR condition. For our first-stage, exploratory analysis of hubs of interest, we set the alpha level for hubs-of-interest at \( \alpha < 0.10 \) which allowed us to capture ROIs that may have important connectivity profiles despite not reaching a conventional level of significance using the degree metric.

In addition, a second phase for validating the conceptual import of each hub was to examine first order (direct) and second order (indirect) connections between the hubs-of-interest. To be included in the HBN-A, hubs not only had to pass the first level of screening based on degree, but also had to show evidence of either first or second order connectivity to one of the other hubs-of-interest. If the hub met these two criteria, it was then considered a node in the HBN-A. Once the nodes of the HBN-A were established, we then compared connectivity in this network for the water condition versus the MR treatment condition. It is important to note that all hypothesis tests conducted were based on paired t-tests and a 0.05 pairwise level of significance.
We used a mixed model ANCOVA to test the effect of the MR manipulation on the food craving questionnaire. In this analysis, we controlled for the assessment taken at the time of the post-breakfast feeding as well as the random subject effect. The outcome of interest was the craving responses taken just prior to conducting the fMRI scans.

Results

Participants (7 males and 7 females) had a mean (SD) age of 71.35 (4.92) years with a Body Mass Index of 30.43 (2.09). Two of our participants were African American and the remaining 12 were Caucasian. The leading comorbidities included arthritis (n = 8) and hypertension (n = 4); these health conditions were followed by cancer (n = 2) and cardiovascular disease (n = 1).

The efficacy of the MR manipulation was supported by data showing that ratings of hunger from the food craving inventory were higher on the day that participants consumed water prior to the scanning procedure, mean (SE) = 9.00 (0.67), as compared to the day they received a MR, mean (SE) = 6.78 (0.75); t = 2.14 (13), p = 0.05.

Craving

As discussed above, a mixed-model ANCOVA was employed to examine how participants’ level of state craving was influenced by the two treatment conditions (MR or water). Analysis of the FCQ\textsubscript{state} measure produced a significant main effect for the MR manipulation (F\textsubscript{1,12} = 6.23, p = 0.028). Cravings were significantly higher in the water than the MR condition, respectively: LS means (SE) = 41.74 (2.41) vs. 33.62 (2.41)

Network analyses
Our initial analysis examined which ROIs served as hubs-of-interest for the hot-state network. This step involved a qualitative comparison of the resting brain networks in the water and MR treatment conditions that was guided by evidence from existing research. We then conducted statistical tests to empirically validate the qualitative findings. Using this method, five hubs met the criteria and were included as nodes of the HBN-A: ACC (p=0.053), the right hippocampus (p=0.030), superior temporal pole (p=0.029), insula (p=0.030), and amygdala (p=0.079). Table 1 provides results for the between condition statistical analyses of these hubs; whereas, Figure 2.2 shows the location of the high-degree nodes that were spatially consistent across individuals.

### Table 2.1 Summary of hubs of the HBN-A

<table>
<thead>
<tr>
<th>Degree Profile</th>
<th>No BOOST ® (Mean, (SE))</th>
<th>BOOST ® (Mean, (SE))</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACC</td>
<td>78.000 (10.689)</td>
<td>50.888 (6.879)</td>
<td>0.053</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>127.152 (24.982)</td>
<td>61.800 (7.900)</td>
<td>0.030*</td>
</tr>
<tr>
<td>STP</td>
<td>120.051 (20.866)</td>
<td>76.790 (8.820)</td>
<td>0.029*</td>
</tr>
<tr>
<td>Insula</td>
<td>73.127 (8.321)</td>
<td>49.703 (5.412)</td>
<td>0.030*</td>
</tr>
<tr>
<td>Amygdala</td>
<td>48.42 (7.103)</td>
<td>34.485 (6.380)</td>
<td>0.079</td>
</tr>
<tr>
<td>Precuneus</td>
<td>83.348 (12.171)</td>
<td>133.551 (24.868)</td>
<td>0.050</td>
</tr>
</tbody>
</table>

STP= superior temporal pole

ACC= Anterior Cingulate Cortex

*= significance

Table one provides the means and standard errors for the average number of connection (i.e. degree) for each hub included for the five hubs of the HBN-A (ACC, hippocampus, STP, insula, and the amygdala). The precuneus was greater in the BOOST ® than NO BOOST ® condition; therefore, it was not included in the HBN-A.
Image maps demonstrating brain areas found to consistently have high degree (i.e. number of connections). The maps demonstrate brain areas found to consistently have high degree (i.e. number of connections). For each subject, the voxels with degree values in the top 20% were identified. The maps shown here represent the overlap of these voxels across subjects in each condition. The consistency of overlap between conditions is indicated by the color bar which represents the percentage of individuals for which each voxel was among the top 20%. On the top left are axial slices (MNI z=54) through the superior temporal pole and amygdala (red circle) and the right hippocampus (green circle). On the top right are sagittal slices (MNI x=139) through the insula (yellow circle). Finally the images on the bottom are a sagittal slices (MNI x=95) through the anterior cingulate (blue circle). Figures 2.3-2.7 are shown with these same slices. The figure highlights that the superior temporal pole, the right hippocampus, the ACC, and the insula have greater connectivity in the water condition than in the MR condition during resting state.
Within Figure 2.2 it is also important to note that the precuneus had higher degree in the MR than the water condition (p=0.05). However, in defining the HBN-A, we were interested in ROIs that were hubs in the water condition; these ROIs did not include the precuneus. Moreover, the precuneus did not have a greater number of connections in the water condition compared to the MR condition or to any of the other hubs-of-interest in the HBN-A.

Figures 2.3-2.6 illustrate the number of direct (upper panel in each figure) and indirect (lower panel in each figure) connections between each of the five nodes identified in Figure 2.2. Supporting data from statistical tests can be found in Tables 2.2 and 2.3. In each case, the number of connections is always higher in the water condition as compared to the MR condition. Also, a large number of direct and indirect connections can be observed within each seed ROI; however, this would be expected. For defining the HBN-A, we were interested in the interconnections from each ROI to every other ROI in the HBN-A.
Table 2.2: Summary of direct connections between nodes of the HBN-A

<table>
<thead>
<tr>
<th>Total Number of Direct Connections</th>
<th>No BOOST ® (Mean, (SE))</th>
<th>BOOST ® (Mean, (SE))</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula- Amygdala</td>
<td>132.371 (26.765)</td>
<td>77.429 (18.798)</td>
<td>0.045*</td>
</tr>
<tr>
<td>Insula - Hippocampus</td>
<td>209.143 (60.357)</td>
<td>51.286 (12.501)</td>
<td>0.029*</td>
</tr>
<tr>
<td>Insula - STP</td>
<td>2610.571 (485.806)</td>
<td>1278.000 (197.122)</td>
<td>0.018*</td>
</tr>
<tr>
<td>Insula - ACC</td>
<td>707.571 (140.501)</td>
<td>285.214 (87.067)</td>
<td>0.021*</td>
</tr>
<tr>
<td>STP - Amygdala</td>
<td>345.857 (77.915)</td>
<td>178.143 (45.532)</td>
<td>0.052</td>
</tr>
<tr>
<td>STP - ACC</td>
<td>593.786 (173.683)</td>
<td>234.143 (83.338)</td>
<td>0.059</td>
</tr>
<tr>
<td>STP - Hippocampus</td>
<td>529.000 (105.903)</td>
<td>220.643 (43.129)</td>
<td>0.016*</td>
</tr>
<tr>
<td>ACC - Hippocampus</td>
<td>57.000 (25.035)</td>
<td>10.214 (3.598)</td>
<td>0.091</td>
</tr>
<tr>
<td>ACC - Amygdala</td>
<td>18.357 (5.990)</td>
<td>9.071 (3.656)</td>
<td>0.180</td>
</tr>
<tr>
<td>Amygdala - Hippocampus</td>
<td>59.286 (16.611)</td>
<td>27.929 (9.765)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

This table summarizes the number of direct connections between each of the ROIs in the HBN-A.

Table 2.3: Summary of indirect connections between nodes of the HBN-A

<table>
<thead>
<tr>
<th>Total Number of Indirect Connections</th>
<th>No BOOST ® (Mean, (SE))</th>
<th>BOOST ® (Mean, (SE))</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insula to Amygdala</td>
<td>2107.286 (399.922)</td>
<td>1257.357 (306.347)</td>
<td>0.050*</td>
</tr>
<tr>
<td>Insula to Hippocampus</td>
<td>3332.143 (765.529)</td>
<td>1308.143 (222.055)</td>
<td>0.030*</td>
</tr>
<tr>
<td>Insula to STP</td>
<td>31331.643 (6325.345)</td>
<td>18182.214 (2618.435)</td>
<td>0.032*</td>
</tr>
<tr>
<td>Insula to ACC</td>
<td>8066.429 (1419.632)</td>
<td>3599.714 (796.690)</td>
<td>0.024*</td>
</tr>
<tr>
<td>STP to Amygdala</td>
<td>2352.071 (403.201)</td>
<td>1479.786 (342.118)</td>
<td>0.051</td>
</tr>
<tr>
<td>STP to ACC</td>
<td>7882.286 (1457.577)</td>
<td>3428.429 (585.329)</td>
<td>0.017*</td>
</tr>
<tr>
<td>Connection</td>
<td>Weight</td>
<td>SEM</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>STP to Hippocampus</td>
<td>3646.000 (752.764)</td>
<td>1599.000 (249.125)</td>
<td>0.027*</td>
</tr>
<tr>
<td>STP to Insula</td>
<td>26656.071 (3678.668)</td>
<td>15925.429 (2047.362)</td>
<td>0.021*</td>
</tr>
<tr>
<td>ACC to Insula</td>
<td>21480.000 (3781.479)</td>
<td>8227.286 (2078.500)</td>
<td>0.009*</td>
</tr>
<tr>
<td>ACC to Hippocampus</td>
<td>2429.143 (772.684)</td>
<td>569.857 (187.532)</td>
<td>0.035*</td>
</tr>
<tr>
<td>ACC to Amygdala</td>
<td>1180.857 (375.519)</td>
<td>346.571 (96.477)</td>
<td>0.041*</td>
</tr>
<tr>
<td>ACC to STP</td>
<td>24000.214 (6547.866)</td>
<td>8839.000 (2725.875)</td>
<td>0.024*</td>
</tr>
<tr>
<td>Amygdala to STP</td>
<td>21729.000 (5951.026)</td>
<td>6780.000 (1412.635)</td>
<td>0.022*</td>
</tr>
<tr>
<td>Amygdala to ACC</td>
<td>3267.643 (1089.861)</td>
<td>866.571 (247.474)</td>
<td>0.053</td>
</tr>
<tr>
<td>Amygdala to Hippocampus</td>
<td>2613.071 (624.028)</td>
<td>724.500 (192.236)</td>
<td>0.015*</td>
</tr>
<tr>
<td>Amygdala to Insula</td>
<td>12309.071 (2931.547)</td>
<td>3983.714 (978.878)</td>
<td>0.016*</td>
</tr>
<tr>
<td>Hippocampus to Amygdala</td>
<td>1615.429 (431.436)</td>
<td>775.786 (261.550)</td>
<td>0.068</td>
</tr>
<tr>
<td>Hippocampus to ACC</td>
<td>4155.786 (1491.771)</td>
<td>793.214 (242.229)</td>
<td>0.048*</td>
</tr>
<tr>
<td>Hippocampus to STP</td>
<td>23542.000 (6573.370)</td>
<td>8037.857 (2161.360)</td>
<td>0.025*</td>
</tr>
<tr>
<td>Hippocampus to Insula</td>
<td>14417.071 (3946.902)</td>
<td>3269.214 (795.160)</td>
<td>0.022*</td>
</tr>
</tbody>
</table>

This table summarizes the number of direct connections between each of the ROIs in the HBN-A.
Inspection of the data in Figure 2.3 shows that the insula had significantly more direct connections with the superior temporal pole (STP) (red circle, p= 0.018), to the anterior cingulate cortex (ACC) (blue circle, p=0.021), the hippocampus (green circle, p = 0.029), and the amygdala (red circle, p= 0.045) in the water than the MR condition. The insula also exhibited greater indirect connections to the same four nodes within the HBN-A during the water condition: the STP (red circle, p=0.032), the ACC (blue circle, p=0.024), hippocampus (green circle, p=0.030) and the amygdala (red circle, p=0.050).
Figure 2.3. Direct and Indirect Connections from the Insula

These maps show the total number of connections from the insula during resting state averaged across individuals. These maps show the total number of connections from the insula during resting state averaged across individuals. The brain images on the top demonstrate the insula’s average number of direct connections with other brain regions. These images demonstrate that the insula has a greater number of direct connections with the ACC, STP, Amygdala, and the hippocampus during the water condition versus the MR condition.

The brain images on the bottom demonstrate the total number of indirect connections from the insula averaged across participants. These images demonstrate that during the water condition, the insula has a greater number of indirect connections with the ACC, STP, amygdala, and hippocampus than during the MR condition.
As shown in Figure 2.4, the STP had significantly more direct connections with the insula (p=0.018) and with the hippocampus (p=0.016) in the water than MR condition. Although the STP appears to have had a greater number of direct connections with the ACC and the amygdala (Figure 2.4), these trends were just below an alpha level of 0.05; that is, the probability values were p=0.059 and p=0.052, respectively. Indirect connections from the STP were also significantly greater in the water condition with the insula (p=0.021), the ACC (p=0.017), the hippocampus (p=0.027), and marginally significant with the amygdala (p=0.051).
The brain images on the top demonstrate the total number of direct connections from the superior temporal pole (STP) averaged across participants. *These images demonstrate that the STP has a greater number of direct connections with the insula and the hippocampus during the water than in the MR condition.* The difference in direct connections with the ACC and the amygdala did not reach significance (p=0.059 and p=0.052, respectively).

The brain images on the bottom demonstrate the total number of indirect connections from the STP averaged across individuals. *These images demonstrate that during the water condition the STP has a greater number of indirect connections to the insula, ACC, and the hippocampus than during the MR condition.* The indirect connections to the insula were marginally significant (p=0.051).
Figure 2.5 illustrates the number of connections from the anterior cingulate cortex (ACC). The ACC had significantly more direct connections with the insula (p=0.021) in the water condition and marginally significant greater direct connections with the STP (p=0.059). However, the ACC had significantly more indirect connections to all four nodes in the water versus the MR condition: insula (p=0.009), STP (p=0.024), amygdala (p=0.041), and the hippocampus (p=0.035).
Figure 2.5 Direct and Indirect Connections from the ACC

The brain images on the top demonstrate the total number of direct connections from the anterior cingulated cortex (ACC) averaged across participants. *These images demonstrate that the ACC has a greater number of direct connections to the insula during the water than in the MR condition.* It appears that the ACC also has greater direct connections with the STP in the water condition; however, this trend did not reach statistical significance (0.059).

The brain images on the bottom demonstrate the total number of indirect connections from the ACC averaged across individuals. *These images demonstrate that during the water condition the ACC has a greater number of indirect connections to the insula, ACC, the amygdala and the hippocampus than during the MR condition.*
Figure 2.6 illustrates the number of connections that the amygdala had within the water versus MR condition. Direct connections were significantly greater with the insula (p=0.045) with trends for the STP (p=0.052) and hippocampus (p=0.062). The amygdala also had a greater number of indirect connections with the insula (p=0.016), with the STP (p=0.022), and with the hippocampus (p=0.015) in the water condition, whereas the effect with the ACC was marginally significant (p=0.053).
Figure 2.6 Direct and Indirect Connections from the Amygdala

The brain images on the top demonstrate the total number of direct connections from the amygdalae averaged across participants. *These top images demonstrate that the amygdala has a greater number of direct connections with the insula during the water than in the MR condition.*

The brain images on the bottom demonstrate the total number of indirect connections from the amygdalae averaged across participants. *These images demonstrate that the amygdala has a greater number of indirect connections to the insula, the hippocampus, and the STP during the water condition than during the MR condition.* Despite visually appearing to have a greater number of connections to the ACC, this trend did not reach statistical significance (*p*=0.053).
Figure 2.7 captures the direct and indirect connections from the hippocampus. The hippocampus had a greater number of direct connections in the water condition with the insula (p=0.029) and STP (p=0.016). Indirect connections from the hippocampus were higher in the water condition with the insula (p=0.022), the STP (p=0.025), and the ACC (p=0.048).
Figure 2.7 Direct and Indirect Connections from the Hippocampus

The brain images on the top demonstrate the total number of direct connections from the right hippocampus averaged across participants. *The top images demonstrate the right hippocampus has a greater number of direct connections with the insula and to the STP in the water condition than in the MR condition.*

The brain images on the bottom demonstrate the total number of indirect connections from the right hippocampus averaged across individuals. *These images demonstrate that the right hippocampus has a greater number of indirect connections to the STP, ACC, and the insula during the water condition than the MR condition.*
Figure 2.8 provides a composite cartoon of both the direct and indirect connections between the nodes of the HBN-A that were greater in the water than the MR condition. Because the direct connections are first order, we refer to this circuit as the primary circuit of the HBN-A, where we use the term secondary circuit to capture the second order or indirect connections. Effect sizes are provided for each connection with both the primary and secondary circuits to provide the reader with a sense of their relative strength. For the direct connections, the effect sizes ranged from 0.38 to 0.74; whereas, for indirect connections, the effect sizes ranged from 0.53 to 0.82. It is important to note that nearly all of these effect sizes are moderate to large in magnitude.

When examining the primary circuit of the HBN-A (Figure 2.8-A), there are several notable features. First, the insula is a hub in the primary circuit as it has direct connections to every other node in the HBN-A. Second, there is also a sub-circuit which includes the insula, the hippocampus, and the STP (Figure 2.8-A). And third, this sub-circuit was reproduced within the secondary circuit which involved indirect connections.
This is a cartoon summary of the direct (8-A) and indirect (8-B) connections among the hubs of the HNB-A that are greater in the water versus the MR condition. The connections are weighed by effect sizes between the two conditions which are shown as part of the arrows.
Whereas the structure of the primary circuit was found to be embedded within the secondary circuit, there were many more indirect connections between the nodes of the HBN-A. The indirect connections or the secondary circuit showed high connectivity between all 4 nodes with the exception of a few connections with the amygdala. Of note, the secondary circuit revealed a greater number of indirect connections with the ACC and the amygdala with the other regions in the HBN-A. Specifically, the ACC had outgoing, indirect connections to every other node in the HBN-A. The amygdala has greater outgoing, indirect connections to the insula, the hippocampus, and the STP. The amygdala was the only ROI in the HBN-A that did not have reciprocal indirect connections to every other ROI in the HBN-A. However, it is important to point out that the amygdala showed a trend of significantly greater indirect connections to both the ACC (p=0.053) and the STP (p=0.051).

Discussion

The consumption of a meal replacement (MR) beverage in a group of older, obese adults after a short-term period of food restraint lowered state cravings and hunger ratings. As we shall see, these shifts observed with the MR were most likely due to the alleviation of the ‘hot-state’ created by the short-term restriction from food. Specifically, consistent with these self-report data, we also demonstrated that consumption of a MR blunted connectivity in the hot-state brain network of appetite (HBN-A). To our knowledge, this is the first paper to show that a MR can modulate brain networks after a short-term period of food restraint. A novel feature is that using graph theory we identified a complex network of indirect connectivity within the HBN-A.

To define the HBN-A, we examined the resting brain network of the experimental trial in which individuals continued to refrain from eating; i.e. the water condition. Qualitatively, we observed that their brain networks had hubs in regions associated with the identification, processing, and the emotional elaboration of visceral food cues. These brain regions were defined as hubs-of-interest
because they had a greater number of connections (degree) in the water versus the MR condition. Once we defined these hubs, we were able to investigate the direct and indirect connections among these hubs between the two treatment conditions to fully characterize the network.

To our knowledge, this is the first study that uses graph theory to build and to study resting brain networks after a short-term period of food restraint. Graph theory is becoming an increasingly popular technique among neuroscientists to study human brain networks using fMRI; however, this technique is radically different from traditional fMRI methodologies as it allows the study of whole brain relationships. Thus, it is difficult to fully integrate this work with previous neuroimaging studies as we are most likely capturing a different network. As a result, we have decided to fully characterize this network and to name it the HBN-A.

In our investigation of the HBN-A, we found that when individuals continued to consume only water, their brain networks had hubs in the following regions: insula, superior temporal pole, hippocampus, ACC, and amygdala (see Figure 2.2). These hubs became the nodes of the HBN-A, having a long history of being identified with visceral sensations and hedonic attribution/elaboration (Gautier et al., 2001; LaBar et al., 2001; Rothemund et al., 2007). Importantly, we do not believe the hubs defined in the HBN-A are the only regions that are active in a ‘hot-state.’ It is more than likely that these regions are the up-stream result of primary, sub-cortical networks that have been well characterized as being important for eating behavior and addiction in animal studies (Kringelbach et al., 2012; Panksepp, 2012; Stice et al., 2013). Moreover, in the current study, there was substantially greater connectivity between these nodes in the water than MR condition. As shown in Figure 2.8, nearly all of these connections had moderate to large effect sizes (Cohen, 1992).

The insula, the hub of the primary circuit (Figure 2.8-A), is well-known for its association with gustation and visceral sensations in general (Critchley, 2004; Kringelbach et al., 2012; Uddin et al., 2013); however, it has also been implicated in the experience of emotions derived from bodily states.
(Rothemund et al., 2007; Kringelbach et al., 2012). The insula is known to have connections with the superior temporal gyrus, the temporal pole, amygdala, hippocampus and the ACC (Shelley and Trimble, 2004; Nagai et al., 2007) which was evident in our data. The centrality of the insula to the HBN-A suggests that this network is viscerally-driven and is truly embodied. In other words, visceral cues from peripheral sensory systems literally drive activity in this network (Rejeski and Gauvin, 2013). To confirm the visceral foundation of the HBN-A, future research using this paradigm should consider evaluating hormones known to be related to appetite and monitor potential changes in the autonomic nervous system.

The temporal pole (TP), the second node of the HBN-A, is considered a paralimbic region that is important for the multisensory processing of auditory, olfactory and visual stimuli (Olson et al., 2007). It has also been implicated in the emotional processing of these multisensory stimuli, and some studies have even argued that the anterior temporal lobe is important for emotional stability (Olson et al., 2007). The temporal pole is not a region that we originally hypothesized to be a hub of the HBN-A; however, it is not surprising since the TP is an important brain region for multisensory and emotional processing.

The hippocampus, another limbic structure and the third node of the HBN-A, is classically known to be important in memory; however, it has also been implicated in eating behavior (Tracy et al., 2001; Squire, 2004; Davidson et al., 2007; Bragulat et al., 2010). There is increasing evidence that the hippocampus is important for hedonic and incentive processes and for sensing the metabolic and hormonal status of the body (Lathe, 2001; Davidson et al., 2007; Bragulat et al., 2010). It is important to note that, in our analysis of the hippocampus, connectivity differences were unilateral and localized to the right-side. Interestingly, a study by Stoeckel and colleagues (2009) found that obese women have significantly greater unilateral activation in the right hippocampus in response to high-calorie foods compared to controls; of note, the obese women also had greater bilateral activations in the amygdala, insula, ACC, and several other brain regions as well.
The fourth node of the HBN-A is the ACC. The ACC has traditionally been associated with regulation of attention to both cognitive (dorsal portion) and emotional processing (ventral portion) and with goal-directed behavior (Devinsky et al., 1995; Bush et al., 2000; Mohanty et al., 2007; Gasquoine, 2013). The ventral (genual or anterior) region of the ACC, the region that was found to be part of the HBN-A in this study (see Figure 2.5), has been shown to be important for emotional processing (Lindgren et al., 2012; Gasquoine, 2013). Specifically it is important for evaluating and encoding the emotional salience (pleasantness/averseness) of various stimuli including the pleasantness of fat-content in drinks and human touch (Grabenhorst et al., 2010; Lindgren et al., 2012; Gasquoine, 2013). The ACC is capable of translating its emotional processing into a behavioral consequence, in part, via the autonomic nervous system (ANS) working in conjunction with the insula (Devinsky et al., 1995; Critchley et al., 2003; Gasquoine, 2013).

The fifth and final node of the HBN-A was the amygdala. The amygdala’s role in fear and aversion is well known; however, the amygdala is now considered to be important for valence attribution, as it responds to both aversive and appetitive stimuli (Ball et al., 2009). The amygdala has connections to the insula in humans (Shelley and Trimble, 2004) and to the temporal pole in marquee monkeys (Olson et al., 2007), connections we observed in our data.

The primary circuit of the HBN-A (Figure 2.8-A) has a sub-circuit that includes the insula, the hippocampus and the STP. This sub-circuit has the capacity to integrate visceral sensation from the insula with memory/metabolic status from the hippocampus and emotional processing of multisensory stimuli from the STP. In addition, this sub-circuit has access to emotional information from the ACC and the amygdala through the hub of the insula.

Importantly, a novel feature of this study is our ability to delineate indirect connections and thereby to create a secondary circuit. With traditional neuroimaging techniques, this would have been impossible and only the primary circuit (i.e. direct connections) could have been elucidated.
However, by using graph theory to define our brain network, we were able to analyze indirect connections leading to the identification of the secondary circuit. This secondary circuit underscores how highly interconnected the ACC and the amygdalae are with the other regions in the HBN-A in the water condition (see Figure 2. 8-B). Using the direct connections only, it would appear that the ACC and the amygdalae must filter their information into the primary circuit through the hub of the insula; however, the identification of the secondary circuit suggests that this is an oversimplification. Many of the indirect connections from the ACC and amygdalae are through the insula; however, the robustness of their indirect connectivity could not have been predicted from the direct connections alone.

The secondary circuit illustrates that the indirect connections among the hubs of the HBN-A are reciprocally related to one another and highly distributed. This gives credence to the idea that brain networks are complex and do not function in a linear manner. Thus, the HBN-A is truly a circuit where sensory stimuli [visceral (insula); multisensory (STP)] and memory/metabolic status (hippocampus) are directly integrated with one another and further elaborated upon emotionally via the secondary circuit involving the ACC and the amygdala. Therefore, graph theory provides novel and important information to our understanding of functional brain network circuitry and should be further explored in future applications of functional connectivity.

Generally speaking, most regions in the HBN-A operate below the level of consciousness; thus, when people are in a “hot state” it is likely that behavior is controlled to a significant degree by automatic processes. Loewenstein (2005) has shown that when people are in “cold states” they overestimate their self-regulatory capacities; in other words, they might believe that they can control their eating behavior immediately after a noon meal, yet fail miserably at controlling consumption by mid-afternoon. Thus, future research should examine the role of the HBN-A on one’s ability to control consumption. It is important to note that since MR beverages are low scoring hedonic products and are often less liked than ‘regular’ food, it is possible that other regions may be important
during active attempts at self-regulation. For instance, after a short term fast, a tasty food product may not only trigger the hubs identified in this study, but also portions of the hedonic system such as the OFC creating a more complex network. In short, at this point in time, we do not know whether the structure of the HBN-A or connectivity within this network is generalizable beyond the realm of meal replacements.

This current study is not without limitations. First, the small sample size makes us sufficiently underpowered to investigate individual differences in the HBN-A. Secondly, the target sample was restricted to an older, overweight and obese population that was not currently dieting. It is possible that differences in brain networks may have been observed if participants had been actively engaged in intentional weight loss. We also did not have a normal weight control group, meaning that these results may be limited to older adults that are overweight or obese. The results of this study should be considered specific to the population under investigation until subsequent studies confirm these effects in younger age-groups and people with more diverse biometric characteristics. Finally, the reader should be aware that we used a per comparison error rate for evaluating the individual connections between hubs of interest and supplemented these analyses with effect sizes. Some may view this approach as too liberal and likely to create type I errors. On the other hand, the small sample sizes often used in imaging studies makes correction for multiple comparisons challenging. We want to underscore the moderate to strong effect sizes for all comparisons conducted between the water and MR conditions providing consistent support for the HBN-A using cutting edge methods from graph theory.

In summary, our qualitative and data-drive approach was successful in defining a well interconnected HBN-A. The plausibility of the HBN-A network is supported by its significant attenuation during the consumption of a MR as compared to water. This network included many regions previous implicated in eating behavior and describes a viscerally-driven network that is rich in valence attribution and incentive-motivated processing. Furthermore, the findings of this study
demonstrate that MR beverages are able to down-regulate the HBN-A, and reduce food craving/hunger following a short-term period of food restraint in older, overweight and obese adults as compared to water. In light of these findings, further research is warranted to examine how the HBN-A is related to peoples’ ability to stick with daily caloric goals typically set in weight management programs. In other words, is the HBN-A a brain signature for self-regulatory failure when attempting weight loss? Does the HBN-A have important relationships with other brain regions, such as the OFC, during active attempts at self-regulation? We are beginning to address some of these questions. Currently, we are examining whether differences in the HBN-A in response to an overnight fast predicts weight loss behavior during an active weight loss intervention. Additionally, we will also investigate if weight loss blunts the HBN-A response post intervention.
References


Kavanaugh, J.F. (2005). Brainism: 'we are not our brains; we are embodied persons'. *America (NY)* 193, 8.


CHAPTER III

NETWORK ANALYSIS: METRICS MATTER!

Brielle M. Paolini, Paul J. Laurienti, and W. Jack Rejeski

In Review at Frontiers in Aging Neuroscience, 2015
Introduction

Within the field of neuroimaging, network science (Bassett and Bullmore, 2006; Bassett and Bullmore, 2009; Bullmore and Sporns, 2009) has become the method of choice for analyzing functional brain imaging data. This methodology allows the creation of highly multivariate functional brain networks. To understand the complex properties of and dynamic changes that occur in these networks, a rapidly expanding number of brain metrics have evolved, see Bullmore and Sporns (2009) and Telesford (2011). While not a comprehensive list, popular metrics include degree, clustering coefficient, modularity, characteristic path length, global efficiency, local efficiency, small-worldness, and network motifs (Rubinov and Sporns, 2010). Although attention is often directed at degree and small-worldness, the conceptual rationale for the choice of these metrics is lacking. It should be emphasized that each metric captures only one feature of the system; thus, no one metric captures the inherent complexity present in a functional brain network nor should data for one metric be taken as evidence for good or bad network topology. In other words, metrics simply describe individual features of networks; however, it may be true that certain network features may be associated with a good or bad phenotype or behavior. In fact, we will demonstrate that two different metrics, degree and global efficiency, are associated with very different states and behaviors. This underscores the importance of adopting a multivariate as opposed to a univariate approach to network analysis; in fact, consideration of multiple metrics is required to more fully capture the complexity inherent in a dynamic system such as a functional brain network.

We recently published a paper identifying a brain network that is highly connected following short-term food restriction (Paolini et al., 2014), a hot-state brain network for appetite (HBN-A). Using a 2 condition, counterbalanced within-subjects design, we had older, obese adults consume a controlled macronutrient diet for breakfast and then monitored their behavior for 2.5h to ensure that they did not consume anything but water. In one condition, participants consumed a liquid meal replacement following the 2.5h period of food restraint, completed measures of craving and hunger,
and then had a 5-min resting MR scan. The second condition was identical except that participants consumed water as opposed to the meal replacement. Using the degree metric, the HBN-A was found to encompass 5 hubs or nodes: the insula, anterior cingulate cortex, superior temporal pole, amygdala and hippocampus. These regions have a long history of being implicated in visceral sensation and hedonic attribution/elaboration (Gautier et al., 2001;LaBar et al., 2001;Rothemund et al., 2007). As expected, there was substantially greater connectivity between these hubs in the water than meal replacement condition. *We thus reasoned that greater connectivity in the HBN-A following short-term food restriction might be a brain signature for people who are prone to self-regulatory failure during intentional weight loss.*

After publishing our finding on the HBN-A network, we wanted to continue to fully characterize this circuit using metrics other than degree. During this period of time, we also uncovered a body of literature suggesting that greater levels of integration across the brain are healthy and adaptive (Siegel, 2007;Cozolino, 2010). It is important to note that if one wants to assess the integrative potential of a network node, global efficiency (GE) is a better measure than degree (Latora and Marchiori, 2001;Rubinov and Sporns, 2010). In an attempt to assess the integration capacity of the HBN-A, we reanalyzed our previously published data using GE (Paolini et al., 2014).

We had two explicit aims in this reanalysis. First, because the HBN-A is a highly interconnected unit and GE is a measure of brain integration, we hypothesized that GE assessed across the 5-hubs of the HBN-A could be captured by a single component score. And second, since brain integration in the face of a challenge has been suggested to be adaptive (Cozolino, 2010), we hypothesized that changes in GE from a resting scan to an active food imagery scan would be inversely related to both ratings of hunger and craving following the food imagery task.

**Approach and Findings**
For this reanalysis, we focused exclusively on the treatment condition in which participants had consumed water prior to the scanning protocol. Participants wore goggles in the scanner that were directly interfaced with a computer screen. They first experienced a resting state scan where they viewed a cross on a computer screen interfaced with their goggles (Resonance Technology, www.mrvideo.com) for a 5 minute period. This was followed by a food-cue visualization task where four of the participant’s favorite food words were presented on the computer screen for approximately 25 s each (total scan time was 5 minutes). The words were presented in a random order with the restriction that each of the four words was at least presented once. Individuals were instructed to visualize the food words with all five senses (Bullins et al., 2013). Immediately following the food imagery task, participants rated their hunger and level of craving using visual analog scales (VAS) ranging from 0 (“not at all”) to 100 (“extreme”/”very well”).

Specifics on the scanning protocol, processing and analysis of the MRI scans have been described previously (Paolini et al., 2014). The GE (Latora and Marchiori, 2001) of the individual network nodes was then assessed. GE is the inverse of the average shortest path-length where $L=$average shortest path-length for every node ( ) and $N=$ size of the network. Thus, this metric ranges from 0 to 1. A node that is directly connected to all nodes in the network has would have a GE of 1 whereas a disconnected node would have a GE of 0. GE was calculated for the brain network from each study participant at the voxel-wise level. We then calculated the mean GE for each of the components of the HBN-A by averaging the GE of all network nodes within those brain regions, or regions-of-interest (ROIs). The ROIs used for our analysis were identical to the ROIs we used to originally define the HBN-A and included the insula, anterior cingulate cortex, superior temporal pole, amygdala and hippocampus (Paolini et al., 2014).

To examine AIM 1, we conducted a principal components analysis (PCA) on the GE scores for the 5 regions of the HBN-A (SPSS, Version 22) during both rest and the food cue imagery. The
results of this analysis supported our hypothesis that examination of GE across the 5 regions of the HBN-A was captured by a single underlying dimension, irrespective of whether the scan was conducted at rest or during the food imagery task. In the analysis of the resting scan, a single statistically meaningful eigenvalue of 4.5 emerged from the PCA; it captured 90.07% of the variance in the GE scores across the 5 regions with individual regions loading between 0.92 and 0.99. For the food imagery scan, the PCA yielded a single statistically meaningful eigenvalue of 4.55 and it captured 90.99% of the variance in the GE scores across the 5 regions with individual regions loading between 0.95 and 0.97.

As part of these analyses, we created a combined factor score for GE both during rest and food imagery, as well as a difference score for this combined factor score—food minus rest. We observed that increases in the GE factor scores from rest to food imagery had strong inverse relationships to both hunger, $r_s = -0.71$, $p = .004$, and craving, $r_s = -.67$, $p = .009$ that immediately followed the food imagery task. In other words, increases in GE during imagery were associated with lower self-report of hunger and craving.

Recall that, in our previously published work, the fasting condition was associated with higher cravings for favorite foods after the initial resting state scan. Moreover, we ran a PCA on the degree metrics for the 5 regions of the HBN-A both during rest and food imagery. The results of the PCA analyses were far less impressive, with the first component capturing just 67% of the variance in the items during rest and only 60.82% during food imagery.

**An Important Perspective in Network Analyses: Metrics Matter!**

The reanalysis of data from our initial publication on the HBN-A led us to two differing hypotheses regarding how degree and GE may be contributing to the brain networks associated with eating behavior. Originally using degree, we found increased connectivity in the regions of the HBN-
A during a short-term fasting state as compared to a fed condition. This suggests that increased degree in the HBN-A is sensitive to the physiological state of fasting. However, our present analysis using the fasting state demonstrated that a greater change in GE from rest to food imagery for the HBN-A was inversely related to both subsequent craving and hunger. This suggests that GE may be sensitive to subjective behavioral experiences of hunger and craving following food cue visualization. Both degree and GE appeared to be capturing very different real-world phenotypes and behaviors underscoring the fact that one metric only captures one dimension of a multivariate system and thus, cannot fully capture the inherent complexity of a functional brain network.

Degree (k) is a measure of how many direct connections a particular node has in a given network. Degree answers the question, “how far is a node’s immediate reach?” Therefore, the degree metric is only dependent on a node’s immediate neighbors. This can be illustrated by looking at node A in Figure 3.1. Node A has a degree of eight as it has eight nodal connections (contained within the black box); thus, only these immediate neighbors of node A affect the score for degree.
Below is a sample network illustrating the differences between degree and global efficiency (GE). Degree is the number of connections of a particular node. In the box on the left, node A’s degree is eight as it has eight connections. GE is calculated as the inverse of the average path length. Degree and GE are calculated for every node in the network and are summarized in the table on the left. GE is highly sensitive to the structure of the entire network. Removing the dashed edged between node C and node D changes the global efficiency for every node in the network as shown in the table on the bottom right.
Global efficiency (GE) is the inverse of the average shortest path length. GE answers the question, “How far is an individual node’s reach throughout the entire network?” Thus, the GE metric is dependent on the edges present in the total network and is highly multivariate in nature. The GE calculation includes every node in the network and is highly dependent on the network’s overall topological organization. Figure 3.1 demonstrates that the nodes with the highest GE (nodes B and C) are centrally located and thus have the shortest path lengths to every other node in the network. It should be stressed that GE is not a property that is intrinsic to a particular node; rather, GE is intrinsic to a node’s position within a particular network. In other words, the GE of any node is not only dependent on its direct connections to other nodes, but also on how every other node in the network is connected. For example, in Figure 3.1 we removed one edge from node C to node D (dashed line) and provide a table with the resulting degree and GE values. Removing this one edge drastically decreased the GE for all nodes in the network; thus, distant connectivity changes can have a profound effect on the GE of a particular node.

Degree and GE are frequently highly correlated because, by definition, nodes with high degree have a large number of connections. Oftentimes these multiple connections provide a node greater access to the whole network and a large GE (see node B in Figure 3.1); however, this is not always true (see node A in figure 3.1). Parenthetically, a node can have high degree and have low GE (i.e. nodes A and E) or can have high GE and low degree (i.e. nodes C and D) depending on the nature of its connections and the topology of the entire network.

So how can a brain region have high degree but a low GE or vice versa? In terms of the HBN-A, the five nodes may have high degree and a low GE because the nodes of the HBN-A have a large number of connections to other regions within the HBN-A and not to the rest of the brain. Moreover, the GE of nodes in the HBN-A can be increased in one of two ways. The nodes of the HBN-A may increase their connections to regions outside the HBN-A, or changes in the network structure outside of the HBN-A may increase the GE of the nodes in the HBN-A without changing the connectivity of
the HBN-A nodes. It is important to note that GE does not differentiate between these two possibilities.

In order to further understand the nature of the HBN-A, we also ran a community structure analysis on the networks. A network’s community structure is defined by subsets of nodes or neighborhoods whose intra-connections are greater than their inter-connections with the rest of the network (Newman and Girvan, 2004). In other words, a community of nodes is more highly interconnected with each other than with other nodes in the network. We performed a community structure analysis using the modularity metric ($Q$—(Newman and Girvan, 2004)) and the Qcut algorithm (Ruan and Zhang, 2008). We then identified the consistency of the module organization across people using scaled inclusivity (SI) (Steen et al., 2011); scaled inclusivity demonstrates how consistently across people a node belongs to a particular module, for further details on group modular structure analysis see Moussa et al. (2012). As demonstrated in Figure 3.2, we found that the community containing the bilateral insula also included the key regions of the HBN-A. These regions are somewhat exclusively and preferentially interconnected as they have more connections to each other than to the rest of the brain. Modularity allows one to determine with whom a node connects; whereas, degree and GE describe how one node fits into the overall network topology. It should be stressed that modularity is not independent of other network metrics. A network’s modular structure is influenced by every node’s degree, GE, and clustering; however, modularity does further extend the analysis of traditional network metrics. For example, modularity can help one understand why GE increases or decreases in a highly interconnected module such as the HBN-A. The GE of the HBN-A nodes can increase due to either increasing these connections with regions outside the HBN-A (i.e. the modular structure of the HBN-A decreases) or as a secondary effect of distant connectivity changes in the network outside the HBN-A (i.e. the modular structure of the HBN-A is maintained).
Figure 3.2 Modularity Maps for the Bilateral Insula

These maps demonstrate brain regions found to be part of the module containing the bilateral insula. Scaled inclusivity demonstrates how consistently across people a node belongs to a particular module. The higher the SI value, the more likely that region is part of the module. On the top left is an axial slice through the superior temporal pole and amygdala (red circle), and the right hippocampus (green circle). On the top right is a sagittal slice through the insula (yellow circle). Finally the image on the bottom is a sagittal slice through the anterior cingulate (blue circle).
It should also be stressed that the magnitude of a network metric does not denote that a network topology is ‘good’ or ‘bad’. Different brain patterns support different brain functions; thus, it is the associated behavior that determines if an increase or decrease in a particular metric is beneficial or detrimental. For example, high GE in the insula may be beneficial for controlling the subjective experience of hunger during a resting state as we show here; however, high GE isn’t inherently beneficial. For example, during a seizure the GE of the brain region that is the seizure’s focus is extremely high, and this is obviously detrimental as it contributes to characteristic network-wide synchronous brain activity (Otte et al., 2012). In short, it is important to remember that different metrics such as degree and GE are capturing very different network properties and, as our data suggest, increases or decreases in these metrics can lead to very different predictions regarding a behavioral outcome such as weight loss.

From our original analysis of the resting state data, degree appears to have been sensitive to the visceral state of not being fed. From our present analysis, GE appears to capture the subjective experience of hunger and craving as greater GE during a food challenge was associated with decreased hunger and craving. GE is a metric of whole brain integration; thus, regions with high GE are poised to integrate their information with the entire brain. Since the regions of the HBN-A are known to process visceral and emotional information, higher GE in these regions facilitates integration of this information with the rest of the brain. This improved information exchange may allow certain individuals to have conscious awareness of their visceral and emotional drives and, thus, improved self-regulatory capacity to act positively upon these drives, a hypothesis that warrants future testing. This idea is supported by the work of Cozolino (2010) and Siegel (2007) who suggest that both horizontal (hemispherical: left/right) and vertical (cortical/sub-cortical: top/bottom) integration is needed for effective self-regulation. This may provide a mechanism for why mindfulness-based therapies designed to increase moment-to-moment awareness appear to be promising in the treatment of eating disorders and obesity (O'Reilly et al., 2014).
In summary, it is important for neuroscientists to better understand various network science metrics used in their analyses, and to be cautious in relying on a single metric to understand complex, dynamic systems. The field of neuroscience has evolved from recognizing which regions become active during certain tasks (i.e. univariate analyses) to understanding the dynamic interactions of the brain as a complex unit (i.e. multivariate analyses). Graph theory and network science methods allow researchers to look at the brain through multiple lens (i.e. metrics) and to understand the complex interrelationships among nodes and regions.

As we advance our understanding of brain networks, it will even become important to move beyond network metrics and evaluate brain as a dynamic system that is continuously changing. It has recently been proposed that fixed structure-function relationships (i.e. one-to-one mapping) should give way to a perspective of the brain where regions are dynamically recruited into ‘mutli-region assemblies’ (Pessoa, 2014). These multi-region assemblies are highly context-dependent; therefore, a given brain region could participate in multiple different assemblies across different contexts (many-to-one mapping) or similar functions could be carried out by diverse regions or assemblies (one-to-many mapping) (Pessoa, 2014). It follows that understanding real-world behaviors, such as eating, will require examination of how brain regions interact to form circuits or multiple region assemblies and how these circuits change dynamically from one context to the next. In this paper we characterize how different metrics provide unique information about the circuit of the HBN-A, however, it is important to remember that this circuit does not exist in isolation. Further research should consider how differential connections to and from circuits, such as the HBN-A, may continuously change and this change may be the key association that is related to a diverse behavioral repertoire.
References


CHAPTER IV

BRAIN NETWORK INTEGRATION AND INCREASING SELF-EFFICACY PREDICT SUCCESSFUL WEIGHT LOSS IN OLDER ADULTS


In Review at Frontiers Aging in Neuroscience, 2015
Introduction

Obesity has become a public health crisis in North America, not sparing a quickly expanding population of older adults (Mathus-Vliegen, 2012; Ogden et al., 2014). Although lifestyle interventions remain the most popular treatment option for obesity, there is considerable variability in weight loss during the intensive phase of treatment and weight regain is common (Kramer et al., 1989; Wing and Hill, 2001; Rejeski et al., 2011). In our previous work (Rejeski et al., 2011), we found that increasing participants’ self-regulatory efficacy related to eating behavior was associated with better weight loss during the intensive phase of treatment. In the current study, we sought to replicate this finding but, more importantly, to examine whether functional brain network global efficiency in network regions responsive to food restriction (Paolini et al., 2014) accounted for additional variability in weight loss during active treatment.

A consistent observation with successful weight loss has been the important role played by restraint and inhibition (Herman and Mack, 1975; Stunkard and Messick, 1985). In 1991 Clark and colleagues developed the Weight Efficacy Lifestyle Questionnaire (WEL) to evaluate self-efficacy for regulating eating behavior in response to both internal and external challenge: negative emotions, social pressure, physical discomfort, when engaging in positive activities, and when food is readily available. Studies have shown that effective weight loss treatment improves participants self-efficacy related to the self-regulation of eating behavior and that this change is related to overall weight loss (Jeffery et al., 1984; Bernier and Avard, 1986; Richman et al., 2001; Linde et al., 2006; Warziski et al., 2008; Rejeski et al., 2011).

While improvement in conscious cognitive control related to eating behavior is undoubtedly important to success with weight loss, as supported by the role that the prefrontal cortex and executive function play in weight management (Yokum et al., 2011; Kishinevsky et al., 2012; Weygandt et al., 2013), research in neuroscience suggests that this focus is too narrow. For
example, in a recent review on hedonic and homeostatic brain regions involved in eating behavior, Stice and colleagues (Lowe and Butryn, 2007;Stice et al., 2013) highlighted involvement of the insula, hippocampus, amygdala, anterior cingulate cortex (ACC), parabrachial nucleus (PBN), the ventral tegmental area (VTA), and the orbitofrontal cortex (OPFC). Concurrent with recognition that multiple areas are involved in eating behavior, we have recently directed our attention to the study of brain networks (Paolini et al., 2012;Rejeski et al., 2012;Bullins et al., 2013;Paolini et al., 2014). Notwithstanding the limitations of fMRI and our inability to study the VTA and PBN identified by Stice and colleagues (2013), we examined highly connected brain regions (hubs) in a resting state network that exhibits connectivity changes after a brief period of food restriction, the hot-state brain network of appetite (HBN-A) (Paolini et al., 2014). As described by Paolini and colleagues, the hubs within the HBN-A overlapped nicely with the areas identified by Stice and colleagues (2013) and, importantly, the hubs were characterized by multiple first and second order connections.

This initial work led us to conclude that high levels of connectivity in the HBN-A following a brief period of fasting was dysfunctional since connectivity in the HBN-A was lower when participants consumed a liquid meal replacement as compared to water (Paolini et al., 2014). Thus, we hypothesized that those who exhibited increased connectivity in the HBN-A following food restriction would struggle with intentional weight loss. However, this hypothesis was called into question during a recent secondary analysis we conducted comparing different network metrics in the HBN-A, specifically degree versus global efficiency (GE) (Paolini et al., Network Analysis: Metrics Matter!, Frontiers in Aging Neuroscience, submitted). Degree is a measure of the number of connections for a network node (Bullmore and Sporns, 2009). GE is a measure of the number of steps it takes to get from a given node to the remainder of the network (Latora and Marchiori, 2001;Bullmore and Sporns, 2009).
Degree was used in the original data analysis, since it is ideal for identifying the most connected network nodes (hubs). However, GE is better suited to characterize distributed processing across the entire network (Rubinov and Sporns, 2010). There is strong evidence that higher levels of integration across the brain are adaptive (Cozolino, 2010) and growing support for the view that higher, simultaneous activity in brain regions known to be involved in eating behavior is predictive of future weight loss (Batterink et al., 2010; Stice et al., 2010; Hege et al., 2013). Interestingly, when we examined GE, there was a strong, negative relationship between change in this metric from rest to the visualization of food with both self-report ratings of hunger and craving (Paolini et al., 2014). In summary, whereas degree increased in a fasting state (Paolini et al., 2014) and has been found to be directed related to ratings of power that food has in the lives of obese, older adults (Rejeski et al., 2012), an increase in GE following the active imagery of food was associated with lower ratings of hunger and food craving. This result supports the hypothesis that high levels of distributed processing throughout the HBN-A, as evident by GE, would be favorable for self-regulation and weight loss (Paolini et al., 2014).

In the current study, we were able to prospectively examine whether activity in the HBN-A during resting state following an overnight period of fasting was related to weight loss during the first 6-months of intensive treatment among a subgroup of obese, older adults with metabolic syndrome or known cardiovascular disease. Our primary hypothesis was that after controlling for baseline weight, sex, age, and residualized change in the WEL scores, higher baseline levels of GE across the HBN-A assessed after an overnight fast would be significantly associated with 6-month weight loss.

Methods

The present study is an ancillary imaging study that is part of an ongoing randomized clinical trial involving weight loss, the Cooperative Lifestyle Intervention Program-II (CLIP-II) (Marsh et al.,
In short, CLIP-II randomized 252 obese, older adults with cardiovascular disease (CVD) or metabolic syndrome (MetS) into a weight loss only treatment (WL), aerobic exercise training (AT) + WL, or resistance exercise training (RT) + WL for 18 months (Marsh et al., 2013). Since our interest in this study was weight loss during the intensive phase of treatment, we restricted our analysis to the first 6-months of treatment and statistically controlled for treatment in the linear model since we did not want to reveal treatment assignment prior to completion of the main study.

Participants

Sixty-six participants who were enrolled during the first year of the CLIP-II study participated in this ancillary imaging project. The cohort and methods for this study have been well characterized (Marsh et al., 2013). Briefly, participants were either overweight or obese (BMI ≥ 28 kg/m² but < 42 kg/m²) community dwelling men and women between the ages of 60 and 79 years with a documented history of cardiovascular disease or an ATP III diagnosis of MetS. Cardiovascular disease included myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), chronic stable angina, or cardiovascular surgery. Participants also had low levels of physical activity (less than 60 min of moderate intensity physical activity per week) and self-reported disability. The Weight Efficiency Lifestyle Questionnaire (WEL) was administered at both the baseline visit and the 6 month follow-up visit for the parent study.

After the participants had completed screening and baseline testing for the parent study, they were contacted via phone to discuss participation in and screening for the ancillary study. In addition to the inclusion/exclusion criteria for the parent study, participants were excluded for (1) active participation in another research study that might interfere with either the study’s procedures or objectives, (2) visual acuity less than approximately 20/40 (corrected), (3) severe hearing loss, (4) claustrophobia, (5) MR-incompatibility (implant or other incompatible foreign object in the body), (6) depression treated by antidepressants unless on a stable regimen for more than 2 months, (5) serious
CNS trauma as defined by the history of acquired sub or epidural hematomas or loss of consciousness for greater than 5 minutes. Following successful completion of the phone screen, participants completed one 2.5 hour experimental session at Wake Forest School of Medicine receiving $50 to compensate for their time. Informed consent was obtained on all participants and the study protocol was approved by the Wake Forest University School of Medicine Institutional Review Board.

Of the original 66 participants that agreed to participate in this study, seven were lost to follow-up and three had missing 6-month weight loss data leaving a final n of 56. Two participants withdrew during the MRI scan due to claustrophobia, two ended up being ineligible for the parent study, and three withdrew from the parent study during the first 6-months of treatment.

Measure of Self-Regulatory Efficacy

*Weight Efficiency Life-Style Questionnaire (WEL):* The WEL, a 20-item measure developed by Clark and colleagues (1991), was employed to assess self-regulatory efficacy related to eating behavior. The measure has five subscales (negative emotions, food availability, social pressure, physical discomfort, and positive activities) but can be scored as a single measure as was done in the current study. Participants rated their confidence to resist the desire to eat using a 10-point scale ranging from 0 (not confident) to 9 (very confident). A total score was calculated by summing all items; therefore, scores range from 20 to 180.

Experimental Protocol for the scanning visits

Once a participant expressed interest in being involved in the imaging study and passed the telephone screening, an MRI appointment was arranged. Participants completed a 2.5 hour visit beginning in the early morning either around 7:15 a.m. or 9:15 a.m. Participants were asked to arrive in a fasting state, having not eaten breakfast or consumed anything other than water. Upon arrival, participants were led to a quiet, private room where informed consent was obtained by the study staff.
The research staff then administered the MRI safety form and led the participant in a practice session of the tasks to be completed during the fMRI.

Three functional trials were completed during the MRI. The first trial was a 5-minute resting state scan during which individuals relaxed with their eyes open viewing a cross on the rear projection screen. The second task was a 5-minute food visualization task where participants viewed their four favorite food words on an MR-compatible rear projection screen; each word was presented for 30 seconds. Participants were instructed to visualize the food with all five senses for the entire time the food word was on the screen. The final trial was a recovery resting state scan identical to the first trial. Originally our plan was to analyze the responses during all three trials; however, when we added global efficiency collected during the food task to our analysis, it did not explain any additional variance beyond what was learned from data collected during the first resting scan. Thus, we limited further discussion to the initial resting state scan.

Scanning Protocol

MRI data were obtained on a Siemens MEGNETOM SKYRA 3T scanner using a 32-channel head coil with a gradient strength equal to 45 mT/m at 200 T/m/sec. The scanning protocol included anatomical imaging, one resting state fMRI, a food-cue fMRI task scan, and a recovery resting state fMRI. Functional images for the network analyses measured the T2*-relaxation rate that is sensitive to blood oxygenation level dependent (BOLD) changes (Ogawa et al., 1990). As brain activity changes, the oxygen content of the blood in the same area also changes. Thus, the T2* signal is an indirect measure of changes in neural activity. All fMRI data was used to create brain networks for each individual in native space. High-resolution (1.0 x 1.0 x 1.0 mm) T1-weighted structural scans were acquired in the sagittal plane using a single-shot 3D MPRAGE GRAPPA2 sequence (Scan time = 5 minutes and 30 seconds, TR = 2.3 seconds, TE = 2.9 ms, TI = 900 ms, flip angle = 9˚). Functional imaging or BOLD-weighted images (3.5 x 3.5 x 5.0 mm) were acquired using a single-shot echo-planar imaging sequence (Scan time = 5 minutes and 20 seconds, TR = 2.0 seconds, TE = 25 ms, flip
angle = 75˚). The scanning planes were oriented parallel to the anterior–posterior commissure line and extended from the superior extent of motor cortex to the base of the cerebellum.

**Imaging Processing and Network Analyses**

Functional and structural data were preprocessed using SMP8 ([www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)). The first 20 seconds of the scan were discarded to allow for signal equilibration. Functional images were then realigned, slice-time corrected and co-registered to a skull-stripped version of the accompanying structural data. During image acquisition, the pre-scan normalize function was turned off for all 60 baseline scans. Pre-scan normalize is used to correct for the inherent bias in tissue signal based on location (i.e. tissue closer to the head coil has higher signal values than deeper brain structures). The built-in inhomogeneity correction function in SPM8 was used to remove this bias in the first volume of the functional data series. The parameters used on the first volume were then applied to the remainder of the functional data volumes images in the series.

Images were smoothed using a 2 voxel (8x8x8 mm) smoothing kernel, band-pass filtered (0.009–0.08 Hz) to limit physiological noise and low-frequency drift, and globally normalized to the image mean. Confounding signals were regressed out of the functional data and included 6 rigid-body transformation parameters generated during the realignment process and 3 mean signals (whole-brain, white matter, and CSF). The mean signals for the three tissue types were determined by masking the functional data with masks of the individual segmented tissue images generated with the unified segmentation in SPM8. The functional data were also motion corrected using a protocol designed to eliminate scan volumes with both excessive frame-wise displacement and BOLD signal change (Power et al., 2012). Networks were created and analyzed in native space to limit further data manipulation providing greater confidence in the time series signal.

For each individual, a correlation matrix was then created by computing Pearson correlations between all possible voxel pairs. This produced symmetrical matrices where each cell (ij) represented
the correlation coefficient between nodes i and j. A threshold was then applied to the correlation matrix, and all cells that surpassed this threshold were assigned a value of 1 while remaining cells were assigned a value of zero. The size of each network ranged from 19,526 and 23,618 nodes based on the subject’s head size. The threshold was defined so that the relationship between the number of nodes and average number of connections at each node was consistent across subjects. This thresholding procedure ensured comparable connection densities regardless of the number of network nodes. Specifically, the relationship $S = \log(N)/\log(K)$ was the same across subjects, with $N =$ number of nodes and $K =$ average degree (Hayasaka and Laurienti, 2010). The threshold $S = 2.5$ was used for this paper. This resulted in connection densities meeting expected values based on network size (Laurienti et al., 2011). All remaining analyses were completed using the binary symmetrical adjacency matrixes.

GE (Latora and Marchiori, 2001) was used to assess the integrative capacity of the network and was calculated for each study participant at the nodal level. GE is the inverse of the average shortest path-length of the node and is defined as $GE = \frac{1}{L}$, where $L =$ shortest path-length from node (i) to every other network node and $N =$ number of nodes in the network. Thus, this metric ranges from 0 to 1. A node that is directly connected to all nodes in the network would have a GE of 1 whereas a disconnected node would have a GE of 0. Since GE incorporates the number of nodes in the network ($N$), the GE metric is readily comparable across networks of different sizes.

**Region-of-interest (ROI) Analysis**

In order to quantify GE for various ROIs, we warped the Automated Anatomical Labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) to each subject’s native-space structural brain image. First, the structural brain image was warped to standard space using the unified segmentation function in SPM8. The inverse warping parameters from the SPM were then applied to the AAL atlas for each
study participant. A nearest neighbor interpolation was used to ensure the boundaries of the atlas regions were respected (i.e. no voxel was included in more than one atlas region).

For our analysis, the following ROIs were used: insula, amygdala, superior temporal pole, anterior cingulate cortex, and parahippocampal gyrus, all defined by the AAL atlas. For each ROI both the left and right corresponding regions were included with the exception of the parahippocampal gyrus which included only the right side. The choice of these ROIs was based on our prior work with the hot-state brain network of appetite (HBN-A) (Paolini et al., 2014); however, there were two exceptions. We used the entire anterior cingulate cortex as defined by the AAL atlas for the present analysis; whereas, we used a sphere with radius of 10mm previously. Secondly, we used the AAL’s region of the parahippocampal gyrus because this region mapped more closely onto the region of interest from our former work compared to the hippocampus (Tzourio-Mazoyer et al., 2002; Maldjian et al., 2003; Paolini et al., 2014). For each study participant, the average GE was calculated for all the network nodes that fell within each of the 5 ROIs.

Statistical Analyses

Means and standard deviations or medians and the 25th and 75th percentiles were used as measures of central tendency and variability. A principal components analysis was applied to the global efficiency data from the 5 regions of the HBN-A with factor scores generated for the subsequent regression analysis. The primary data analytic technique was linear regression, regressing baseline weight, treatment assignment, age, sex, self-regulatory efficacy, and the factor scores for GE on change in weight from 0 to 6 months. We originally conducted analyses examining the difference in GE from rest to food imagery, however, the data did not differ whether we used GE during the resting state or change in GE from rest to food imagery. Thus, we chose to report data on GE during the resting state only. The variance inflation factor (VIF) was used to check for co-linearity. All analyses were conducted using SPSS version 22.
Results

The study sample consisted of 14 men and 42 women with 20 African Americans and 36 non-Hispanic Whites. Participants had a mean (SD) age of 67.55 (5.06) years with a Body Mass Index of 34.38 (3.69) kg/m². The average Montreal Cognitive Assessment (MOCA) was 25.96 (2.39).

The five regions of the hot-state brain network of appetite (HBN-A) are depicted in Figure 4.1’s cartoon. The mean (SD) GE scores for the 5 regions of the HBN-A were as follows: insula 0.1308 (0.0233), ACC 0.1369 (0.0291), amygdala 0.1361 (0.02602), STP 0.1044 (0.0227), and the parahippocampal gyrus 0.1262 (0.0263). The GE values of these 5 regions during the baseline resting-state scan were highly integrated as evidenced by Principal Component Analysis. Specifically, the 5 regions loaded on a single dimension with an eigenvalue of 3.94 that accounted for 79% of the variance in the GE scores from the 5 regions. The loadings for all 5 regions were high and ranged from 0.6 to 0.7. Across the 6-months, the mean (SD) weight loss was 8.08 (4.76) kg.
Above is a cartoon depicting the five regions of the HBN-A: the anterior cingulate cortex (ACC), the insula, the superior temporal pole (STP), the amygdala, and the parahippocampal gyrus (PH). Important all ROIs were bilateral with the exception of the PH which only included the right side.
Table 4.1 presents the results of the linear regression analysis. The overall model including covariates had an $R^2$ of $0.46$, $F_{7,48} = 5.80$, $p < .0001$. None of the variables had a VIF $>1.7$ removing any concern for co-linearity. Examination of the standardized beta weights and the partial correlations revealed that, after controlling for the effect of treatment, the amount of weight lost during the intensive phase of the intervention was greater for (a) men than women, (b) older than younger participants, (c) individuals who were heavier at baseline, and (d) those who experienced increased efficacy for control over eating. More important, in the presence of these known determinants, additional variance in weight loss was explained by higher levels of GE across the regions of the HBN-A.
Table 4.1 Results from Linear Regression

<table>
<thead>
<tr>
<th>Effect</th>
<th>Standardized Beta</th>
<th>t Value</th>
<th>p-value</th>
<th>Partial r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment* Vector 1</td>
<td>-.36</td>
<td>2.67</td>
<td>0.10</td>
<td>-.36</td>
</tr>
<tr>
<td>Vector 2</td>
<td>-.04</td>
<td>0.30</td>
<td>0.76</td>
<td>-.04</td>
</tr>
<tr>
<td>Baseline Weight</td>
<td>-.42</td>
<td>3.39</td>
<td>0.001</td>
<td>-.44</td>
</tr>
<tr>
<td>Age in Years</td>
<td>-.25</td>
<td>2.00</td>
<td>0.052</td>
<td>-.28</td>
</tr>
<tr>
<td>Sex</td>
<td>.32</td>
<td>2.40</td>
<td>0.018</td>
<td>.33</td>
</tr>
<tr>
<td>Self-Efficacy</td>
<td>-.48</td>
<td>4.30</td>
<td>0.000</td>
<td>-.52</td>
</tr>
<tr>
<td>Global Efficiency</td>
<td>-.31</td>
<td>2.80</td>
<td>0.007</td>
<td>-.38</td>
</tr>
</tbody>
</table>

*Dummy coding was used to control for the 3 treatments, thus requiring 2 vectors. Because treatment was used as a covariate and we did not want to reveal treatment assignment prior to completion of the main study, we do not discuss the treatment effect any further.

The table contains the result from the linear regression analysis. Examination of the standardized beta weights and the partial correlations revealed that, after controlling for the effect of treatment, the amount of weight lost during the intensive phase of the intervention was greater for (a) men than women, (b) older than younger participants, (c) individuals who were heavier at baseline, and (d) those who experienced increased efficacy for control over eating. More important, in the presence of these known determinants, additional variance in weight loss was explained by higher levels of GE across the regions of the HBN-A.
**Discussion**

This is the first study to prospectively demonstrate that integration in the HBN-A during resting state following an overnight period of food restriction is predictive of weight loss that occurs during the intensive phase of weight loss therapy. This effect was observed among obese, older adults participating in a community-based weight loss trial who had either MetS or known CVD. We observed a significant association between higher levels of GE in the HBN-A, as measured by a summary score from a principal component analysis (PCA), and weight loss after a 6-month intervention. This effect was true after accounting for the effects due to baseline weight, sex, age, treatment assignment, and improvement in self-regulatory efficacy related to eating behavior.

These findings support the position of Rubinov and Sporns (2010) that GE is an important metric for capturing network integration. From our perspective, the findings support the adaptive nature of maintaining distributed processing across the HBN-A when exposed to a homeostatic and hedonic challenge related to food—in this instance, an overnight period of food restriction. The regions of the HBN-A are all limbic or paralimbic and are important for processing visceral cues and related emotional elaboration of these sensations (Paolini et al., 2014). Thus, we propose that increased GE in the HBN-A ensures that this circuit has increased integration of information from throughout the brain. This provides superior awareness and processing of information flow related to both visceral cues and emotional regulation, a hypothesis that is consistent with Cozolino’s (2010) work in psychotherapy. Interestingly, in previous work, we found that, following recovery from a food challenge (i.e. visualization of food cues), individuals high in trait mindfulness had greater GE in the insula relative to individuals lower on trait mindfulness (Paolini et al., 2012). Collectively, these data suggest that higher levels of integration across the HBN-A are important in understanding impulsive eating and the violation of dietary goals during intentional weight loss. Confirmation of this hypothesis awaits further controlled study.
While the results replicate previous work of ours showing that change in self-efficacy related to eating behavior is related to weight loss (Rejeski et al., 2011), the effect for GE across the HBN-A at baseline held even after controlling for residualized change in WEL scores. This result reinforces a review by Stice and colleagues (2013) which emphasizes that multiple inputs throughout the brain, both homeostatic and hedonic, determine food consumption and obesity. Moreover, many of these inputs are below the level of conscious awareness. Future intervention research for weight loss will need to be more cognizant of the complex relationship between the body, brain, environment, and behavior (Sporns, 2012; Rejeski and Gauvin, 2013). The current demonstration that self-regulation of weight is associated with integration of visceral and emotional brain centers lends support to recently developed methods that capitalize on the embodied nature of the mind and the importance of integration across the brain (Cozolino, 2010; Seigel, 2013).

There are several studies in the literature that have examined baseline brain activity and subsequent success or failure during an active weight-loss intervention, although this body of evidence has not employed state-of-the-art methods in network science. Weygandt and colleagues (2013) using fMRI found that higher activity in two regions of the frontal cortex, the VMPFC and DLPFC, during a food-related task requiring executive function predicted subsequent weight loss in a twelve-week low calorie dietary intervention. Moreover, greater functional connectivity between these two regions was associated with greater dietary success and impulse control (Weygandt et al., 2013). Given the focus on the prefrontal cortex, this investigation most likely captured the role that conscious self-regulatory mechanisms play in weight loss. In the current study, we found that change in a self-report measure of self-regulatory efficacy for eating behavior was related to weight loss; however, this effect was independent of the effect due to the distributed connectivity of the limbic and paralimbic regions of the HBN-A. Also, using magnetoencephalography (MEG), Hege and colleagues (2013) investigated whether baseline brain activity during a one-back visual memory task with food and nonfood cues differed between individuals who either succeeded or failed at weight
loss during a lifestyle intervention. Interestingly, those who were successful with weight loss had greater activation in the right temporal areas, including the hippocampus and the fusiform gyrus.

Finally, in line with others (Comings and Blum, 2000), Stice and colleagues (2010) propose that individuals who exhibit less activation in the reward circuitry of the brain may overeat to compensate for reward deficit. Two recent studies offer support for this line of thinking. One study examined brain activation while viewing food cues both before and after treatment with NB32, a new drug therapy under investigation for the treatment of obesity (Greenway et al., 2010); it combines 32 mg of naltrexone-sustained release (SR) with 360 mg of buproprion SR. The authors found that NB32 enhanced activation in reward regions important in eating behavior (anterior cingulate cortex, insula, hippocampal, and several other regions) (Wang et al., 2014). Interestingly, a second study demonstrated that obese participants, as compared to healthy weight controls, exhibited less activation in several areas of the brain (anterior cingulate cortex, ventromedial prefrontal cortex, posterior cerebellar lobe, precuneus, and cuneus) when viewing food related commercials (Gearhardt et al., 2014).

The current study is not without limitations. While we feel that GE is an ideal metric for capturing the experience of hunger and thus self-regulation during weight loss, it would have been ideal to replicate the findings of our original study on the HBN-A circuit using degree (Paolini et al., 2014). We were unable to do so for two reasons. First, as we demonstrated in that original work, degree appears to be associated with the state of being fed or unfed (i.e. regions of the HBN-A have higher degree in the unfed versus the fed condition). Unfortunately, this study was not designed to replicate these findings as we did not have a fed condition. Secondly, the present network analyses were performed in native space; thus, each network had a different number of nodes and connections making it impossible to use the same analysis employed in the original study. A second limitation is that we were limited to studying the intensive phase of weight loss in a group of older adults who were overweight/obese and had either MetS or CVD. Thus, it is unclear whether the brain signature
associated with GE across the HBN-A would apply to weight regain during long-term follow-up. It is well recognized that weight regain following the intensive phase of weight loss therapy is a significant barrier in treating obesity (Kramer et al., 1989; Wing and Hill, 2001; Rejeski et al., 2011). Finally, the age and disease status of the cohort restricts the external validity of the study findings.

In summary, after controlling for important covariates, this study demonstrated that distributed connectivity of the HBN-A was an important determinant of weight loss in obese, older adults with MetS or CVD. The HBN-A is comprised of limbic and paralimbic regions important in processing visceral cues and emotional elaboration of these sensations. Lower levels of activity in this network may well be linked to the reward deficit hypothesis for overeating espoused by Stice and colleagues (2010). We would argue that improved integration between the HBN-A and the rest of the brain supports superior awareness of food-related cues and improved emotional regulation. Indirect evidence for this position was provided in another publication of ours in which higher levels of GE across the HBN-A were inversely related to hunger and craving immediately following active imagery of favorite snack foods (Paolini et al., Network Analysis: Metrics Matter!, Frontiers in Aging Neuroscience, submitted). Further research is warranted to examine whether a targeted intervention can improve brain integration across the regions of the HBN-A in those who have compromised function and whether these changes augment the success of weight loss therapy.
References


CHAPTER V

DISCUSSION

Brielle M. Paolini, W. Jack Rejeski, and Paul J. Laurienti
Discussion

5.1 Summary of Findings and Introduction

The purpose of this thesis was to elucidate a brain network that might help to identify the variability in weight lost during an intensive phase of weight loss treatment. The supposition is that brain networks are related to the self-regulatory of eating-related behaviors that are important in weight loss. Three primary objectives were defined: 1) characterize the brain network associated with a hot-state following short-term food restriction; 2) tie this circuitry to real-world behavioral consequences; and 3) test whether the resulting brain network was predictive of weight loss during an intensive 6-month weight-loss intervention. To achieve these objectives, we first performed a study comparing the brain networks of older adults in a hot state (i.e. fasting) to a cold one (i.e. fed). From this analysis, we identified a hot-state brain network of appetite (HBN-A). A second analysis of this same data-set showed that the level of integration of the HBN-A with the remainder of the brain was related to hunger and craving. Separately, baseline brain network analysis was performed on 66 individuals prior to a randomized clinical trial involving weight loss, to determine whether the established brain network was predictive of weight loss; it was! We identified exciting and novel findings regarding the neuroscience of eating behavior in older adults.

Generally speaking, brain networks are vulnerable to “hot-states,” which individuals move in and out of dynamically through the course of the day due to changes in visceral cues or affect (Loewenstein, 2005). In chapter two, a resting-state brain network circuitry associated with the vulnerable hot-state of brief food restriction was characterized among fourteen older, overweight or obese adults. Specifically, participants arrived at our laboratory on two different occasions. On one visit, they consumed a liquid meal replacement (MR) following a short fast and on the other visit they consumed water. The participants then underwent both a resting-state and a food-cue fMRI scan. For this first aim, we characterized the brain network of the initial resting-state scan using an exploratory, data-drive approach to identifying hubs relevant to the HBN-A. The degree metric was used for
evaluation, measuring how many direct connections a particular node has in a given network (with high degree nodes being considered network hubs). The resulting network included five hubs: the insula, the anterior cingulate cortex (ACC), the superior temporal pole (STP), the amygdala and the hippocampus. The regions of the HBN-A had a greater number of connections (i.e. increased degree) in the fasting (hot-state) versus the meal replacement (cold-state) condition.

Overall, these five regions are highly involved in memory and in processing body sensations and emotions. The temporal pole (TP), the hippocampus and the amygdala are all limbic or paralimbic regions important for the processing of emotionally-related stimuli (Shelley and Trimble, 2004; Olson et al., 2007; Ball et al., 2009). The ventral portion of the ACC, included in the HBN-A, is known to be key for more cognitive processing and elaboration of emotion as well as for valence attribution (i.e. deciding whether a particular stimuli is good or bad) (Mohanty et al., 2007; Gasquoine, 2013). The insula was found to be a central hub in the HBN-A, and is well-known for its association with body-related sensation and the experience of emotions derived from bodily states (Critchley, 2004; Kringelbach et al., 2012; Uddin et al., 2013). The centrality of the insula to the HBN-A suggests that this network is viscerally-drive and truly embodied.

In light of existing literature suggesting higher levels of overall brain-integration are healthy and adaptive (Siegel, 2007; Cozolino, 2010), we also looked at the integrative ability of the HBN-A using global efficiency (GE), a choice metric for studying brain integration (Latora and Marchiori, 2001; Rubinov and Sporns, 2010). Since our first study indicated that the HBN-A was a highly-connected unit during the fasting condition, we focused our secondary analysis on this state (chapter three). We found that all five regions of the HBN-A, during both resting-state and food-cue visualization, were highly integrated as demonstrated by principal component analysis (PCA). We also found that increased integration from rest to a challenge (i.e. food cue visualization task) among the regions of the HBN-A was inversely related to both subsequent hunger and cravings. In other words, the more integrated the HBN-A was with other regions of the brain, the less hunger and craving were
experienced after the food-cue visualization task. While the measurement of degree (chapter two) was sensitive to the fasting versus fed state, GE appeared to be sensitive to the subjective experience of hunger and craving. Degree and GE were capturing very different real-world phenotypes and behaviors. This finding underscored the importance of using multiple metrics to fully characterize highly-multivariate systems, such as a functional brain networks. One metric will only ever capture one dimension of the network and can never completely summarize the network’s inherent complexity.

Since greater HBN-A integration was associated with lower craving and hunger scores, as measured by GE, we hypothesized that greater baseline HBN-A integration would be predictive of weight loss after 6-months of the intensive phase of a weight-loss intervention. To test this hypothesis, baseline resting-state brain scans were obtained on 66 individuals prior to a randomized clinical trial involving weight loss. At the time of baseline testing, MRI scans were conducted following an overnight fast. We found that five regions of the HBN-A were highly integrated as evidenced by PCA, and higher levels of GE were indeed associated with greater weight loss even after controlling for baseline weight, age, sex and self-regulatory efficacy. This prospective study design demonstrated, for the first time, that integration in the HBN-A during resting state following an overnight fast is predictive of weight loss during the intensive phase of weight loss therapy.

Taken together, these findings have helped advance our understanding of the neuroscience of eating behavior among older adults. Specifically, this work was the first to move beyond traditional activation studies of food-cue stimuli and use graph-theory to build a complex brain network present at rest. The benefits of this evolution will be addressed in detail in subsequent sections. Also, the idea that the HBN-A may operate as an unconscious driver of food consumption will be introduced along with how improved integration of the HBN-A with the rest of the brain may help make this unconscious processing more conscious. Two theories from behavioral psychology, the psychosomatic and deregulation theories, will be presented and will provide some insight into how
poor integration of visceral signals (i.e. HBN-A) with the rest of the brain may lead to disordered eating. Mindfulness offers promise as a teachable technique to help individuals integrate and become more self-aware of bodily cues and emotions. Mindfulness and mindfulness-based interventions (MBI) will be examined in detail, but in brief, mindfulness is characterized by attending to one’s moment-to-moment experiences in an open, non-judgmental fashion, making it an ideal intervention for disordered eating characteristic of dissociative, automatic processes. Finally, this discussion will explore ideas for future work emphasizing the steps needed to translate findings into personalized care for weight loss.

5.2. Neuroscience of Eating Behavior

Over the past several decades, the majority of theories regarding the neurobiology of eating behavior, including both the homeostatic and hedonic regulation of food-intake, were based on findings from animal studies (Berthoud, 2007; Berridge et al., 2010; Richard et al., 2013; Stice et al., 2013). Recently, the literature has expanded to include brain-imaging studies of eating behavior in humans; however, this more-recent work has been limited to activation studies identifying brain regions with an increased or decreased response to food-cues.

Some of these studies tie obesity and weight gain to increased activation (Rothemund et al., 2007; Stoeckel et al., 2008; Bruce et al., 2010), while, others show the opposite pattern (Cornier et al., 2009). Pessoa (2014) and Laurienti (2014) argue that regional activations are less important than the differences in dynamic connectivity among them. Regional activation studies, do not deal with the dynamic connection changes among regions—which may be the primary driver of brain function. Moreover, traditional fMRI activation studies use very dissimilar food-cue related tasks. For example, certain studies compare brain activation involving food-cues to neutral stimuli (i.e. desk); whereas, other studies compare highly appetizing foods to less appetizing ones. Still other studies focus exclusively on executive function tasks that involve food (i.e. n-back or go nogo). The problem in this
approach is that the various tasks all lead to activation of different brain regions making the identification of a common brain-network important to eating behavior challenging, if not impossible.

In short, the literature heretofore operates from two critical assumptions: (1) the “turning-on” or independent activation of singular brain regions regulates eating behavior; and (2) eating-behavior is driven by stimulus-response reactions to food. These assumptions about “independent activation” and “stimulus-response reactions” limit the learning that flows from the study of eating behavior. They should be considered only in the broader context of recent advances in neuroscience, such as network science analysis and resting-state brain function, which together provide insight into the brain’s intrinsic patterns of connectivity.

5.2.1. Network science and the HBN-A

Pessoa and others (Laurienti, 2014; Pessoa, 2014) have suggested that individual brain regions do not work in isolation and that the brain dynamically recruits distinct “multiple region assemblies” in various contexts (i.e. physiological states or tasks). Brain regions do not show one-to-one mapping with function (Schaefer et al., 2014). According to Pessoa a single brain region is not solely responsible for a particular response. There is no single “apple” neuron firing in response to seeing an apple. Instead, many different brain regions are recruited to comprehend an apple and these same brain regions may be recruited for other tasks (one-to-many) (Pessoa, 2014).

One circuitry may comprehend an apple as an appetizing treat while an altered one may view it as less desirable. Imagine a circuit of three brain regions. When this circuit is active, the brain perceives an apple as an appetizing treat, but when the connection strength to one region is altered, the brain may no longer view the apple as appetizing. Each state, task, or context recruits a different “multiple region assembly” of brain regions (Pessoa, 2014; Schaefer et al., 2014), however, it is not the group of brain regions that garner understanding but, instead, the dynamic connectivity patterns among them. Network science uniquely provides insight into these patterns of connectivity.
This thesis used network science to characterize the HBN-A and to identify its connections and relationships within both itself and the other regions of the brain. It led to the conclusion that the HBN-A was comprised of five regions including the insula, hippocampus, amygdala, STP and the ACC. The insula was the primary hub within the HBN-A, since it had direct connections to all four regions in the fasting state. It allowed for cross-talk among the regions. For example, the ACC and the amygdala have the potential for sharing information with each other through the insula, a mutual neighbor, despite not having a direct connection with one another. The overall functional interplay is much more important than identification of regions affected. During our secondary analysis of the HBN-A circuit (chapter three), we also demonstrated that HBN-A had greater connections within itself than with the remainder of the brain giving credence to the notion that the circuit works as a functional unit or “multiple region assembly.”

We also found that the more of an increase in HBN-A integration an individual experienced from baseline to food challenge, the lower his/her cravings and hunger. In fact, individuals with networks most adaptable to the food-challenge received the greatest benefit. The HBN-A not only exists as a “multiple region assembly” but it also is highly context-dependent and dynamic. This finding underscores the notion advanced by Pessoa (2014) that understanding real-world behaviors, such as eating, will require examination of how brain regions interact to form circuits and how these circuits change dynamically from one context to another.

5.2.2. Resting State and the HBN-A

Whilst part of what we perceive comes through our senses from the object before us, another part (and it may be the larger part) always comes out of our own head. - William James (1890)

This prescient comment by William James in his work The Principles of Psychology (James, 1890) captures the second critical difference between the foundational work in eating behavior using food-cues and the work discussed in this thesis defining resting-state brain networks. Foundational
work operated from the notion that brain function is primarily reflexive, driven by stimuli and demands in the environment. Consequently, food-cue tasks became the standard for studying the neuroscience of eating behavior.

Over the past decade, network science has gone beyond “reflexive analysis” to studying the brain’s intrinsic connectivity patterns at rest. Evaluation of such resting-state offers the benefit of easier comparison across studies than cognitive behavioral task analysis (Cao et al., 2014). It also allows insight into the default patterns of brain activation independent of the influence of external stimuli in the environment.

We have all had the experience on a long car ride or sitting in a boring lecture when our minds drifted off to simulate past experience, plan for an upcoming event, and reflect on our lives and relationships (Andrews-Hanna et al., 2014). The “drift” flows from the absence of attention to external stimuli, causing our resting minds to turn to self-generated thoughts, which are both complex and heterogeneous (Buckner and Carroll, 2007; Andrews-Hanna et al., 2014). However, the brain network supporting this activity is somewhat homogeneous and is called the default network, or DMN (Shulman et al., 1997; Raichle et al., 2001; Buckner et al., 2008; Andrews-Hanna et al., 2014). The DMN is often the most prominent brain network during rest. It consists of several core regions, which most commonly include the medial prefrontal cortex, posterior cingulate/precuneus, and inferior parietal lobe (Buckner et al., 2008; Moussa et al., 2012; Andrews-Hanna et al., 2014).

Of course, if the DMN is associated with resting-state brain networks, it begs the question: why didn’t our data-driven characterization of the HBN-A include regions of the DMN? There are several potential reasons for this result. First, the DMN is not the only brain network active during resting-state (Moussa et al., 2012; Andrews-Hanna et al., 2014). Multiple sub-networks are present during rest (Moussa et al., 2012), and different physiological states, such as acute alcohol consumption or
experimentally induced low back pain, have been shown to alter these resting-state networks (Spagnolli et al., 2013; Telesford et al., 2013; Zhang et al., 2014).

Second, we did see emergence of the DMN in our ‘fed’ condition of the original study. The precuneus, a common hub of the DMN (Raichle et al., 2001; Hagmann et al., 2008; Moussa et al., 2012), had higher degree in the condition that received a liquid meal replacement than the fasting condition (Paolini et al., 2014), suggesting that the consumption of a MR potentiated expression of the DMN. The precuneus was not included as a node in the HBN-A analysis because, in defining the nodes of the HBN-A, only those regions that were hubs during the fasting condition were included (not the fed condition).

With several modules or “multiple-region-assemblies” simultaneously present during rest, and the brain having a finite amount of processing power, one theory for the lack of DMN during the fasting-condition is that the brain dynamically allocates its resources to different sub-networks in different contexts or states (Garcia-Garcia, 2012). For instance, in a fasting state, the limbic-driven HBN-A circuit may hijack the brain’s limited resources leaving fewer resources for DMN activity. The fed state may free up some of the brain’s resources and permit more DMN activity, as demonstrated by the greater degree of the precuneus, a DMN hub, after MR (Paolini et al., 2014).

It is important to note that the DMN is still likely active in the hot-state of short term food restriction. As Aleksander R. Luria eloquently expressed on page 99 in The Working Brain (Luria, 1973), “Each form of conscious activity is always a complex functional system and takes place through the combined working of all... brain units, each of which makes its own contribution.” From a big picture standpoint, the brain is comprised of multiple sub-networks (i.e. modules or assemblies) such as the DMN and the HBN-A, and many of these sub-networks are present during the experience of different states and contribute to the overall result. These sub-networks are dynamic, therefore, they each may contribute more or less depending on the state or condition. A recent review on the
DMN suggests that self-generated thought is supported by such sub-networks within the brain and that it is the dynamic relationship between the DMN and these other sub-networks, such as the HBN-A, that is most important for understanding self-generated thought (Andrews-Hanna et al., 2014). In the studies presented here, the experimental conditions were such that the HBN-A was the sub-network most sensitive to the fasting condition. The sub-network of the DMN likely is still active in this state; however, it is less responsive to the state difference of being fed versus unfed.

In any event, it is important to note that the HBN-A was characterized in older, overweight or obese adults. Its ability to be the prominent sub-network during the fasting state may be compromised by either aging or excess weight. In fact, the functional integrity of the DMN is diminished in healthy older adults (Andrews-Hanna et al., 2007; Damoiseaux et al., 2008; Spreng and Turner, 2013) and obesity (Garcia-Garcia, 2012). A decrease in DMN activity with aging and excess weight may make it easier for the HBN-A to deplete the brain’s resources and become the prominent network during resting-state. To fully characterize the nature of a hot-state, future work should further investigate the dynamic connections and exchanging of resources between the HBN-A and the DMN during rest.

5.2.3. HBN-A and conscious awareness

The HBN-A includes many of the regions previously implicated in eating behavior in response to food-cues. What is surprising about the present investigation was the prominence of the HBN-A in the absence of any food-related stimuli. Different physiological states (i.e. fed versus unfed) provided enough of a physiological stimulus to alter brain connectivity at rest exclusive of the introduction of food-cues. During identical resting-state scans, older, overweight or obese adults had brain networks that were significantly changed from a fed to fasted state (i.e. cold versus hot state). Considering the stark difference between these two states, it is possible that older, overweight adults perceive and experience the world in fundamentally different ways depending on short-term dietary status. An individual’s experience with the world is not static (Siegel, 2007; Rejeski and Gauvin, 2013). Brain
networks, and likely our experiences and self-regulatory capacity, change dynamically as contexts transform (Siegel, 2007; Rejeski and Gauvin, 2013).

The HBN-A is a viscerally-driven network rich in valence attribution and incentive-motivated processing. Generally speaking, most regions within it operate below the level of consciousness, such that behavior during a “hot state” is likely controlled to a significant degree by automatic processes (Berridge, 1996; Paolini et al., 2014). With dynamic change occurring at an “automatic,” unconscious level, it follows that older, overweight adults likely are not consciously aware of the fact that their brain networks and consequent mental processes, including perceptions of the world, have dramatically changed after short-term food restriction.

So unconscious processing of physical sensations and emotions may affect people’s ability to self-regulate eating behavior and lose weight without their awareness. During a hot-state of food restriction, cognitive resources may get tied up in visceral processing, leaving fewer resources to be allocated to other important networks and tasks (i.e. DMN and self-referential thinking or frontal circuits and executive function). This could be akin to driving while not knowing the emergency brake is on. Limbic and visceral processing may affect one’s world experience, decisions, and behavior without his/her cognitive awareness. Currently, it is not known if the presence of this network during a hot-state contributes to self-regulatory failure as it pertains to eating behavior. If, in fact, it proves to be a driver of food-consumption or a predictor of such self-regulatory failure, then an effort to avoid a hot state through the use of MR beverages or multiple small meals throughout the day may be beneficial. Notably, behavioral data exist demonstrating that both of these practices are advantageous for active weight loss (Bellisle et al., 1997; Heymsfield et al., 2003; Kulovitz et al., 2014).

If the HBN-A network is replicated in normal, healthy individuals, it can also have implications for improvement in learning and peak performance. If an individual is in a hot-state of food
restriction, resources may be driven towards the HBN-A and not towards learning centers making consolidation of new material challenging: the same may be true for athletes. If they experience a visceral hot-state, they may not have the cognitive resources or motor integration to perform at a peak level. Again, if hot-states do prove to affect performance at an unconscious level in normal, healthy individuals, a conscious effort to avoid entering a hot-state by consuming snacks and small meals may be advantageous.

5.3. Integration of the HBN-A

But not all hot-states are created equal. We found that hot-state integration of the HBN-A with the remainder of the brain is the most significant predictor of hunger, cravings and weight loss. It appears that greater HBN-A integration is beneficial for self-regulation of eating behavior among older, overweight or obese adults experiencing a hot-state created by food restriction. These findings give credence to the idea put forth by Cornier (2011) that the fundamental issue in obesity is not an individual metabolic, autonomic and/or hormonal signal, but is instead the manner in which these homeostatic and non-homeostatic signals are integrated centrally (Paolini et al., 2012).

5.3.1. HBN-A integration and body-awareness

What benefit does integration of the HBN-A with the rest of the brain provide? As mentioned before, the HBN-A is comprised of limbic and para-limbic regions important for processing visceral sensations and for their related emotional elaboration and regulation (Paolini et al., 2014). Improved integration of these regions allows limbic information to be shared across the brain and to reach higher, cortical areas, potentially allowing unconscious information to reach a conscious level. Global sharing of visceral and limbic information may provide individuals with superior body and emotional awareness and improved processing capabilities, resulting in enhanced self-awareness of hunger levels, emotional state, and visceral drives. Such improved capabilities may lead to successful self-regulation of eating behavior and subsequent weight loss.
Our findings in chapter three and four offer indirect evidence for this position. In chapter three, higher levels of integration across the HBN-A were shown to be inversely related to hunger and craving following active imagery of favorite snack foods; in chapter four, greater baseline GE across the HBN-A was associated with greater weight loss during treatment. These findings support the work of Cozolino (2010) and Siegel (2007) suggesting that both horizontal and vertical brain integration is required for effective self-regulation.

5.3.2. Poor body-awareness and dysregulated eating

Two theories of food-consumption from psychology, the psychosomatic theory and the dysregulation model, provide potential explanations for how poor body-awareness and affect-regulation may be associated with failed self-regulation and weight-gain. According to psychosomatic theory, individuals overeat in response to their emotions because they are unable to distinguish the difference between emotional arousal and physical hunger (Bruch, 1964; O'Reilly et al., 2014). They confuse emotional arousal with physiological hunger. Such limited coping skills cause overeating in many individuals to pacify or avoid unpleasant feelings. Often such patterns of disinhibited eating occur in both an automatic and dissociative fashion (Kristeller and Hallett, 1999; Byrne et al., 2003; Dalen et al., 2010). Since many regions of the HBN-A are important for emotional regulation, some regions of the HBN-A may be important for both emotional and hunger processes or circuitry (many-to-many mapping). It follows that a behavior such as eating which satisfies the needs of one circuit (i.e. hunger) may also satisfy the needs originating from another circuit (i.e. emotion). Consequently, some individuals may overeat as a form of self-medication satisfying the brain’s emotional needs, but leading to disordered food consumption.

The dysregulation model of obesity purports that poor awareness of satiety signals and physical hunger may lead to the inability to self-regulate eating behavior (Craighead, 1996; Dalen et al., 2010; O'Reilly et al., 2014). Obese individuals are thought to have lost the ability to recognize or
respond to internal cues of hunger, taste, satiety and fullness (Lowe, 1993; Craighead, 1995; Kristeller, 2003; Dalen et al., 2010). Consistent with this theory, it may be true that some obese individuals have dysfunctional HBN-A processing. In other words, the HBN-A successfully drives people to eat, however, it may also fail to attenuate with eating. This failure of the HBN-A to respond to food may contribute to an overactive drive for consumption.

Both of these theories suggest that poor integration of limbic (i.e. emotional) and visceral (i.e. hunger/satiety) information with the rest of the brain may contribute to poor self-awareness and subsequent disordered eating. Consequently, it is possible that less HBN-A integration with the rest of the brain may result in confusing (i.e. psychosomatic theory) or limited (i.e. dysregulation model) limbic and visceral information reaching conscious awareness contributing to disordered eating. So, HBN-A integration may provide a possible mechanism for self-regulatory failure and it may also offer a potential way to identify individuals at risk for disordered eating. Treatments targeting improved self-awareness and subsequent brain integration, such as mindfulness-based interventions (MBI), may improve weight-loss success in at-risk individuals. However, the nature of these treatments may differ based on the origin of the over-consumption (i.e. psychosomatic or dysregulated). Individuals who eat in response to emotional stimuli may need MBI interventions targeting improved awareness and integration of emotional information; whereas, individuals with dysfunctional HBN-A circuits may need MBI training on becoming more conscious of satiety cues and hunger.

5.3.1. Mindfulness, self-awareness and network integration

Although a relatively new construct in the Western psychological literature, the last decade of research has established mindfulness as an important quality of conscious experience playing a central role in psychological function (Brown, 2007; Williams, 2010; Keng et al., 2011). Mindfulness is described as continually attending to one’s moment-to-moment experiences, sensations, thoughts
and emotions in an open, non-judgmental manner (Baer et al., 2006; Ludwig and Kabat-Zinn, 2008; Paolini et al., 2012; O’Reilly et al., 2014).

By promoting awareness of emotional states and physiological signals, mindfulness meditation may increase one’s self-awareness to bodily cues. Our previous work, not included in this thesis, provided some indirect evidence for this idea. We found that older adults, who scored high on a scale of trait mindfulness, had enhanced brain integration as compared to less mindful older adults (Paolini et al., 2012). Specifically, we found that following recovery from a food challenge (i.e. visualization of food cues), individuals high in trait mindfulness (i.e. high scores on the Mindfulness Attention and Awareness Scale, MAAS) (Brown, 2003) had greater GE in the insula relative to individuals who scored lower. Since the insula is a key brain structure for processing visceral sensation (LeDoux, 2003), higher GE in the insula is indicative of improved visceral integration with the rest of the brain—and perhaps greater self-awareness to bodily cues.

In a mindful state, individuals are also less apt to be trapped by intrusive thoughts and conditioned responses. Cognitive flexibility reduces automatic and impulsive reactivity to thoughts and behavior (Brown, 2003; Boderick, 2005; Brown, 2007; Weinstein, 2009). Consequently, mindfulness practice is thought to result in greater self-awareness, acceptance and improved ability to make adaptive choices when responding to difficult conditions (Baer et al., 2006). A few studies have shown that scores on the MAAS are directly related to dispositional self-control (Barnes et al., 2007; Lakey et al., 2007).

In our previous work, we also found that mindful older adults had better self-confidence in their ability to control eating following food challenge than those low in trait mindfulness (Paolini et al., 2012). Interestingly, however, the low and high mindful sub-groups did not differ in either state craving or hunger ratings, suggesting that mindful individuals are not spared from the physical experience of craving and hunger, but rather they have the cognitive flexibility to act more
consciously on these sensations than “low-mindful” individuals. As a matter of fact, a key characteristic of mindfulness is the ability to witness one’s physical sensations in an accepting rather than a reactive manner (Kabat-Zinn, 1990).

Our previous work also demonstrated that trait mindfulness was a powerful moderator of brain connectivity following a food-visualization task. Specifically, mindful older adults (i.e. high MAAS scorers) were able to return to the DMN, as indicated by greater GE in the precuneus, during a rest-period following food-cue exposure. There was a trend for a stronger effect in the fed (MR) versus the fasting condition (water). This was not the case for less mindful older adults. Instead of returning to DMN, these individuals had brain networks that were characterized by pre-occupation with, and elaboration of, food-cues (Paolini et al., 2012). This result was most dramatic for the low mindful group during fasting (i.e. water condition).

Mindfulness practice is known to foster greater non-judgmental awareness of physical sensations and emotions providing response plasticity (Baer et al., 2006). Consequently, mindful individuals may have greater cognitive flexibility allowing for reallocation of resources to the DMN following a food challenge; whereas, those low in mindfulness are unable to reallocate their resources and, as a result, remain trapped in a visceral-elaboration network.

Interestingly, the consumption of a MR beverage not only helped mindful individuals return to DMN, but also attenuated the reactive brain network in the less mindful ones. Thus, our prior work suggests that older, mindful adults have improved brain integration, better perceived control relating to eating behavior, and enhanced network flexibility (Paolini et al., 2012). The greater awareness and self-control afforded by mindfulness make interventions designed to actively cultivate mindful qualities ideal for conditions, such as disordered eating, that involve automatic, dissociative behaviors. Mindfulness-based treatments relating to eating behavior may uniquely restore an individual's ability to detect and respond to natural cues of hunger and satiety (Dalen et al.,}
2010; Kristeller and Wolever, 2011), and may provide the tools to make more and better adaptive choices when responding to difficult contexts—such as a hot-state of short term food restriction.

In recent years, the efficacy of mindfulness-based interventions (MBI) have gained attention and have garnered increasing empirical support for many conditions (O'Reilly et al., 2014) including chronic pain (Chiesa and Serretti, 2011a; Zeidan et al., 2012), depression (Piet and Hougaard, 2011; Klainin-Yobas et al., 2012), anxiety disorders (Roemer and Orsillo, 2007; Treanor, 2011), stress reduction (Chiesa and Serretti, 2009), cancer care (Smith et al., 2005; Ott et al., 2006; Musial et al., 2011; Shennan et al., 2011), psychiatric disorders (Chiesa and Serretti, 2011b), speech pathologies (Boyle, 2011), and substance use disorders (Zgierska et al., 2009). Although the application of mindfulness approaches to problematic eating behaviors and to weight-loss therapies is somewhat limited, early results have been promising (Baer et al., 2006; Daubenmier et al., 2011; Hepworth, 2011; Kristeller and Wolever, 2011; Wanden-Berghe et al., 2011; Daubenmier et al., 2012; O'Reilly et al., 2014). Improved visceral and limbic integration may be a mechanism for understanding why mindfulness-based therapies designed to increase moment-to-moment awareness appear to be promising in the treatment of eating disorders and obesity (Wanden-Berghe et al., 2011; Godsey, 2013; O'Reilly et al., 2014).

5.5 Limitations of presented findings

The presented research is not without limitations. First, the HBN-A was characterized in older, overweight and obese adults. Since we did not have a normal weight control group, the HBN-A should be considered specific to this population until subsequent studies confirm these effects in younger age-groups and people with more diverse biometric characteristics. Secondly, the HBN-A was defined in a study population of only fourteen individuals with limited demographic variability. Future work should seek to confirm its presence in larger, more heterogeneous sample sizes.
Our second study which found that greater HBN-A integration was associated with subsequent weight loss was also restricted to overweight or obese, older adults who were additionally metabolically compromised. Specifically, the inclusion criteria involved the presence of either metabolic syndrome (MetS) or history of a cardiovascular event (CVD); therefore, this finding has limited population validity. Moreover, we only predicted weight loss during six months of the intensive phase of weight loss. We did not relate baseline HBN-A integration to either weight maintenance or weight regain.

It should also be noted that during image acquisition for the weight loss study (chapter four), the pre-scan normalize function was turned off for all 60 baseline scans. Pre-scan normalize is used to correct for the inherent bias in tissue signal based on location (i.e. tissue closer to the head coil has higher signal values than deeper brain structures). The built-in inhomogeneity correction function in SPM8 was used to remove this bias. In order to limit data manipulation, the network analysis for this study (chapter 4) was performed in native space; whereas, the original study data (chapter two and three) was warped and analyzed in template space. One limitation of native space networks is that each network has a different number of nodes and connections. This characteristic makes certain metrics, such as degree (k), that are highly dependent on the number of nodes and connections in the network confusing if not uninterruptable.

5.6 Future directions

Findings from the studies presented in this thesis raise a number of important questions for the study of eating behavior. While the HBN-A was shown to be an important network during the hot-state of short-term food restriction in older, overweight adults, this finding should be replicated in both younger and healthier populations. Moreover, it is currently unknown if HBN-A’s prominence during resting-state in older, overweight adults is attributable to the physiological stress of short-term food restriction, to the metabolic pressures and consequent network changes relating to excess weight,
or to a decrease in DMN function. Analysis of resting-state networks during food-restriction in more diverse samples may begin to help answer these questions. Some individuals may, in fact, be more vulnerable to short-term food restriction and, consequently, have greater HBN-A reactivity and connectivity than others. Genetic, biochemical and/or long-term dietary factors could contribute to such variability (Stice et al., 2010). And, there may be gender differences in the HBN-A. If the HBN-A’s prominence during short-term food restriction is replicated across multiple sample demographics, it may become important for future neuroimaging studies to control for nutritional energy status.

Since self-generated thoughts characteristic of resting-state are often heterogeneous, it begs the question: what are the predominant thoughts during resting-state when individuals are also experiencing a hot-state of food restriction? As most of the regions of the HBN-A theoretically operate below the level of conscious awareness, it is possible that individuals in this state are not experiencing food-related thoughts but, instead, continue to be influenced by normal thoughts that are self-generated? A recent review of the DMN suggests that such self-generated thought is multifaceted, including multiple component processes supported by different sub-networks within the brain. And, they claim that the dynamic interactions of the DMN with these other sub-network systems are vital for understanding the regulation of internal thought (Andrews-Hanna et al., 2014). Future work should investigate how the DMN and the HBN-A dynamically interact and how these interactions relate to the content of self-generated thoughts during resting-state.

The liquid MR, BOOST®, used in this study reduced connectivity of the HBN-A. It would be interesting to study what factors of the MR are most responsible for this attenuation. For example, it is known that protein is highly satiating (Journel et al., 2012). Would a MR beverage with greater protein content provide a further reduction in HBN-A connectivity? Along this line of reasoning, sugar is known to be highly addictive and to stimulate brain regions associated with reward (Ahmed et al., 2013; Lennerz et al., 2013). Would a high-sugar drink fail to reduce HBN-A connectivity? It is
also conceivable that more hedonically-appealing food may be superior in attenuating connectivity in the HBN-A.

Since HBN-A integration assessed prior to an intervention was predictive of weight-loss, it follows that self-regulation and weight-loss themselves might be powerful-enough stimuli to change the integration of the HBN-A with the remainder of the brain. It is possible that as individuals are taught to monitor food consumption and they begin to practice self-awareness skills and positive regulatory behaviors they may become more conscious of their emotions and their physical sensations (i.e. hunger and cravings)? Over a period of time, these active changes may actually improve HBN-A integration-- a hypothesis certainly warranting future investigation.

Integration of the HBN-A with the rest of the brain was predictive of six-month weight loss during the intensive phase of an intervention; however, it is unknown whether HBN-A integration is important for long-term weight maintenance. Weight regain remains a significant problem as an estimated 80% of individuals who lose weight return to or exceed their initial weight within three to five years (Byrne et al., 2003). Knowledge of a baseline, brain signature predictive of weight regain would be invaluable. It could help identify individuals at risk and provide them with more targeted, aggressive treatment which potentially could have a significant impact in reducing their secondary morbidity and mortality.

Mindfulness based interventions (MBIs) relating to eating behavior may uniquely restore an individual's ability to detect and respond to natural cues of hunger and satiety. According to the dysregulation and psychosomatic theories of eating behavior, enhanced awareness to such visceral and emotional cues is fundamental to self-regulatory success (Bruch, 1964;Craighead, 1996;Dalen et al., 2010;O'Reilly et al., 2014). Future research is warranted to examine whether a targeted MBI can improve brain integration across the regions of the HBN-A in those who have compromised function and whether these changes augment the success of weight-loss therapy and subsequent maintenance.
Importantly, the work included in this thesis lays the foundation for future analysis in relation to the personalization of weight loss treatment. Prior to treatment, a baseline MRI may help identify individuals at risk for self-regulatory failure, recognize their driving factors for over-consumption, and assist in triaging them into personalized interventions. For instance, it is possible that individuals who have poor attenuation of the HBN-A following food consumption (i.e. dysregulated eating) (Bruch, 1964; O'Reilly et al., 2014), may benefit from MBIs emphasizing hunger and satiety cues; whereas, other individuals who overeat due to emotional distress (i.e. psychosomatic eating) (Craighead, 1996; Dalen et al., 2010; O'Reilly et al., 2014) may benefit from MBIs focusing on emotion awareness.

It is critical that post-intervention analyses examine how the HBN-A responds to weight loss interventions and determine if changes in connectivity are associated with weight loss. Such knowledge could provide insight into why people fail to lose weight and could help redirect them into more successful treatment. For example, if an individual fails to lose weight and also does not achieve the brain changes characteristic of successful treatment with a particular intervention, it is possible that he/she may simply not have engaged fully in the intervention. Alternatively, if another individual fails to lose weight but does achieve the characteristic brain changes, he/she may have a secondary reason for being unable to lose weight warranting further investigation and informed redirection.

The ultimate goal is to translate these findings into personalized medicine for weight loss among older adults. In the not too distant future, it may be possible use brain network analysis to identify the root cause of over-consumption of food, to personalize treatment, and to help redirect unsuccessful individuals into more promising interventions while significantly reducing secondary morbidity and mortality in this vulnerable population.
References


CURRICULUM VITAE

NAME  Brielle M. Paolini

ADDRESS  Laboratory for Complex Brain Networks
          Wake Forest University School of Medicine
          2000 West 1st Street, Suite #701
          Winston-Salem, North Carolina 27104
          Telephone: (336) - 716-0473
          E-mail: bpaolin@wakehealth.edu

EDUCATION

Graduate:  Wake Forest University School of Medicine
          2011 – present
          Winston-Salem, North Carolina
          Ph.D. in Neuroscience
          Dissertation Title
          Self-Regulatory Brain Networks of Eating Behavior
          W. Jack Rejeski, Ph.D. (Co-mentor)
          Paul J. Laurienti, M.D. / Ph.D. (Co-mentor)

College:  College of William and Mary
          2006 – 2010
          Williamsburg, VA
          B.S. in Neuroscience

PROFESSIONAL APPOINTMENTS & ACTIVITIES

MD/PhD Student  2010 – present
Wake Forest University School of Medicine - Winston-Salem, NC

Combining Clinical and Research Careers in
Neuroscience Symposium Participant – Washington D.C.

Ad Hoc Scientific Reviewer  2012 – present
          NeuroImage
          Experimental Brain Research
          Journal of the American Society of Nephrology
          Plos One
MD/PhD Student Association – President  
Wake Forest University School of Medicine - Winston-Salem, NC  
2012 – 2013

Integrative Medicine Interest Group – Founder and President  
Wake Forest University School of Medicine - Winston-Salem, NC  
2010 – 2013

Student Wellness Center – Co-founder  
Wake Forest University School of Medicine - Winston-Salem, NC  
2010

MD/PhD Summer Research Rotation  
Brain Networks and Eating Behavior – Laboratory for Complex Brain Networks (LCBN)  
Wake Forest University School of Medicine - Winston-Salem, NC  
2011

MD/PhD Summer Research Rotation  
Neuro-imaging of Pain Response – Laboratory of Robert C. Coghill, PhD  
Wake Forest University School of Medicine - Winston-Salem, NC  
2010

Medical Student Participant  
Leadership and Education Program for Students in Integrative Medicine (LEAPS into IM) – Kirpalu, NY  
2011

Summer Research Institute Participant  
The Mind and Life Institute – Garrison, NY  
2010, 2012

SROP (Student Research Opportunities Program) Researcher  
Wake Forest University School of Medicine – Winston-Salem, NC  
2008

Computational Modeling of Alzheimer’s Disease Researcher  
College of William and Mary – Williamsburg, VA  
2009 – 2010

Division I Varsity Women’s Golf Team  
College of William and Mary – Williamsburg, VA  
2006 – 2010

**HONORS AND AWARDS**

WFU Graduate School Research Day, 1st place  
2014

*Poster presented*

International Symposium of Contemplative Science Travel Award  
2014

WFU Center for Integrative Medicine Travel Award  
2014

WFU Medical Student Research Day, 1st place  
2012

*Poster presented*

Integrative Medicine Micro-Grant for Educational Development  
2011
Awarded by the American Medical Student Association
MD/PhD Academic Merit Scholarship 2010
Goldwater Scholar 2009
Cornel Award- top premedical student at William and Mary 2010
Robert Usry Award 2010

Highest GPA among all student-athletes at William and Mary
College of William and Mary – Summa Cum Laude 2010
Student Presentation Awards – Runner Up 2008

Poster Presented at Conference of Biomedical Sciences and Instrumentation
William and Mary Athletic Golf Scholarship 2006 – 2010

PROFESSIONAL MEMBERSHIPS

American Medical Association 2010 – present
Society for Neuroscience 2010 – present
Western North Carolina Society for Neuroscience 2010 – present
Phi Beta Kappa Society 2010 – present

PUBLICATIONS


SCIENTIFIC PRESENTATIONS & TALKS


