Brain Functional Connectivity in Childhood Obesity

By

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In memory of my sister Cathy. I miss you.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

Introduction

Childhood obesity in the US has nearly doubled over the past 30 years; among adolescents obesity has tripled (Ogden *et al.*, 2010; Ogden *et al.*, 2012). In 2012, one-third of children and adolescents were overweight or obese, totaling more than 24.6 million children in the US (childStats.gov, 2014; Ogden *et al.*, 2014). Seventeen percent of children were obese (Ogden *et al.*, 2014). Worldwide, 42 million children were either overweight or obese in 2013 (World Health Organization, 2015). Approximately 67% of children who are overweight and obese remain obese as adults (Juonala *et al.*, 2011). The efficacy of long-term weight loss among adults is poor (Fildes *et al.*, 2015), with up to 90% returning to baseline weight within 3 years of behavioral treatment (Cooper *et al.*, 2010; Butryn *et al.*, 2011). Therefore the understanding, treatment, and prevention of childhood obesity are critical for the treatment of adult obesity.

Physiological comorbidities.

Children who are overweight or obese have a higher risk of metabolic syndrome (Weiss *et al.*, 2004), type 2 diabetes (Juonala *et al.*, 2011; Park *et al.*, 2012; Halfon *et al.*, 2013), hypertension (Reinehr and Wunsch, 2010; Wake *et al.*, 2010; Juonala *et al.*, 2011; Park *et al.*, 2012), hyper-lipidemia (Reinehr and Wunsch, 2010), cardiovascular disease (Park *et al.*, 2012; Ayer *et al.*, 2015), stroke (Field *et al.*, 2005; Lawlor and Leon, 2005; Falkstedt *et al.*, 2006; Baker *et al.*, 2007; Li *et al.*, 2007; Ford *et al.*, 2008; Lambert *et al.*, 2008; Virdis *et al.*, 2009; Reilly and Kelly, 2010), sleep apnea/snoring (Marcus *et al.*, 2012), musculoskeletal disorders and pain (Backstrom *et al.*, 2012; Sabharwal and Root, 2012; Adams *et al.*, 2013), and nonalcoholic fatty

liver disease (Koot *et al.*, 2015). Obesity during childhood is associated with earlier and accelerated development of coronary atherosclerosis (McGill *et al.*, 2002) and can lead to coronary artery disease by mid-adulthood (Raghuveer, 2010). Being overweight during childhood is significantly associated with premature mortality in adulthood (Reilly and Kelly, 2010). On average, 58% of obese 15-year-old males, and 84% of obese 15-year-old females, are obese in their 40s (Goldhaber-Fiebert *et al.*, 2013). Overweight children have increased lifelong risk for diabetes, hypertension, stroke, and coronary heart disease (Field *et al.*, 2005; Lawlor and Leon, 2005; Falkstedt *et al.*, 2006; Baker *et al.*, 2007; Li *et al.*, 2007; Ford *et al.*, 2008; Lambert *et al.*, 2008; Virdis *et al.*, 2009; Reilly and Kelly, 2010).

Psychological comorbidities.

In addition to these medical risks, the most prevalent, and perhaps most disruptive, co-morbidities related to childhood obesity are psychosocial (Dietz, 1998). Shockingly, self-reported quality-of-life for children who are obese is comparable to children receiving chemotherapy (Schwimmer, 2003). The stigma of obesity can create feelings of low self-esteem (Mustillo *et al.*, 2012), social isolation (Goffman, 2009), and victimization (Robinson, 2006). Qualter *et al.*, showed an association between increased adiposity and victimization among girls (Qualter *et al.*, 2015). Furthermore, they reported that increased victimization contributed to increased weight gain, thereby creating a vicious cycle. Compared to children who are healthy weight, children who are obese have higher rates of depression (Halfon *et al.*, 2013). Adolescents who are obese are 80% more likely to have thoughts of suicide compared to healthy weight adolescents (Zeller *et al.*, 2013). Obese boys are more likely to be bullied and to carry weapons (Farhat *et al.*, 2010). Children who are obese, compared to healthy weight children, are more likely to have internalizing problems (*e.g.*, feels worthless/inferior); have externalizing problems (*e.g.*, disobedient,

argumentative, bullies); repeat a grade in school; and miss more school days (Halfon *et al.*, 2013). Women who were obese as adolescents are almost three times as likely to develop depression, and four times as likely to develop anxiety, compared to women who were healthy weight children (Anderson *et al.*, 2007).

Costs.

The economic cost of childhood obesity is substantial. The direct costs of childhood obesity, which includes outpatient costs, emergency department visits, and drug prescriptions, is $14 billion (Trasande and Chatterjee, 2009), and inpatient costs is $238 million (Trasande *et al.*, 2009). Finkelstein *et al.*, estimate that the lifetime direct medical cost of an obese 10-year old child relative to a 10-year old child who remains at a healthy weight throughout adulthood is greater by $19,000 (Finkelstein *et al.*, 2014). This estimated lifetime cost is greater by $12,600 when compared to a healthy weight 10-year old who gains weight through adulthood. Janicke *et al.*, reported a greater yearly total cost for children who are obese ($3,042) compared to healthy weight children ($2,578) (Janicke *et al.*, 2009).

Physiology of energy balance.

Body weight is determined via energy balance, which is achieved when caloric intake equals energy expenditure. Caloric intake is the number of calories ingested. Energy expenditure is the number of calories burned due to resting metabolism (basal metabolic rate), thermogenesis, and physical activity. When caloric intake is greater than energy expenditure, excess calories are stored. When caloric intake is less than energy expenditure, stored calories are used. Body weight is maintained via homeostatic mechanisms (Leibel *et al.*, 1995) as well as non-homeostatic eating (Berthoud and Levin, 2012). Both mechanisms are described below.

Managing homeostatic and non-homeostatic mechanisms necessitates communication about the internal and external environment to the brain (Berthoud and Levin, 2012). The brain responds to external cues about the availability and palatability of food. The brain also responds to internal signals from nutrients (*e.g.*, glucose, fatty acids, amino acids), hormones (*e.g.*, insulin, leptin, and ghrelin), and neural substrates (*e.g.*, glutamate, gamma-aminobutyric acid [GABA], adenosine triphosphate [ATP], transporters, signaling pathways]) (Berthoud and Levin, 2012).

Homeostatic regulation

Homeostatic eating is coordinated by neuroendocrine feedback loops involving nutrient and hormonal signals indicating energy store levels to the hypothalamus and hindbrain. Robust and redundant biological systems have developed to defend energy supply (Berthoud and Morrison, 2008). Kennedy suggested, in 1953, that this defended energy supply is controlled by a "set point" signal originating from adipose tissue that is monitored by the brain (Kennedy, 1953). This set point theory became more widely accepted with the discovery of leptin, the "satiety hormone," which is secreted primarily by adipose tissue. The dysregulation of this set point often promotes an increase in adiposity with an accompanying increase in the set point. Prentice and Jebb argued that the development of robust fat storage in humans confers a biological advantage (Prentice and Jebb, 2004). They therefore hypothesized that the hunger mechanism, which promotes caloric intake, is more powerful than the satiety mechanism, which curbs eating. Therefore, when an individual loses weight, homeostatic mechanisms work to regain the previous, elevated adiposity-driven set point (Levin, 2010).

Non-homeostatic eating

Non-homeostatic eating, *i.e.*, eating in the absence of energy need, may also contribute to body weight. Non-homeostatic eating can be initiated via complex neural systems (Berthoud and Levin, 2012). Brain regions involved in non-homeostatic eating include: the hippocampus, associated with memory and spatial orientation; ventral striatum, associated with reward and reward-motivated behavior; dorsal striatum, associated with habit learning; and the amygdala, associated with emotional learning (Berthoud and Levin, 2012). The prefrontal cortex (PFC) is another significant brain region involved in non-homeostatic eating. The PFC is well positioned to integrate emotional, cognitive, homeostatic, and environmental information leading to eating choices and decisions (Berthoud and Levin, 2012). Brain regions associated with response inhibition, impulsivity, motivation, and reward, are increasingly recognized as potent modulators of non-homeostatic eating (Fields *et al.*, 2013; Johnstone *et al.*, 2013).

Given the central role of the brain in homeostatic and non-homeostatic eating, and the lack of efficacious long-term obesity treatment, our hypotheses stem from the overarching position that weight loss maintenance involves not only a change in eating habits and physical activity, but also a change in brain function. Our research contributes to the understanding of the neurobiological underpinnings of childhood obesity that involves the interplay among response inhibition, impulsivity, motivation, and reward, and their relationship with weight and eating habits.

Brain network vs. discrete brain regions.

Although discrete brain regions have been associated with homeostatic and non-homeostatic eating, the brain functions as a network. Brain regions engage in specialized functions and continually communicate with each other, thereby creating an efficient and powerful network.

Studying the brain as a network will provide valuable insight into neural connectivity, communication, and integration. As detailed in Chapter 2, the examination of brain networks has been used to identify brain disorders and track brain development. As such, we therefore investigated the functional connectivity between pairs of discrete brain regions associated with non-homeostatic eating. In broad terms, functional connectivity is a methodology that quantifies the communication and integration of regions of the brain that may be anatomically and spatially distinct.

Hypotheses.

We posited that brain networks of children who are overweight or obese are biased toward increased drive to eat and away from cognitive control. As such, we hypothesized that the functional connectivity associated with the drive to eat, *e.g.*, motivation and impulsivity, would be greater than the functional connectivity associated with cognitive control, *e.g.*, response inhibition. Our hypotheses were built upon the idea that, while discrete brain regions may (or may not) exhibit healthy function, the relative balance among these regions may provide additional information about childhood obesity.

Hypotheses for task-based psychophysiological interaction study

In our psychophysiological interaction (PPI) study we examined the functional connectivity between two pairs of brain regions: (1) the basolateral amygdala (BLA) and nucleus accumbens (NAc); and (2) the rostral anterior cingulate cortex (rACC) and NAc. The BLA is associated with motivational drive; the rACC is associated with response inhibition; and the NAc is associated with reward-motivated behaviors. We detail the function and relationships of these regions in Chapter 3. We hypothesized that increased functional connectivity associated with motivational

drive to eat and/or decreased functional connectivity associated with inhibition is related to increased adiposity and increased unhealthy eating habits. Our PPI study used a food cue task-based magnetic resonance imaging (MRI) paradigm in which children viewed images of high calorie food, low calorie food, and nature. Images of food are prevalent in the daily lives of children. Therefore our visual food cue task is an ecologically valid method to probe putative differences in the communication and organization of the young obese brain.

Hypotheses for resting state study

In our resting state study we examined the functional connectivity between: (1) the frontal pole and NAc; and (2) the inferior parietal lobe (IPL) and NAc. The frontal pole is associated with impulsivity; the IPL is associated with response inhibition. We detail the function and relationships of these regions in Chapter 4. We hypothesized that increased functional connectivity associated with impulsivity and/or decreased functional connectivity associated with response inhibition are associated with increased adiposity and increased unhealthy eating habits. We hypothesized that even during quiet rest, in the absence of overt food-related stimuli, functional connectivity continues to exhibit a bias toward drive with increasing adiposity.

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CHAPTER 2

Background, Concepts, and Methodology

Measures of eating behaviors.

To quantify eating behaviors in our PPI functional connectivity study, we used the Dutch Eating Behaviour Questionnaire for Children (DEBQ-C). For our resting state functional connectivity study, we used the Child Eating Behaviour Questionnaire (CEBQ). These questionnaires were developed to measure eating styles among children through self-reporting (Braet *et al.*, 2008). Self-reported behavioral measures are preferable to retrospective food recall as recalls often result in an underestimate of food consumption due, in part, to memory bias and social expectations and pressure (Ahmed *et al.*, 2006). While a 24-hour recall may be more accurate compared to a retrospective recall, food consumption can vary greatly from day to day such that a single day may not be representative (Block, 1982). Self-report instruments, such as DEBQ-C and CEBQ, identify eating habits rather than actual food intake. Both questionnaires have good factorial validity and external validity (van Strien *et al.*, 1986; Schlundt, 1995; Braet and van Strien, 1997; Wardle *et al.*, 2001; Carnell and Wardle, 2007).

Dutch Eating Behaviour Questionnaire for Children (DEBQ-C)

The DEBQ-C is a validated 20-item questionnaire that measures three aspects of eating behavior: external eating (six questions), restrained eating (seven questions), and emotional eating (seven questions) (van Strien and Oosterveld, 2008; van Strien *et al.*, 2012). External eating is eating in response to the sight or smell of food. Restrained eating is eating less to lose or maintain weight.

Emotional eating is eating in response to negative emotions (van Strien and Oosterveld, 2008). Each DEBQ-C item is rated on a Likert scale from 1 to 3 where 1 = no; 2 = sometimes; and 3 = yes. In our research, we explored the relationship of external eating and restrained eating with brain functional connectivity and weight status. We did not include emotional eating in our assessments because the initial validation study noted that young children had difficulty comprehending questions about emotional eating (van Strien and Oosterveld, 2008). Furthermore, the literature shows mixed results between self-reported emotional eating and laboratory-based measures of emotional eating (Domoff *et al.*, 2014).

Studies reported a statistically significant increase in restrained eating scores when comparing children who are overweight compared to healthy weight children (Braet *et al.*, 2008; van Strien and Oosterveld, 2008). Braet *et al.*, reported lower external eating scores among children who are overweight compared to healthy weight children but increased external eating scores with increased body dissatisfaction (Braet *et al.*, 2008). Van Strien *et al.*, reported increased restrained eating scores with decreased snacking (van Strien and Oosterveld, 2008). They also reported increased external eating scores with increased time viewing screen media (watching television or using computer).

Child Eating Behaviour Questionnaire (CEBQ)

The CEBQ is a validated 35-item questionnaire that measures eight aspects of eating behavior (Wardle *et al.*, 2001):

1. **DD** Desire to Drink indicates frequent drinking;
2. **EF** Enjoyment of Food indicates an overall interest in food;
3. **EOE** Emotional Overeating indicates increased eating under negative emotions;
4. **EUE** Emotional Undereating indicates decreased eating under negative emotions;
5. **FF** Food Fussiness indicates rejection of both new and familiar foods;

6. **FR** Food Responsiveness assesses eating in response to food cues;
7. **SE** Slowness in Eating assesses reduced eating due to low interest and/or enjoyment of food; and
8. **SR** Satiety Responsiveness assesses how well a child controls the amount he/she eats in response to eating recently.

Each item is rated on a Likert scale from 1 (never) to 5 (always). "Food approach" behavior is indicated by increasing DD, EF, EOE, and FR scores, whereas "food avoidance" behavior is indicated by increasing EUE, FF, SE, and SR scores (Wardle *et al.*, 2001). Food approach behaviors have been positively associated with increased weight among children and food avoidance behaviors have been negatively associated with increased weight (Carnell and Wardle, 2008; Sleddens *et al.*, 2008; Webber *et al.*, 2009; Spence *et al.*, 2011; Svensson *et al.*, 2011). The CEBQ was administered only to children younger than 12 years old.

Measure of adiposity.

Among adults, body mass index (BMI) is a convenient proxy measure for adiposity. However, because body composition changes throughout childhood, a measure of adiposity that accounts for changes in body composition during childhood growth is needed. Age- and sex-specific BMI percentile is one such common measure. However, while BMI percentiles are easier to use in the clinical setting, they are not ideal for statistical analyses. For example, percentiles at the extremes, *e.g.*, ≥ 99%, are non-linear as this category can include a wide range of weights. Instead, BMI *z*-scores are a continuous measure and therefore not subject to the non-linearity problem seen with BMI percentiles. Therefore BMI *z*-scores are better suited for statistical analyses (Wang and Chen, 2012). However, BMI *z*-scores can be more difficult to explain to the public. While BMI *z*-scores are not a direct measure of adiposity, they are more strongly associated with percentage of body fat, as measured by dual-energy X-ray absorptiometry, than

BMI percentiles (Heo *et al.*, 2014). We therefore used BMI *z*-scores as a proxy measure for childhood adiposity. We calculated an age- and sex-specific BMI *z*-score for each child using LMS transformation parameters *lambda*, *mu*, and *sigma* (CDC; Kuczmarski *et al.*, 2002).

For our resting state functional connectivity study, we used continuous BMI *z*-scores in all statistical analyses. However, when reporting summary statistics, children were classified as healthy weight with (-1.64 ≤ BMI *z*-scores < 1.04); overweight with (1.04 ≤ BMI *z*-scores < 1.64); and obese with (BMI *z*-scores ≥ 1.64) (Wang and Chen, 2012). For our PPI functional connectivity study, only children who were healthy weight or obese were enrolled in the study; children who were overweight were excluded.

MRI.

Brief basics of MRI physics

Hydrogen, made up of a single proton, has a positive charge and spin (*i.e.*, angular momentum of the nucleus) and can act as a tiny magnet. As with all charged particles, hydrogen generates a magnetic field. The nuclear magnetic moment is proportional to its magnetic field. The nuclear magnetic moment of this hydrogen ion, also referred to as a "proton," will align parallel or anti-parallel with a strong magnetic field, such as 3 Tesla found in many research MRI scanners. This strong external magnetic field is called B0. When an individual is placed in an MRI scanner, nuclear magnetic moments from many, many protons in the body will align parallel or anti-parallel with B0. A state of equilibrium is achieved in which a slight majority of nuclear magnetic moments align parallel with B0 compared to those aligning anti-parallel (Figure 2.1A). This proton equilibrium is called longitudinal magnetization. The introduction of additional energy via a radio frequency (RF) pulse, called B1, is orthogonal to B0 and disrupts the

equilibrium (Figure 2.1B). The protons absorb this additional energy and are knocked out of alignment with B0 (Figure 2.1C). The *xy*-component of this disrupted equilibrium is called transverse magnetization. When B1 is turned off, the misaligned protons "relax" back to the equilibrium state, that is, they realign with B0. This relaxation occurs in two forms. T1 relaxation is the regrowth of longitudinal magnetization along B0 (Figure 2.1D). T2 relaxation is the loss of transverse magnetization (Figure 2.1F). The energy that was absorbed from the B1 pulse is released during relaxation. This released energy is the basis of magnetic resonance imaging.

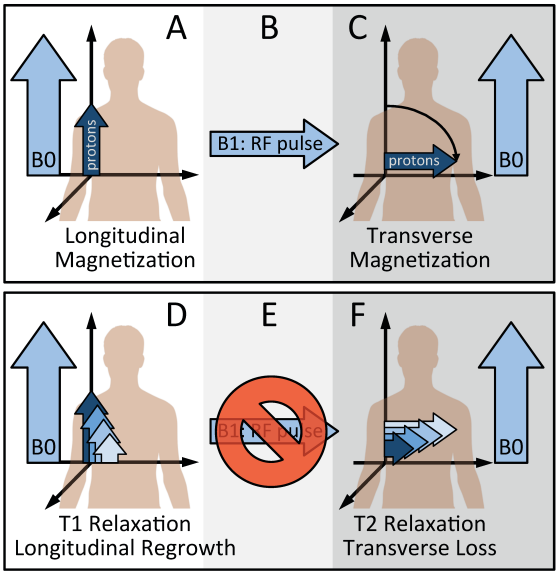

Figure 2.1: Basics of MRI physics. (A) Protons in the body align with external magnetic field B0 and achieve a state of equilibrium called longitudinal magnetization. (B) Additional energy is introduced into the system via a radio frequency (RF) pulse, called B1, orthogonal to B0. (C) The energy from the B1 pulse disrupts equilibrium and the protons fall out of alignment with B0. This disrupted equilibrium is called transverse magnetization. (E) When B1 is turned off, the misaligned protons "relax" back to the equilibrium state, that is, they realign with B0. This relaxation occurs in two forms. (D) T1 relaxation is the regrowth of longitudinal magnetization along B0. (F) T2 relaxation is the loss of transverse magnetization along B1.

The loss of transverse magnetization, T2, is called spin-spin relaxation. Each proton, also referred to as a "spin," experiences a slightly different magnetic field due to differences in its local chemical environment. For example, some protons are in water (H_2O); some protons are in

hydroxyl groups (-OH); some protons are in methyl groups ($-CH_3$); *etc*. An additional type of relaxation is due to magnetic field inhomogeneities, *e.g.*, inhomogeneities in B0. The combination of spin-spin relaxation and relaxation due to magnetic field inhomogeneities is called T2* (pronounced "T2-star"). T2* relaxation loses transverse magnetization faster than T2 relaxation.

Brief biology of functional MRI

The human brain weighs approximately 2% of body weight but it consumes approximately 20% of total glucose utilization (Clarke and Sokoloff, 1999). Glucose is the primary energy source of the brain, yet the brain does not have a local store of glucose. Glucose is delivered to the brain by the blood via vascularization. Huettel *et al.*, succinctly state, "Neuronal activity has metabolic consequences" (Huettel *et al.*, 2004). As neuronal activity increases, metabolism increases. Increased metabolism is accompanied by increased glucose utilization and increased oxygen consumption (Figure 2.2). Glucose and oxygen are replenished via increased blood supply. Blood carries oxygen more efficiently when oxygen is bound to hemoglobin. Therefore increased blood supply is accompanied by increased oxygenated hemoglobin. Although seemingly counterintuitive, increased neuronal activity with its increase in oxygen consumption, ultimately results in a net gain of oxygenated hemoglobin. This net gain is due to increased flow of oxygenated blood is greater than the initial increase in oxygen consumption.

Blood Oxygenation-Level Dependent (BOLD) MRI contrast

The functional MRI scans used in our studies are blood oxygenation-level dependent (BOLD) contrast images. This contrast takes advantage of the chemical properties of hemoglobin. Hemoglobin is made up of four subunits, each of which contains a heme group. Each heme group contains iron (Fe^{2+}). Blood carries oxygen more efficiently when oxygen binds to the

heme group, resulting in oxygenated hemoglobin. Oxygenated hemoglobin is diamagnetic whereas deoxygenated hemoglobin, with its unbound iron ion, is paramagnetic. Deoxygenated hemoglobin increases blood magnetic susceptibility effects and increases magnetic field inhomogeneities. Therefore, the amount of oxygen in blood affects a magnetic field. Hence, MRI BOLD contrast is dependent on the level of oxygen in the blood. Recall that T2* relaxation is due to magnetic field inhomogeneities. Because the level of oxygen in blood affects the magnetic field, BOLD contrast is a T2* phenomenon. The T2* BOLD signal decreases faster with increased paramagnetic deoxygenated hemoglobin. Conversely, the BOLD signal increases with increased diamagnetic oxygenated hemoglobin, as seen with increased neuronal activity (Figure 2.2).

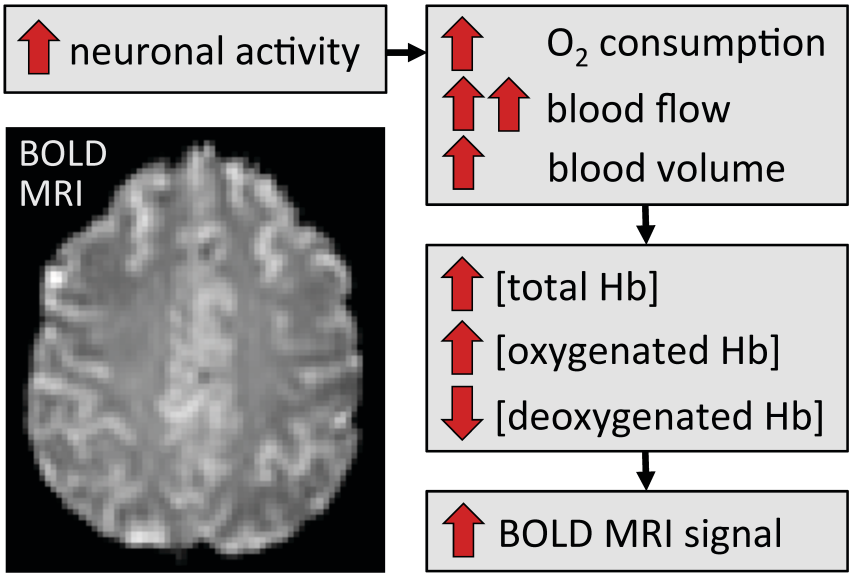

Figure 2.2: Biology of MRI. Increased neuronal activity results in increased in oxygen (O_2) consumption, increased blood flow, and increased blood volume. These increases result in increased concentration of total hemoglobin (Hb), increased oxygenated Hb, and decreased deoxygenated Hb. The blood oxygenation-level dependent (BOLD) signal increases with increased oxygenated hemoglobin.

It is important to note that BOLD MRI does not measure neuronal activity directly. Rather, BOLD contrast is a *correlate* of neural activity. While neuronal activity can be measured directly with electrodes, electrode placement is invasive. Neuronal activity can also be measured via

electroencephalography (EEG) and magnetoencephalography (MEG), which are non-invasive and have excellent temporal resolution, on the order of nanoseconds. However, EEG and MEG have poor spatial resolution. BOLD MRI is non-invasive, has good spatial resolution, but poorer temporal resolution, on the order of seconds.

BOLD functional MRI paradigm for food cue study

Functional MRI (fMRI) data is time series data, *i.e.*, data that are collected over time. For our PPI study, fMRI data of the whole brain is collected every 2 sec over 5 min (Figure 2.3A). The fMRI time series data is the intensity of the BOLD signal at a specific location in the brain acquired at each 2-sec interval (Figure 2.3B). Our PPI study uses a task-based paradigm: BOLD signals are acquired from the brain while the participant performs a particular task of interest, viewing pictures. Figure 2.3C shows a mean BOLD time course from the nucleus accumbens acquired during the food cue task. The BOLD signal acquired during this task is compared to the BOLD signal acquired during a baseline task, thereby quantifying the change in blood oxygen-ation during the task of interest. For example, we investigated the change in BOLD signal in the nucleus accumbens when children viewed images of high calorie food compared to the baseline task of viewing images of low calorie food. Cognitive tasks, such as viewing images of food, result in changes in neuronal activity and its associated increase in blood supply to brain regions such as the nucleus accumbens. This increase in blood supply results in increased oxygenated hemoglobin that is quantified as an increase in the BOLD fMRI signal.

BOLD functional MRI paradigm for resting state study

For our resting state study, fMRI data of the whole brain is collected every 1.4 sec over 9.4 min. Resting state paradigms do not include an explicit task. Instead, participants are asked to rest

quietly, typically with eyes closed, to stay awake, and to think about nothing in particular. Neuronal activity exists even in the absence of an explicit task. This neuronal activity throughout the "resting" brain is not random noise and can therefore be quantified via fluctuations in the BOLD signal. Biswal *et al.,* observed that while participants were resting quietly, BOLD signals from the motor cortex were correlated with signals in other brain regions associated with motor function (Biswal *et al.*, 1995). As a result, Biswal *et al.*, suggested that the brain, even at rest, exhibits meaningful neuronal activity and that this neuronal activity reflects inherent brain function along with its attendant networks (Fox and Raichle, 2007).

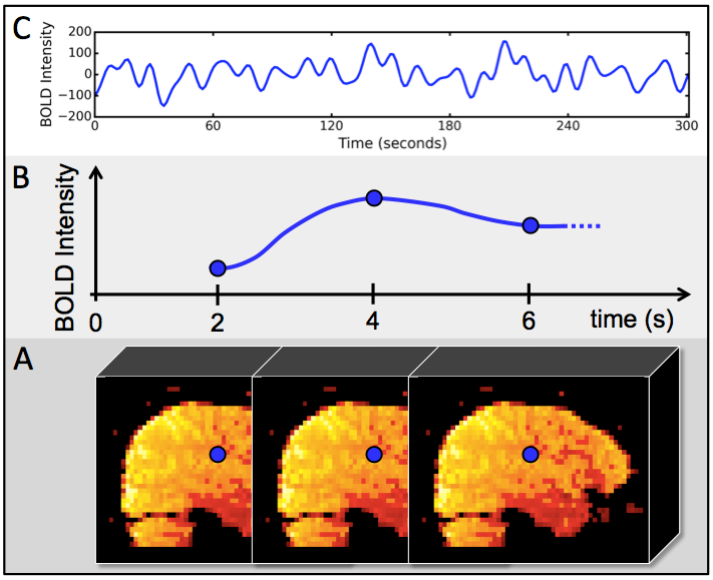

Figure 2.3: BOLD functional MRI. (A) Functional MRI (fMRI) data of the whole brain is collected every 2 sec. (B) FMRI time series data is the intensity of the BOLD signal at a specific location in the brain (blue dot) acquired at each 2-sec interval. (C) A mean BOLD time course from the nucleus accumbens acquired over 5 min.

Functional connectivity

Donald Hebb, a neuropsychologist known for his work in associative learning, put forth the idea that cells that fire together, wire together (Hebb, 2005). The underpinnings of functional connectivity exploit this idea and flip it around: cells that wire together, fire together. However, given

the spatial resolution of MRI, we cannot image individual neuronal cells. Instead we examine brain regions that are made up of hundreds of thousands of neurons. The basis of fMRI functional connectivity becomes: brain regions that wire together, fire together. Note, however, that functional connectivity can exist between brain regions that are not structurally wired together as each brain region may be wired to or influenced by a third, common region. Detailed examples are discussed below.

We quantify "fire together," and therefore functional connectivity, via statistical correlation. If the BOLD signal from Region A is strongly correlated with the BOLD signal from Region B, then we are more confident that Region A and Region B fire together and are functionally connected (Figure 2.4). However, if the BOLD signal from Region A is not correlated with the BOLD signal from Region C, then we are not confident that Region A and Region C fire together. We therefore have low confidence that Region A and Region C are functionally connected (Figure 2.4).

This method of determining functional connectivity is called seed-based correlation analysis (Cole *et al.*, 2010). In our example, Region A is the seed. We create a functional connectivity map where each location in the map corresponds to a voxel location in the brain (Figure 2.5). The values in the functional connectivity map are correlation coefficients quantified by the correlation of the BOLD signal from seed Region A with the BOLD signal from each voxel location in the brain.

Other methods that identify functional connectivity are: independent component analysis (ICA), frequency domain analysis, regional homogeneity (ReHo) analysis, and graph theoretic analysis. For excellent reviews of functional connectivity and its associated analysis methods, see Cole *et*

al. (Cole *et al.*, 2010) and van den Heuvel and Hulshoff Pol (van den Heuvel and Hulshoff Pol, 2010).

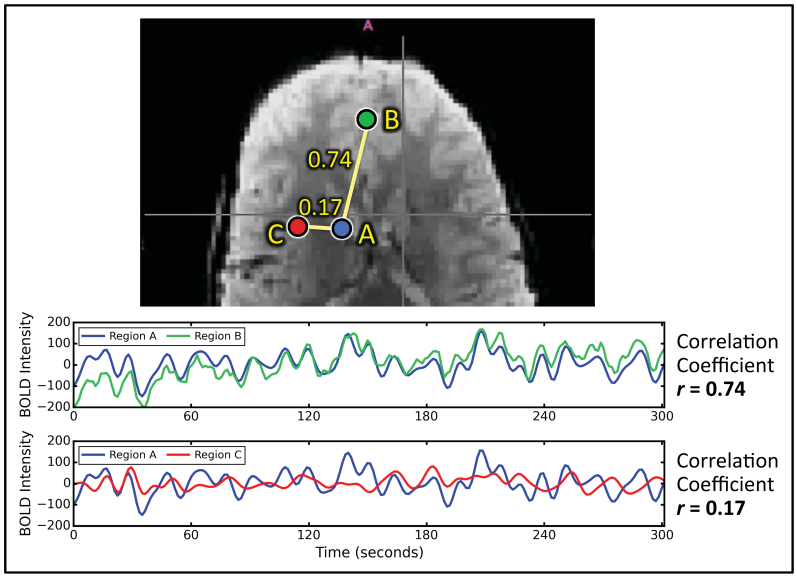

Figure 2.4: Functional connectivity. We quantify functional connectivity via the statistical correlation. Region A and Region B are strongly correlated ($r = 0.74$) and therefore functional connected. Region A and Region C (red dot; red curve) are not correlated ($r = 0.17$) and therefore not functional connected.

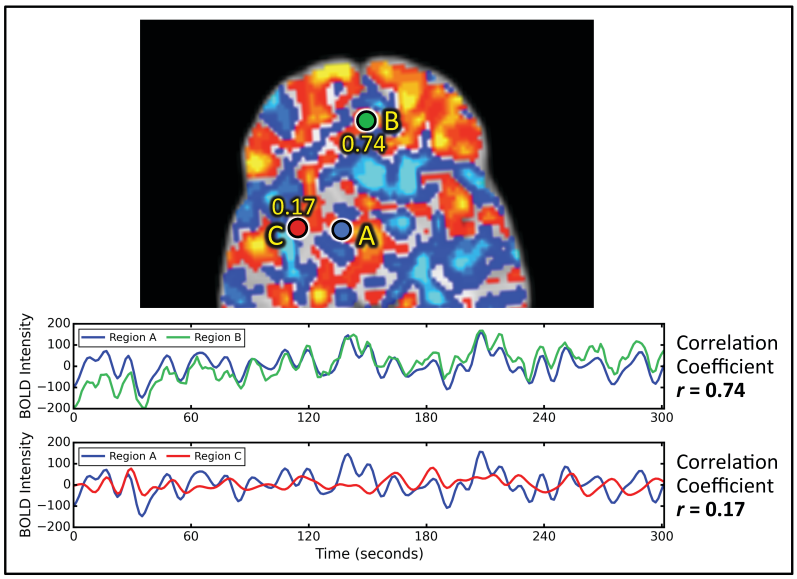

Figure 2.5: Functional connectivity map. The values in the functional connectivity map are the correlation coefficients. Region A is the seed. Regions B and C are targets. The value of voxel B is 0.74, the correlation between Region A and Region B (green dot; green curve). The value of voxel C is 0.17, the correlation between seed Region A and Region C (red dot; red curve).

Resting state functional connectivity

Resting state functional connectivity has been used to:

1. Assess interactions among brain regions;
2. Identify brain networks;
3. Detect brain disorders; and
4. Track brain development.

Resting state functional connectivity has been used to assess interactions among brain regions. Greicius *et al.*, demonstrated that resting state functional connectivity reflects structural connectivity between regions associated with episodic memory processing (Greicius *et al.*, 2009). O'Reilly *et al.*, revealed a complex relationship between functional and structural connectivity by examining resting state interhemispheric functional connectivity before and after severing the corpus callosum in rhesus monkeys (O'Reilly *et al.*, 2013). They found that corpus callosotomy significantly reduced interhemispheric functional connectivity. However, this effect was blunted if the anterior commissure was left intact, resulting in near normal functional connectivity. The work by O'Reilly *et al.*, and others (Hagmann *et al.*, 2008; Tyszka *et al.*, 2011; Hermundstad *et al.*, 2013), suggests that functional connectivity, while strongly associated with structural connectivity, is a superset of structural connectivity. That is, functional connectivity can be found between brain regions that do not have direct white matter axonal connections.

Resting state functional connectivity has also been used to identify brain networks. Beckmann *et al.*, noted functional networks that are consistent across subjects at rest (Beckmann *et al.*, 2005). Interestingly, some of these resting state functional networks resemble task-based functional networks such as the visual systems, auditory system, sensory-motor system, and regions associated with executive control (Beckmann *et al.*, 2005). Raichle *et al.*, suggested a "default mode" to describe the non-random neural activity associated with the resting state (Raichle *et al.*, 2001).

Greicius *et al.*, demonstrated decreased neural activity in the default mode network (DMN) during task-based (also called "task-positive") cognitive processing (Greicius *et al.*, 2003). The DMN is a network of brain regions that are more active during rest, when not focused on the outside world and external stimuli. The DMN has been attributed to a low-level state of watchfulness (Buckner *et al.*, 2008). The DMN has also been associated with the maintenance of one's sense of self via cognitive processes such as self-reflection, retrieving autobiographical memories, gauging the perspectives of others, daydreaming, and thinking about the future (Buckner *et al.*, 2008; Raichle, 2015).

Resting state functional connectivity has been used to detect brain disorders. Neurological or psychiatric pathology in one area of the brain can produce network dysfunction, thereby producing a unique neural signature when compared to healthy individuals. When comparing children with autism to typically developing children, Uddin *et al.*, reported increased functional connectivity during resting state within several brain networks (Uddin *et al.*, 2013). These resting state networks included the DMN, salience, motor, and visual networks. Furthermore, the authors showed that differences in resting state functional connectivity in the salience network identified children with autism from typically developing children with a classification accuracy of 78%, with 75% sensitivity, and 80% specificity. Rashid *et al.*, were able to classify individuals with schizophrenia, bipolar disorder, or as healthy controls based on features within resting state functional connectivity maps (Rashid *et al.*, 2015). Their most accurate method had a classification accuracy of 89%, with 89% sensitivity, and 94% specificity. Hafkemeijer *et al.*, reported differences in functional connectivity when comparing resting state scans from patients with Alzheimer's disease to patients with behavioral variant frontotemporal dementia (Hafkemeijer *et*

al., 2015). Both disorders are different forms of early-onset dementia with overlapping symptoms such as memory difficulties and behavioral issues.

Resting state functional connectivity has been used to track brain development. Betzel *et al.*, have demonstrated age-related changes in both functional and structural connectivity (Betzel *et al.*, 2014). They found that functional connectivity within resting state networks decrease while functional connectivity between resting state networks increases. They also reported a decrease in structural connectivity, quantified by the density and weight of white matter axon tracts, with increasing age. Qin *et al.*, reported differences in functional connectivity patterns of the basolateral amygdala (BLA) and centromedial amygdala (CMA) when comparing children with adults (Qin *et al.*, 2012). The BLA is associated with perception, evaluation, and regulation of emotionally salient stimuli. The CMA is associated with the expression of fear. They reported stronger similarity and fewer distinctions between BLA and CMA resting state functional connectivity networks in children compared to adults. They also showed greater functional connectivity between the BLA and CMA in children compared to adults.

In our resting state functional connectivity study, we investigated functional connectivity while children were quietly resting with their eyes closed. We measured functional connectivity among three brain regions: (1) inferior parietal lobe (IPL), associated with response inhibition (Garavan *et al.*, 2002; Swick *et al.*, 2011; Steele *et al.*, 2013; van Belle *et al.*, 2014); (2) frontal pole, associated with impulsivity (Coccaro *et al.*, 2007; Jimura *et al.*, 2013; Weygandt *et al.*, 2015); and (3) nucleus accumbens (NAc), associated with reward and reward-motivated behaviors (Cardinal *et al.*, 2002; Kalivas and Volkow, 2007). Chapter 4 details the methodology of this study.

Psychophysiological interaction (PPI) functional connectivity

Psychophysiological interaction (PPI) measures the change in functional connectivity between two brain regions during different psychological contexts (Friston *et al.*, 1997). PPI analysis reveals which regions have a more or less similar activity pattern, *i.e.*, functional connectivity, with the seed region *as a function of a specific task.* The physiological aspect of PPI shows that neural activity will fluctuate "in synch" if two brain regions are functionally connected. The psychological aspect of PPI shows that this "in synch" fluctuation may depend on the task.

We used a food cue task as the psychological aspect of our PPI study. We investigated the change in functional connectivity when children viewed images of high calorie food compared to the baseline task of viewing images of low calorie food. We measured the change in functional connectivity among three brain regions: (1) rostral ACC (rACC), associated with response inhibition (Goldman-Rakic, 1987; Kiehl *et al.*, 2000; Etkin *et al.*, 2006; Langenecker *et al.*, 2007; Hwang *et al.*, 2010; Goldstein and Volkow, 2011); (2) basolateral amygdala (BLA), associated with motivational drive (Talmi *et al.*, 2008; Stuber *et al.*, 2011; Britt *et al.*, 2012; Prevost *et al.*, 2012); and (3) nucleus accumbens (NAc), associated with reward and reward-motivated behaviors (Cardinal *et al.*, 2002; Kalivas and Volkow, 2007). We discuss the definition, function, and relation of these regions in Chapter 3.

We based our PPI analysis on the guidelines by O'Reilly *et al.* (O'Reilly *et al.*, 2012). To explain PPI analysis, we will hypothesize that two regions, the BLA and NAc, interact more while participants view high calorie food images compared to viewing low calorie food images. If true, we expect PPI analysis to show that the BOLD signal from the BLA will be more strongly correlated with the BOLD signal from the NAc when participants view high calorie food images,

and less correlated when viewing low calorie food images. We designated the NAc as the seed region. First, we created a psychological time course to define the high calorie *vs.* low calorie contrast, [HICAL > LOCAL]. We set this psychological time course to +1 when participants viewed high calorie food images, and set to -1 when they viewed low calorie food images (Figure 2.6A; grey sticks). Next, this [HICAL > LOCAL] psychological time course was convolved with the canonical hemodynamic response function (HRF) (Figure 2.6A; red curve). The canonical HRF is a theoretical model that characterizes the neural response to a stimulus, *e.g.*, viewing an image of food. Recall that our images are derived from BOLD contrasts where intensity values are dependent on the level of oxygen in the blood. Put simply, the canonical HRF simulates the effects of increased blood flow in response to neural activity. One characteristic of the HRF is a 4-6 sec lag, as seen in the resulting model of the psychological time course convolved with the HDF (Figure 2.6A). We then created the physiological time course by extracting the mean BOLD signal during the 5-min food cue scan from the NAc seed region (Figure 2.6B; blue curve). If the neural response of the NAc increases while viewing high calorie food images, then we would expect the NAc-based physiological time course to be correlated with the convolved [HICAL > LOCAL] psychological time course. Next, we created an interaction time course that is the term-by-term multiplication of the psychological time course with the physiological time course (Figure 2.6C; green curve).

Note that PPI analysis is correlation analysis. However we performed this correlation using the general linear model (GLM) rather than using simple correlation. Using the GLM mechanism allowed us to include additional regressors to better describe our model. Specifically, we included as regressors of no interest the time courses from which the interaction regressor was created: the mean time course from the seed region NAc and the [HICAL > LOCAL] time course.

By adding these regressors of no interest, the variance explained by the interaction regressor is over and above what is explained by the main effects of seed-related functional connectivity and the psychological task. We also included [HICAL AND LOCAL] as a regressor of no interest to explain the shared variance between HICAL and LOCAL.

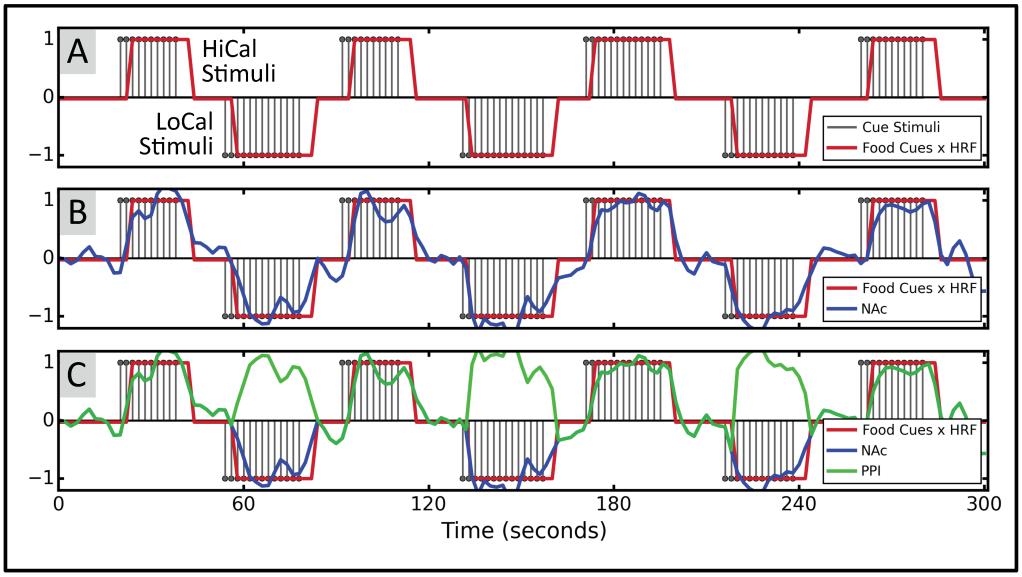

Figure 2.6: Psychophysiological interaction (PPI) term. (A) The psychological time course that describes the high calorie vs. low calorie contrast. Grey vertical sticks represent times at which food cues were displayed. We convolved the psychological time course by the canonical hemodynamic response function (HRF) to simulate the effects of increased blood flow in response to neural activity (red curve). (B) The physiological time course is the mean BOLD signal from the NAc seed region (blue curve). (C) The interaction time course (green curve) is the term-by-term multiplication of the psychological time course (red curve) with the physiological time course (blue curve).

We created a "PPI map" using GLM in which the interaction time course was designated as the seed time course. The GLM operation computed the association of this interaction time course with the BOLD signal from each voxel location in the brain. Voxels with larger correlation coefficients represent brain regions that exhibit neural responses that are in sync, *i.e.*, functionally connected, with that of the NAc when participants viewed high calorie food images.

We cannot infer directionality from functional connectivity analysis, *i.e.*, we do not know if Region A influences Region B, or vice versa. Therefore PPI functional connectivity analysis may be interpreted two ways (Friston *et al.*, 1997). One interpretation is a context-sensitive change in connectivity (Friston *et al.*, 1997). This interpretation suggests that functional connectivity between two regions, *e.g.*, BLA and NAc, is modulated by the psychological context, *e.g.*, viewing high calorie food images (Figure 2.7A). Another interpretation is the modulation of context-specific response (Friston *et al.*, 1997). This interpretation suggests that the responsiveness of the BLA to the psychological context of viewing high calorie food images is modulated by the seed region, NAc (Figure 2.7B). This implies that the BLA is modulated via afferents from the NAc. Note that both interpretations are mathematically plausible, however one interpretation may be more biologically plausible. For example, if the BLA did not receive efferent connections from the NAc, the second interpretation would be less likely.

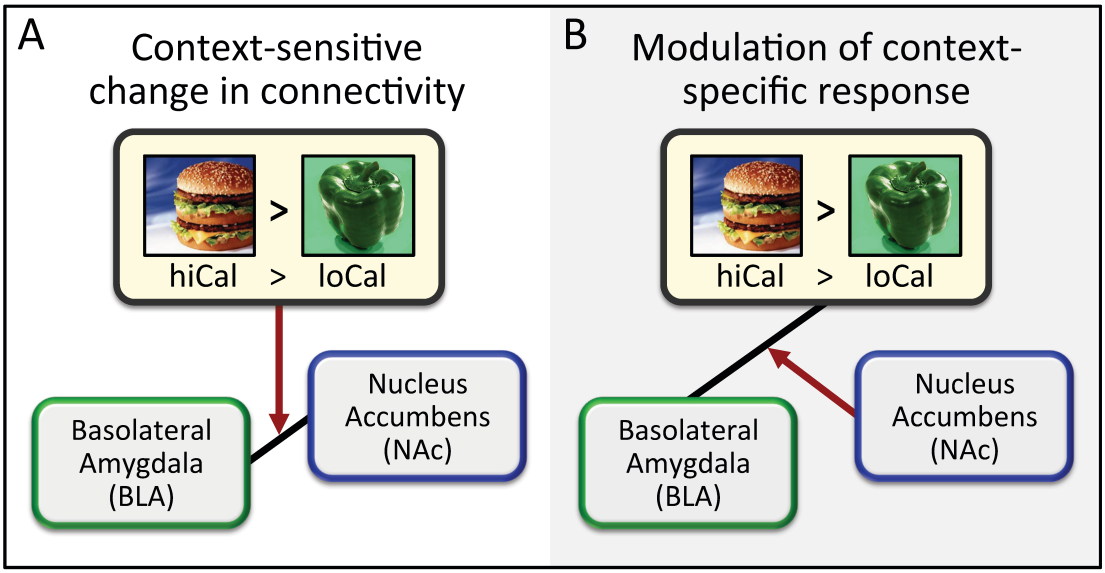

Figure 2.7: Two possible interpretations of PPI functional connectivity results. (A) Context-sensitive change in connectivity. (B) Modulation of context-specific response.

Neuroimaging of childhood obesity.

Why study children?

Neuroimaging studies among adults have contributed tremendous insight into obesity; for review see (Carnell *et al.*, 2012). However, as brain structure and function change throughout development (Luna *et al.*, 2001; Giedd, 2004), our understanding of neural mechanisms in adults may not apply to children. Indeed, neurological maturation continues into early adulthood (Giedd, 2004; Lenroot and Giedd, 2006), with the prefrontal cortex, a brain area associated with executive control, maturing later than the limbic system, associated with drive and reward (Lenroot and Giedd, 2006). The most common behaviors associated with the immature adolescent brain are impulsive behaviors.

Brief review of childhood neural activation studies

There are many foundational studies that elucidate the neural underpinnings of childhood obesity by identifying discrete brain regions. For a review of childhood obesity fMRI activation studies, see (Bruce *et al.*, 2011); for more recent studies, see (Batterink *et al.*, 2010; Yokum *et al.*, 2011; Bruce *et al.*, 2013); and for studies examining neural response to actual food intake, see (Stice *et al.*, 2008; Stice *et al.*, 2010; Stice *et al.*, 2011). Taken together, these studies have identified differences between children who are obese and healthy weight within discrete brain regions associated with response inhibition (*e.g.*, anterior cingulate cortex, inferior parietal lobe), impulsivity (*e.g.*, inferior frontal gyrus, superior frontal gyrus), motivation (*e.g.*, amygdala), and reward (*e.g.*, striatum, orbitofrontal cortex, insula).

Review of childhood functional connectivity studies

However, the brain is made up of networks of brain regions (Seeley *et al.*, 2009). Functional connectivity analyses generate inferences about brain networks thus providing new insight into the communication and organization of the brain (van den Heuvel and Hulshoff Pol, 2010). There are many functional connectivity studies comparing adults who are obese with healthy weight adults (Stoeckel *et al.*, 2009; Kullmann *et al.*, 2012; Nummenmaa *et al.*, 2012; Garcia-Garcia *et al.*, 2013a; Garcia-Garcia *et al.*, 2013b; Kullmann *et al.*, 2013; Carnell *et al.*, 2014; Tuulari *et al.*, 2015). However, to date there are only three functional connectivity studies investigating childhood obesity, reviewed below.

Olde Dubbelink, *et al.*, examined resting state functional connectivity in girls, ages 9-12 years, using magnetoencephalography (MEG) (Olde Dubbelink *et al.*, 2008). They reported increased synchronization in the delta and beta frequency bands among girls who were severely obese compared to healthy weight girls. Using fMRI, Zhang *et al.*, examined resting state functional connectivity among children with Prader-Willi syndrome compared to their healthy weight siblings (Zhang *et al.*, 2013). They reported decreased functional connectivity in the default mode network and the motor sensory network, and both increased and decreased functional connectivity in the prefrontal cortex network among children with Prader-Willi syndrome. Also using fMRI, Black *et al.*, examined resting state functional connectivity among severely obese children compared to healthy weight children (Black *et al.*, 2014). They reported increased functional connectivity among regions associated with cognitive control and reward anticipation among children who were obese. We discuss our results in relation to these studies in Chapter 5.

All three studies investigated resting state functional connectivity. All three studies compared children who were severely obese with healthy weight children. No studies looked at children across a continuous range of adiposity, which includes children who are overweight, as we do in our resting state functional connectivity study. No studies examined task-based PPI functional connectivity as we do in our food cue task-based study.

Neurosynth.

Overview of Neurosynth's meta-analysis technique

We used Neurosynth (`neurosynth.org`) (Yarkoni *et al.*, 2011) to identify *a priori* brain regions associated with phenotypes of interest, *e.g.*, impulsivity, response inhibition, or motivation. Neurosynth is an aggregator that automatically synthesizes results from published neuroimaging studies, performs automated meta-analyses, and creates probabilistic mappings between phenotypes and neural states (Yarkoni *et al.*, 2011).

An overview of Neurosynth's aggregator function, built on text mining and machine learning, is as follows (Figure 2.8). First, Neurosynth extracts coordinates of peak neural activity from published neuroimaging papers (Figure 2.8A). For each peak coordinate, Neurosynth creates an "activation map" where each voxel in the brain is set to 0 or 1. A voxel is set to 1 if it is within some user-defined distance from the peak activation, e.g., 10 mm from the extracted peak coordinate. Otherwise, the voxel is set to 0. Next, Neurosynth parses text from these published papers and identifies terms of high frequency, e.g., "response inhibition" (Figure 2.8B). Neurosynth then generates a list of terms that occur in 20+ papers (Figure 2.8C). For each paper, Neurosynth creates a "feature map" of these terms where each cell is set to 0 or 1 (Figure 2.8D). A cell is set to 1 if the paper contains the term or 0 if it does not.

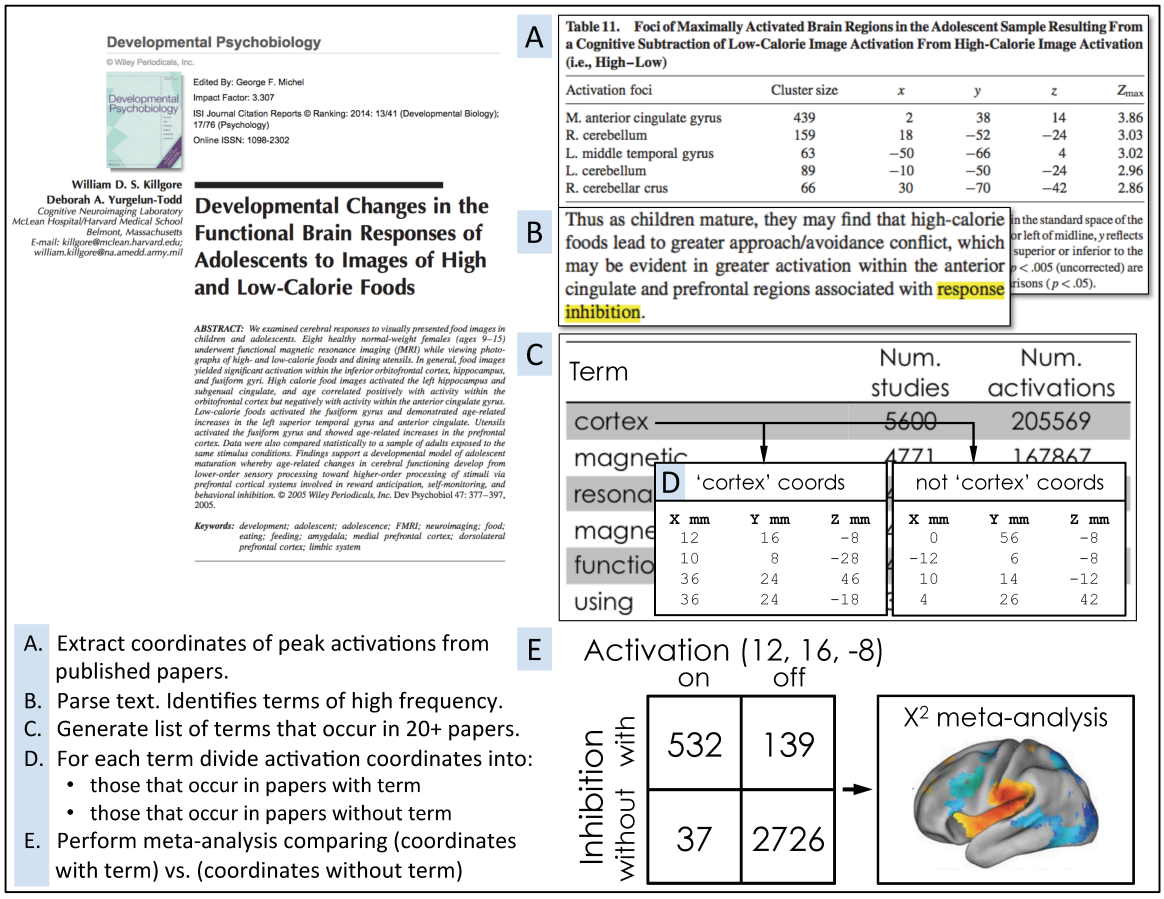

Figure 2.8: Overview of Neurosynth's aggregator function.

Neurosynth then creates a 2·2 contingency table of counts for each term for each voxel (Figure 2.8E). The count is the number of papers that have "activity" at this voxel for this term. With this table, Neurosynth performs a chi-squared test of independence comparing (coordinate with term) *vs.* (coordinate without term). If statistically significant, then a dependency exists between the activity at that coordinate and the term. From this statistical analysis, Neurosynth provides both forward and reverse inference maps. The forward inference map indicates the probability of activation in a particular brain region given a particular term, *i.e.*, $Pr(activation|term)$. The reverse inference map indicates the probability of finding a specific term given activation a particular brain region, *i.e.*, $Pr(term|activation)$. The reverse inference map provides inference about which brain regions are selectively, and not just consistently, associated with a particular term.

Use of Neurosynth

We investigated the functional connectivity between regions associated with specific phenotypes: response inhibition, impulsivity, motivation, and reward. We performed Neurosynth meta-analyses to identify neural regions associated with these phenotypes. We then localized regions of interest via Neurosynth's resulting inference maps. Using the resulting inference map, we selected a voxel located at a peak *z*-score and created a spherical ROI with radius 5 mm (volume = 648 mm^3 = 81 voxels).

Of note, Neurosynth does not allow for additional filters in the specification of its meta-analyses, such as limiting its analyses to "children-only" studies. However, its resulting inference maps are in Montreal Neurological Institute (MNI)-space, a common coordinate system used in MRI brain image analysis. We spatially normalized the brain scans of our participants to a child-specific brain template (Fonov *et al.*, 2009; Fonov *et al.*, 2011), also in MNI-space, thereby allowing the use of Neurosynth results in our cohort of children. As noted previously, our understanding of adult neurofunctionality may not apply to children. However, using functional regions identified by Neurosynth is one way to contribute to the limited study of functional connectivity in childhood obesity by objectively building upon the vast corpus of neuroimaging research.

Research model and neural model.

The aim of our research was to better understand neural functional connectivity between regions of the brain associated with non-homeostatic eating in relation to eating habits and adiposity among children (Figure 2.9, upper). We therefore defined a neural model comprised of three *a priori*-defined regions. For our PPI functional connectivity analysis, the three regions were: (1) rostral anterior cingulate cortex (rACC), associated with response inhibition (Goldman-Rakic,

1987; Kiehl *et al.*, 2000; Etkin *et al.*, 2006; Langenecker *et al.*, 2007; Hwang *et al.*, 2010; Goldstein and Volkow, 2011); (2) basolateral amygdala (BLA), associated with motivational drive (Talmi *et al.*, 2008; Stuber *et al.*, 2011; Britt *et al.*, 2012; Prevost *et al.*, 2012); and (3) nucleus accumbens (NAc), associated with reward and reward-motivated behaviors (Cardinal *et al.*, 2002; Kalivas and Volkow, 2007) (Figure 2.9; lower). The selection and function of these regions are detailed in Chapter 3.

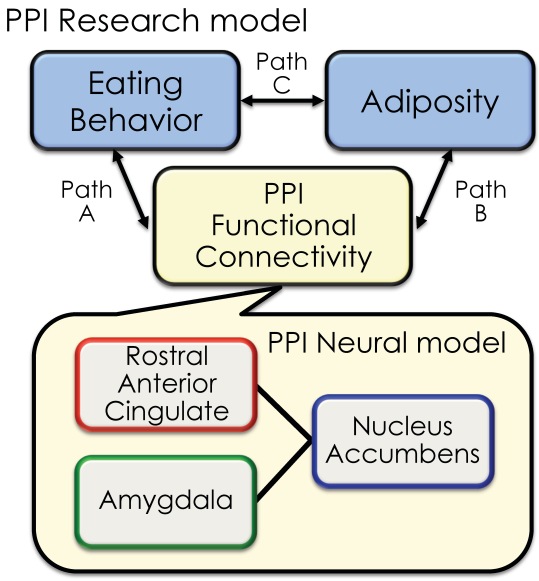

Figure 2.9: Research and neural models for psychophysiological interaction (PPI) study. Upper: Research model showing relationships among PPI functional connectivity, eating behavior, and adiposity. Lower: Neural model made up of three regions: (1) rostral anterior cingulate cortex; (2) amygdala; and (3) nucleus accumbens.

For our resting state functional connectivity analysis, the three regions we examined were: (1) inferior parietal lobe (IPL), associated with response inhibition (Garavan *et al.*, 2002; Swick *et al.*, 2011; Steele *et al.*, 2013; van Belle *et al.*, 2014); (2) frontal pole, associated with impulsivity (Coccaro *et al.*, 2007; Jimura *et al.*, 2013; Weygandt *et al.*, 2015); and (3) nucleus accumbens (NAc), associated with reward and reward-motivated behaviors (Figure 2.10; lower). The selection and function of these regions are detailed in Chapter 4.

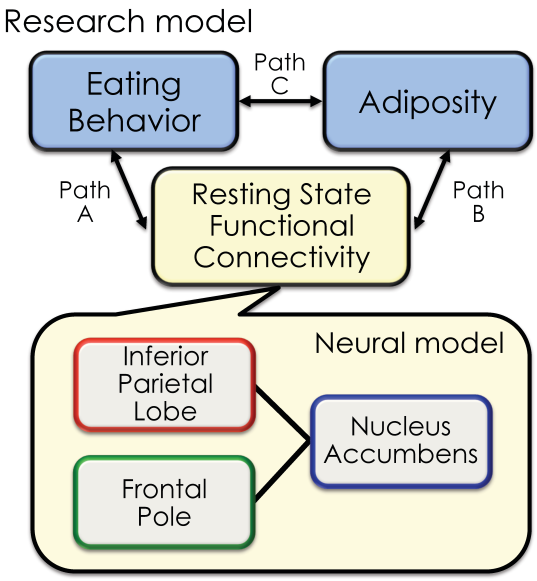

Figure 2.10: Research and neural models for resting state study. Upper: Research model showing relationships among resting state functional connectivity, eating behavior, and adiposity. Lower: Neural model made up of three regions: (1) inferior parietal lobe; (2) frontal pole; and (3) nucleus accumbens.

Mediation analysis.

Since changing one's eating habits alone does not typically produce long-lasting weight loss, we hypothesized that neurobiological factors are also at play. Specifically, we posit that functional connectivity within a brain network mediates the relationship between eating behaviors and adiposity. Statistically, a mediator helps explain *how* or *why* a relationship exists between two variables (Baron and Kenny, 1986). In contrast, a moderator explains *when* certain effects will occur, thereby affecting the strength or direction of a relationship (Baron and Kenny, 1986).

Because robust mediation analysis requires large sample sizes (Fritz and MacKinnon, 2007), we did not perform mediation analysis in our food cue task PPI functional connectivity analysis data. However, we did perform mediation analysis in our resting state functional connectivity data as an exploratory analysis, in advance of additional data. Mediation can be assessed when statistically significant relationships are found in all three Paths A, B, and C, shown in Figure

2.10, where the relationships between pairs of pathways are adjusted for the third pathway (Taylor and MacKinnon, 2012; Valeri and VanderWeele, 2013). If our brain network is a mediator, then the association between eating behavior and adiposity (Path C, Figure 2.10) will decrease after controlling for the effects of neural resting state functional connectivity (Baron and Kenny, 1986).

For our mediation analysis model, we selected eating behavior as the independent variable, *X*; adiposity as the dependent variable, *Y*; and resting state functional connectivity as the mediator, *M* (Figure 2.11). Note that because we do not know if preexisting neural conditions predispose an individual toward unhealthy eating behaviors and/or increased adiposity, or if increased adiposity modifies the brain to promote unhealthy eating behaviors, or some combination of both, our designation of each variable is subjective. The solid blue arrows along Path A and Path B in Figure 2.11 depict the indirect relationship between eating behaviors and adiposity as mediated by neural functional connectivity. In mediation terminology, this indirect relationship is also called the (*ab*) path. Path C (Figure 2.11; dotted black line) is the relationship between eating behaviors and adiposity when *not* controlling for the contribution from functional connectivity. However, Path C', not shown, is the relationship between eating behaviors and adiposity after controlling for, and thereby removing, the contribution from functional connectivity. In mediation parlance, Path C' is called the direct relationship. If neural functional connectivity is a mediator, then the relationship along Path C' will be less than the relationship along Path C.

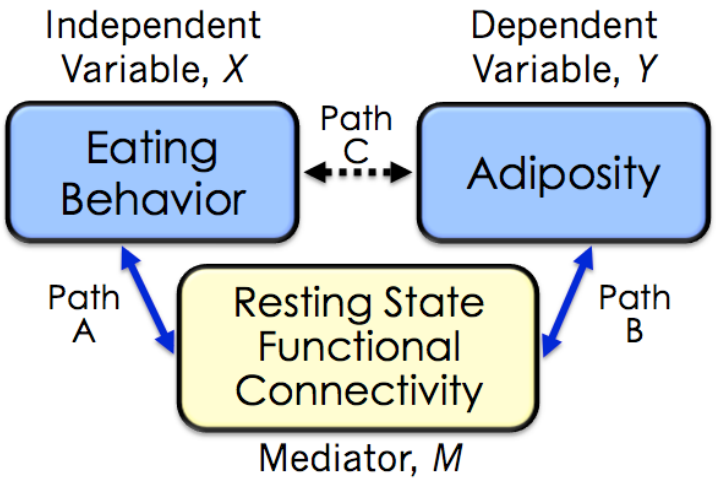

Figure 2.11: Mediation analysis model. Eating behavior is the independent variable, *X*; adiposity is the dependent variable, *Y*; and resting state functional connectivity is the mediator, *M*. The combination of Path A and Path B (solid blue lines) represent the indirect relationship between eating behaviors and adiposity as mediated by functional connectivity. Path C (dotted black line) represents the relationship between eating behaviors and adiposity when not controlling for functional connectivity.

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CHAPTER 3

Differences in Response Inhibition-Associated and Motivational Drive-Associated Functional Connectivity in Childhood Obesity: A Psychophysiological Interaction Functional Connectivity Study

ABSTRACT

Background and Hypothesis

Childhood obesity in the US has nearly doubled over the past 30 years; among adolescents obesity has tripled. Given critical differences in neural function between adults and children, we studied children to better understand the developing neurobiology of obesity. Successful long-term weight loss may be undermined by non-homeostatic eating. Non-homeostatic eating is influenced by impulsive drive to eat and inhibition of this drive. We hypothesized that unhealthy eating habits and overeating are associated with disrupted neural functional connectivity.

Methods

We used psychophysiological interaction (PPI) functional connectivity analysis to quantify brain network integrity between brain regions associated with response inhibition (rostral anterior cingulate cortex), motivational drive (basolateral amygdala), and reward (nucleus accumbens). We acquired functional magnetic resonance images (fMRI) from 34 children (female = 16; obese = 17; mean age = 10.3 [std = 1.3] years) at 3 Tesla while viewing high calorie and low calorie food images. Visual food cues affect eating behavior, and more so for children who are obese. We assessed the relationships of functional connectivity with external and restrained eating

behaviors, as measured by the Dutch Eating Behaviour Questionnaire for Children (DEBQ-C), and with adiposity, quantified by BMI *z*-score.

Results

Our results suggest that ineffective response inhibition-associated PPI functional connectivity, when viewing high calorie compared to low calorie food images, is characteristic of obesity in children, ages 8-12 years old. Furthermore, response inhibition-associated functional connectivity, more so than motivational drive-associated functional connectivity, may be a key functional difference between children who are obese compared to healthy weight children. For example, among children who are healthy weight, decreased external eating was associated with increased response inhibition-associated PPI functional connectivity. However, among children who are obese, increased external eating was associated with increased response inhibition-associated PPI functional connectivity, suggesting that integration of response inhibition-associated PPI functional connectivity is ineffective. There were no significant associations between external eating and motivational drive-associated PPI functional connectivity for either weight class.

Conclusions

These findings suggest that, in addition to changing eating habits and physical activity, strategies that overcome altered neural functional connectivity which influence non-homeostatic eating are needed to maintain a healthy weight status. Strengthening response inhibition-associated functional connectivity may contribute to novel, efficacious obesity treatment.

Background.

Homeostatic control of eating behavior is largely regulated by the hypothalamus and brainstem (Schneeberger *et al.*, 2014). However, eating is also a reward-mediated behavior driven, in part, by the balance between motivational drive to eat and inhibition of this drive. Brain regions associated with motivational drive, response inhibition, and reward, are increasingly recognized as potent modulators of non-homeostatic eating habits (Shin and Berthoud, 2013). Our overarching hypothesis is that increased motivational drive to eat and/or decreased inhibition of this drive is associated with obesity.

Additional non-homeostatic factors that influence adiposity status include environmental (LeBlanc *et al.*, 2015), genetic (Early Growth Genetics (EGG) Consortium, 2012), cultural (Pena *et al.*, 2012), and familial (Latzer and Stein, 2013) factors. One such environmental factor, visual food cues, is prevalent in the daily lives of children via the internet, television, and print media. Exposure to food cues increases food intake (Polivy and Herman, 2014). Moreover, children who are overweight have a stronger behavioral response to food cues than do healthy weight children, leading them to eat more following cue exposure (Jansen *et al.*, 2003). Given that visual food cues affect eating behavior, a visual food cue task is a powerful and ecologically valid method used to probe putative differences in the communication and organization of the obese brain.

Research model

The aim of this study was to better understand neural functional connectivity in childhood obesity in response to viewing images of high calorie food compared to low calorie food. Functional connectivity is at the forefront of neuroimaging analysis, however its use in discovering organizational principles underlying brain function remains largely untapped in

regard to childhood obesity. Because functional connectivity can vary as a function of task (Friston *et al.*, 1997), psychophysiological interaction (PPI) analysis assesses whether the functional connectivity between two brain regions changes from one task to another. We used PPI functional connectivity analysis to examine the relationship of adiposity status, eating behaviors, and brain functional connectivity within the context of a visual food cue task (Figure 3.1; upper). To the best of our knowledge, there are no other published studies that investigate childhood obesity using PPI functional connectivity analysis.

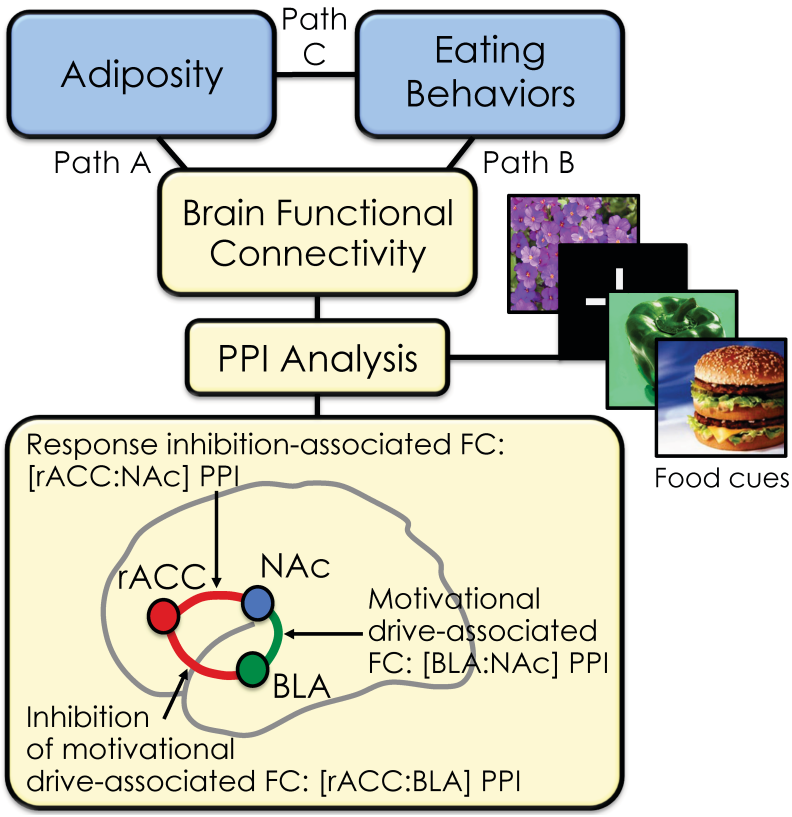

Figure 3.1: Model to probe relationship of adiposity status, eating behaviors, and brain functional connectivity. Upper: Path A: relationship between functional connectivity and adiposity status; Path B: relationship between functional connectivity and eating behaviors; Path C: relationship between adiposity status and eating behaviors. Lower: Neural model included: (1) rostral anterior cingulate cortex (rACC), associated with response inhibition; (2) basolateral amygdala (BLA), associated with motivational drive; and (3) nucleus accumbens (NAc), associated with reward-motivated behaviors. PPI: psychophysiological interaction; [rACC:NAc] PPI: functional connectivity between rACC and NAc; [rACC:BLA] PPI: functional connectivity between rACC and BLA; and [BLA:NAc] PPI: functional connectivity between BLA and NAc.

Functional neural model

We defined a functional neural model comprised of three *a priori*-defined regions (Figure 3.1; lower): (1) basolateral amygdala (BLA), associated with motivational drive (Talmi *et al.*, 2008; Stuber *et al.*, 2011; Britt *et al.*, 2012; Prevost *et al.*, 2012); (2) rostral anterior cingulate cortex (rACC), associated with response inhibition (Goldman-Rakic, 1987; Kiehl *et al.*, 2000; Etkin *et al.*, 2006; Langenecker *et al.*, 2007; Hwang *et al.*, 2010; Goldstein and Volkow, 2011); and (3) the nucleus accumbens (NAc), associated with reward-motivated behaviors (Cardinal *et al.*, 2002; Kalivas and Volkow, 2007). The NAc also integrates inputs from the prefrontal cortex (PFC) and limbic regions (Mogenson *et al.*, 1980; Goto and Grace, 2008; Floresco, 2015). There are direct glutamatergic projections from the amygdala to the NAc and from the rACC to the NAc as well as reciprocal glutamatergic projections between the rACC and amygdala (Cardinal *et al.*, 2002; Kalivas and Volkow, 2007).

Motivational drive is the degree to which one wants and chooses to engage in a particular behavior (Mitchell, 1982). In mice, optical stimulation of the pathway from BLA to NAc increased motivational drive for self-stimulated sucrose delivery (Stuber *et al.*, 2011) and self-stimulation of the NAc (Britt *et al.*, 2012). Pavlovian-instrumental transfer (PIT) demonstrates that Pavlovian conditioning transfers motivational significance onto instrumental conditioning (Talmi *et al.*, 2008). An fMRI PIT paradigm among humans showed neural response in BLA increased as PIT effects increased, that is, the instrumental condition was performed more often in the presence of the conditioned stimulus than in its absence (Talmi *et al.*, 2008; Prevost *et al.*, 2012). These studies suggested that increased BLA neural response is associated with increased behavioral motivation. Temple *et al.*, reported that motivation to work for food, as measured by performance on an operant response computer game that used a progressive ratio schedule of

reinforcement, among overweight 8-12 year old children was greater compared to their healthy weight peers (Temple *et al.*, 2008). Working with even younger children, Rollins *et al.*, similarly showed that increased motivation to work for food was associated with increased weight among preschoolers, ages 3-5 years (Rollins *et al.*, 2014). In a 1-year longitudinal study of children ages 7-10 years, Hill *et al*., showed that increased motivation to work for food was predictive of increased weight gain (Hill *et al.*, 2009). Taken together, this evidence suggests that increased adiposity is associated with increased motivational drive, which in turn is associated with increased neural response in the BLA.

Response inhibition is the ability to suppress an inappropriate and/or unwanted action that would otherwise interfere with one's goals (Barratt *et al.*, 1994; Mostofsky and Simmonds, 2008). Hest and Garavan reported that cocaine users, compared to drug-naïve controls, showed decreased neural activity in the rACC with decreased response inhibition during a Go-No Go task (Hester and Garavan, 2004). Similarly, Li *et al.*, reported that male cocaine users, compared to healthy controls, showed decreased neural activity in rACC with decreased response inhibition during a Stop Signal Task (Li *et al.*, 2007). These studies suggest that decreased neural response in the rACC is associated with decreased response inhibition. Studies have shown that increased BMI is associated with decreased response inhibition in children via the Stop Signal task (Nederkoorn *et al.*, 2006), the Child Behavior Questionnaire (Anzman and Birch, 2009), and the Go-No Go task (Batterink *et al.*, 2010; Kamijo *et al.*, 2012a; Kamijo *et al.*, 2012b). Taken together, this evidence suggests that increased adiposity is associated with decreased response inhibition, which in turn is associated with decreased neural response in the rACC.

FMRI studies have shown that increased activity in the rACC is associated with a reduction in amygdala activity during an emotional conflict Stroop task (Etkin *et al.*, 2006; Egner *et al.*,

2008); among individuals with post-traumatic stress disorder (Etkin and Wager, 2007); among individuals with phobias in response to phobia-related images (Hermann *et al.*, 2007); and in individuals with phobias compared to non-phobic controls (Schienle *et al.*, 2007). This modulatory relationship is consistent with studies in rats demonstrating that the stimulation of the prefrontal cortex decreases amygdala activity (Quirk *et al.*, 2003) and that the inactivation of the rACC enhances amygdala-dependent fear-conditioned learning (Bissiere *et al.*, 2008). This evidence suggests that increased neural response in the rACC decreases neural response in the amygdala.

The NAc is associated with reward-motivated behaviors and integrates input from PFC and limbic regions. Studies have demonstrated the importance of the NAc in reward-motivated behaviors such as response inhibition and motivational drive. For example, in rats, Terregrossa and Taylor determined that the influence of the ACC in cocaine cue extinction was due to its projections to the NAc (Torregrossa *et al.*, 2013). As noted previously, optical stimulation of the pathway from BLA to NAc increased motivational drive for self-stimulated reward (Stuber *et al.*, 2011; Britt *et al.*, 2012) in mice.

In light of the above evidence, we designated the NAc as a region that mediates reward-motivated behaviors (Figure 3.1). We designated the BLA as a region that produces motivational drive to eat via direct projections to the NAc. And we designated the rACC as a region that inhibits response to eating via two proposed mechanisms by: (1) directly inhibiting reward-motivated behaviors via projections to the NAc; and (2) indirectly inhibiting motivational drive via projections to the BLA which, in turn, projects a "weaker input" to the NAc. Our model highlights the considerable influence that response inhibition may contribute to adiposity status.

Hypotheses.

Overarching hypothesis

Our overarching hypothesis is that increased motivational drive to eat and/or decreased inhibition is associated with obesity. That is, it is the imbalance between drive to eat and inhibition of over-eating that determines, in part, feeding behavior and resultant adiposity status. Our specific hypotheses are informed by the premise that increased functional connectivity reflects increased functional integration (Friston *et al.*, 1997). We hypothesized that increased response inhibition-associated functional connectivity between rACC and NAc, or between rACC and BLA, will be associated with decreased unhealthy eating habits and decreased adiposity. We also hypothesized that increased motivational drive-associated functional connectivity between BLA and NAc will be associated with increased unhealthy eating habits and increased adiposity.

We investigated the functional connectivity of three circuits within our neural model (Figure 3.1): (1) motivational drive-associated PPI functional connectivity between BLA and NAc, denoted as [BLA:NAC] PPI; (2) response inhibition-associated PPI functional connectivity between rACC and NAc, denoted as [RACC:NAC] PPI; and (3) inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA, denoted as [RACC:BLA] PPI. We used an ecologically valid food cue paradigm, which approximates the real-life experience of viewing pictures of food, to probe the functional connectivity between these regions. Additionally, we quantified relationships between functional connectivity by adiposity status with eating behaviors as empirical corroboration of our hypotheses.

We formed a series of testable hypotheses, depicted in Figure 3.2.

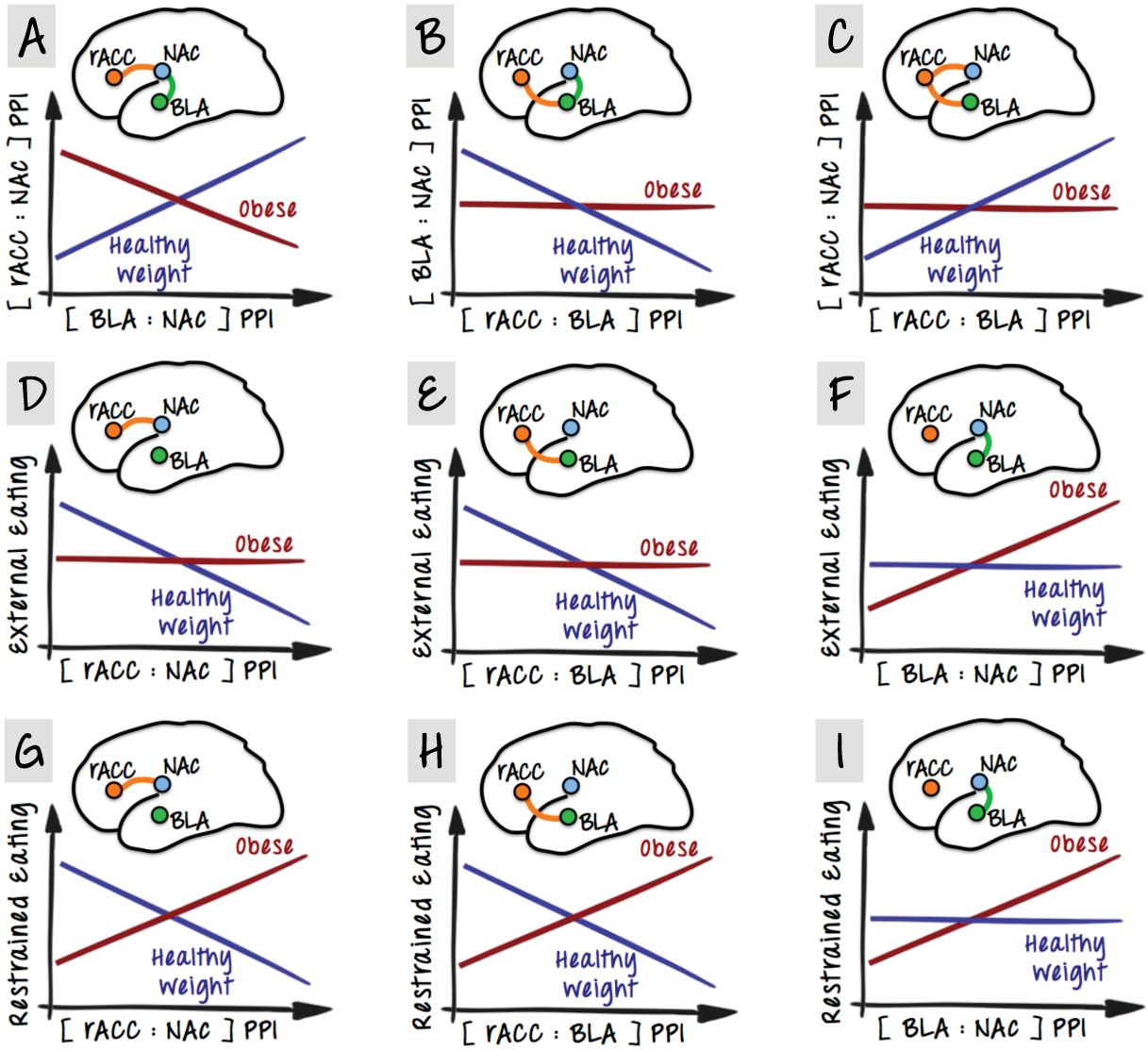

Figure 3.2: Hypotheses. (A) Relationship of [rACC:NAc] *vs.* [BLA:NAc] PPI functional connectivity by adiposity status; (B) Relationship of [BLA:NAc] *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (C) Relationship of [rACC:NAc] *vs.* [rACC:BLA] PPI functional connectivity by adiposity status. (D) Relationship of external eating scores *vs.* [rACC:NAc] PPI functional connectivity by adiposity status; (E) Relationship of external eating scores *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (F) Relationship of external eating scores *vs.* [BLA:NAc] PPI functional connectivity by adiposity status; (G) Relationship of restrained eating scores *vs.* [rACC:NAc] PPI functional connectivity by adiposity status; (H) Relationship of restrained eating scores *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (I) Relationship of restrained eating scores *vs.* [BLA:NAc] PPI functional connectivity by adiposity status. PPI: psychophysiological interaction; NAc: nucleus accumbens; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala.

Hypotheses about PPI functional connectivity and adiposity status (Figure 3.1 Path A and Figures 3.2 A-C)

We hypothesized that among children who are healthy weight (HW), in response to viewing food cues, increasing [BLA:NAc] PPI will be associated with a concomitant increasing [rACC:NAc] PPI (Figure 3.2A). In other words, response inhibition-associated functional connectivity will keep pace with motivational drive-associated functional connectivity among HW. In contrast, we hypothesized that among children who are obese (OB), increasing [BLA:NAc] PPI will be associated with decreasing [rACC:NAc] PPI. In other words, among OB, response inhibition-associated functional connectivity will not only fail to keep pace with motivational drive-associated functional connectivity, but it will be blunted.

When considering functional connectivity between rACC and BLA, we hypothesized that among HW, in response to viewing food cues, increased [rACC:BLA] PPI will be associated with decreased [BLA:NAc] PPI (Figure 3.2B). While, among OB, increased [rACC:BLA] PPI would have no significant association with [BLA:NAc] PPI as we speculate that this functional system of checks and balances is faulty. Continuing to consider increased [rACC:BLA] PPI, we hypothesized that [rACC:NAc] PPI would increase among HW, whereas among OB, [rACC:NAc] PPI would have no significant association (Figure 3.2C).

Hypotheses about PPI functional connectivity and external eating behaviors (Figure 3.1 Path B and Figures 3.2 D-F)

Unhealthy eating behaviors include external eating, defined as eating in response to the sight or smell of food. Increased BMI is associated with an increase in external eating (Braet and van Strien, 1997; Burton *et al.*, 2007). In turn, increased external eating is associated with decreased response inhibition (Jasinska *et al.*, 2012) and increased motivational drive (Nijs *et al.*, 2009).

We therefore hypothesized that, among HW, increased response inhibition-associated [RACC:NAC] PPI, and increased [RACC:BLA] PPI, will be associated with decreased external eating (Figures 3.2D and 3.2E). We also hypothesized that, among OB, response inhibition-associated functional connectivity is ineffective. Therefore we posited that no statistical relationship between [RACC:NAC] and [RACC:BLA] PPI and external eating (Figures 3.2D and 3.2E). However, we hypothesized that, among HW, external eating will not be significantly associated with increased [BLA:NAC] PPI, whereas, among OB, external eating will increase with increased [BLA:NAC] PPI (Figure 3.2F).

Hypotheses about PPI functional connectivity and restrained eating behaviors (Figure 3.1 Path B and Figures 3.2 G-I)

Restrained eating is defined as eating less to lose or maintain weight. Counterintuitively, increased restrained eating is associated with increased BMI (Braet and van Strien, 1997; Provencher *et al.*, 2003). This non-intuitive relationship is believed to be due to an eventual loss of restraint thereby leading to disinhibited overeating (Shunk and Birch, 2004). Restrained eaters also showed decreased response inhibition compared to healthy controls during the Stop Signal Task (Nederkoorn *et al.*, 2004). Taken together, these studies suggest that increased BMI is associated with increased restrained eating which, in turn, is associated with decreased response inhibition. We hypothesized that, among HW, increased [RACC:NAC] PPI, and increased [RACC:BLA] PPI, will be associated with decreased restrained eating (Figures 3.2G and 3.2H). However, among OB, we hypothesized that increased restrained eating will be associated with increased [RACC:NAC] PPI and increased [RACC:BLA] PPI, suggesting ineffective response inhibition (Figures 3.2G and 3.2H). Furthermore, we hypothesized that, among HW, restrained eating will not be significantly associated with increased [BLA:NAC] PPI (Figure 3.2I).

However, among OB, we hypothesized that restrained eating will increase with increased [BLA:NAC] PPI because increasing motivational drive to eat is associated with increased BMI, which, in turn, is associated with increased restrained eating.

Hypothesis about adiposity status and eating behaviors (Figure 3.1 Path C)

In agreement with previous studies (Braet and van Strien, 1997; Shunk and Birch, 2004; Braet *et al.*, 2008; van Strien and Oosterveld, 2008) we hypothesized that children who are obese will exhibit increased external eating and increased restrained eating habits compared to healthy weight children.

Materials and Methods.

Participants

Thirty-four children, in the age range [8-12] years old, were selected from a larger observational study of childhood obesity investigating how the brain influences appetite in children who are healthy weight or obese. All children assented to the study. A legal guardian provided written informed consent after receiving a written description of this study. Participants received financial compensation. This study was approved by the Institutional Review Board at Vanderbilt University and conformed to the provisions of the Declaration of Helsinki (World Medical Association, 2013). All participants had normal visual acuity. Only right-handed participants were enrolled to minimize laterality variations in brain function. Girls were included only if they had not yet reached menarche. All subjects were screened for psychiatric, neurological, chronic medical illnesses, and for MRI safety considerations. All participants ate a standardized meal approximately 30 min prior to MRI scanning. Each participant rated his or her hunger prior to scanning using a visual analog scale.

Adiposity and weight status

We used BMI *z*-scores as a proxy measure for childhood adiposity. Only children who were healthy weight or obese were enrolled in the study; children who were overweight were excluded. Children were classified as healthy weight for (-1.64 ≤ BMI *z*-scores < 1.04); and obese for (BMI *z*-scores ≥ 1.64) (Wang and Chen, 2012). We calculated an age- and sex-specific BMI *z*-score for each child using LMS transformation parameters *lambda*, *mu*, and *sigma* (CDC; Kuczmarski *et al.*, 2002).

Eating behaviors

We used the Dutch Eating Behaviour Questionnaire for Children (DEBQ-C) to measure two aspects of eating behavior: external eating and restrained eating (van Strien and Oosterveld, 2008; van Strien *et al.*, 2012). External eating is eating in response to the sight or smell of food. Restrained eating is eating less to lose or maintain weight. In our study, we explored the relationship of external eating and restrained eating with brain functional connectivity and weight status.

Functional neural model

ROSTRAL ANTERIOR CINGULATE CORTEX (RACC) / RESPONSE INHIBITION.

We used Neurosynth (`neurosynth.org`) (Yarkoni *et al.*, 2011) to identify an *a priori* rACC region associated with response inhibition. We performed a Neurosynth meta-analysis for the term "response inhibition" from 176 studies (Figure 3.3A). We located the rACC in the resulting forward inference map and noted the MNI-coordinate of the peak *z*-score. We created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]) in the right rACC centered on a peak *z*-score of 5.84 at (6, 42, 12) in Montreal Neurological Institute (MNI)-space.

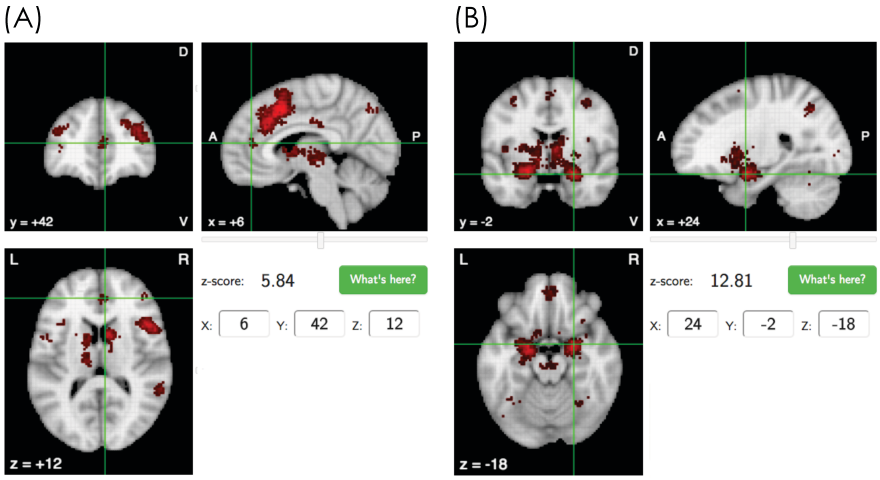

Figure 3.3: Neurosynth meta-analysis inference maps. (A) Results from meta-analysis of term "response inhibition." (B) Results from meta-analysis of term "motivation."

BASOLATERAL AMYGDALA (BLA) / MOTIVATIONAL DRIVE.

We used Neurosynth to identify an *a priori* BLA region associated with the term "motivation" from 135 studies (Figure 3.3B). We located the BLA in the resulting forward inference map and noted the MNI-coordinate of the peak *z*-score. We created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]) in the right BLA centered on a peak *z*-score of 12.81 at (24, -2, -18) in MNI-space.

NUCLEUS ACCUMBENS (NAC) / REWARD-MOTIVATED BEHAVIORS.

The maximum *z*-scores for the rACC and BLA regions, defined above, were in the right hemi-sphere. To be consistent with other fMRI studies reporting an almost wholly right-lateralized network involved in response inhibition (Casey *et al.*, 1997; Garavan *et al.*, 1999; Kiehl *et al.*, 2000; Braver *et al.*, 2001; Swick *et al.*, 2011; Criaud and Boulinguez, 2013), we selected the right NAc for our functional model. We used the right NAc region from the Harvard-Oxford subcortical atlas (Frazier *et al.*, 2005; Desikan *et al.*, 2006; Makris *et al.*, 2006; Goldstein *et al.*, 2007). The right NAc has a volume of 472 mm^3 (59 voxels).

Note that rat studies are able to distinguish between the NAc shell and its core. However, given the spatial resolution of these fMRI scans, we were unable to resolve the NAc shell from the core. The NAc shell is associated with reward salience, wanting, and positive reinforcement (Pecina and Berridge, 2005). The NAc core is associated with motor function related to reward (Malenka *et al.*, 2009).

Task paradigm.

We displayed sets of food and nature images during fMRI scanning (Figure 3.4). Each food image was classified according to its energy density (kcal/g), which remains constant regardless of portion size. We defined high-energy dense foods as those with an energy density greater than 200 kilocalories per 100 grams. All high-energy dense food images depicted food with a minimum estimated total caloric content of 500 kilocalories (based upon a standard serving size). We defined low-energy dense foods as those with an energy density less than 100 kilocalories per 100 grams. All low-energy dense food images depicted foods with a maximum estimated total calorie content of 200 kilocalories. The determination of energy density and caloric content were based on nutritional information from the U.S. Department of Agriculture and nutritional information provided by the manufacturers of commercially prepared foods. In addition to the primary characteristic of energy density, we chose food images that were visually appealing and common in a typical diet for an American child. High-energy dense food images included cheeseburgers, pizza, and chocolate cake. Low-energy dense food images included colorful fruits and vegetables such as bananas, oranges, and carrots. For the rest of this study, we will refer to high-energy dense foods as high calorie foods (HICAL) and low-energy dense foods as low calorie foods (LOCAL). All images were selected from the public domain and were matched for

luminosity, resolution, and size. For baseline comparison, we displayed an image composed of a black background with a white fixation cross in the center (BASELINE).

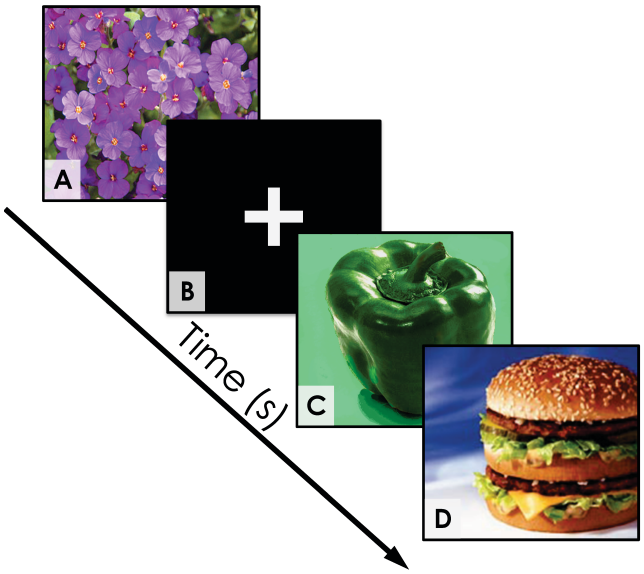

Figure 3.4: Visual food cue paradigm. (A) Nature image; (B) Fixation cross, used as baseline; (C) Low calorie food image; and (D) High calorie food image.

Each scanning session included four 5-min scans. We used an event-related trial design in which participants passively viewed 122 images during each 5-min scan. Each scan included 72 food images, 18 nature images, and 32 fixation cross images. Image types were intermixed with no duplicate food or nature images. Each food and nature image was displayed for 2 sec; fixation cross images were displayed for 2-6 sec. The pseudo-random presentation of images was determined via `optseq2` (`http://surfer.nmr.mgh.harvard.edu/optseq`), a tool that maximizes the power of a task paradigm by scheduling events to reduce unwanted noise and increase statistical efficiency. Images were presented via E-Prime (Psychology Software Tools, Pittsburgh, PA) and displayed on an Epson DLP projector onto a screen at the back of the scanner. Participants viewed the projector via a mirror mounted on the MRI head coil.

Magnetic resonance image acquisition.

MRI scans were acquired on a Philips Intera Achieva 3 Tesla scanner at Vanderbilt University. For each subject, a single high-resolution anatomical T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) scan was acquired with 256 mm field of view; 170 slices; and voxel size = $(1 \cdot 1 \cdot 1)$ mm^3. Each subject participated in four 5-min blood oxygenation level-dependent (BOLD) T2*-weighted echo planar image (EPI) scans, each acquired with repetition time (TR) = 2 s; echo time (TE) = 35 ms; flip angle = 79 degrees; 152 dynamics; 33 slices; and voxel size = $(1.7 \cdot 1.7 \cdot 4.0)$ mm^3 providing whole brain coverage.

MRI image processing and analysis.

We processed MRI datasets with FMRIB Software Library (FSL) v6.00 (Jenkinson *et al.*, 2012). Preprocessing included removal of non-brain tissue (Smith, 2002); spatial smoothing using a Gaussian kernel with full-width at half maximum (FWHM) 6.0 mm; 4D grand-mean intensity normalization; highpass temporal filtering using Gaussian-weighted least-squares straight line fitting with sigma = 60 sec; motion correction (Jenkinson *et al.*, 2002); motion scrubbing (Power *et al.*, 2012; Yan *et al.*, 2013); and linear and nonlinear spatial normalization (Jenkinson *et al.*, 2002; Andersson *et al.*, 2007; Andersson *et al.*, 2008; Greve and Fischl, 2009) to an age-appropriate MRI brain atlas for ages [7.5-13.5] years old (Fonov *et al.*, 2009; Fonov *et al.*, 2011). We discarded data from six children because they moved more than 2 mm in three or more fMRI scans.

Psychophysiological interaction (PPI).

Functional connectivity helps characterize how brain regions work together as a network (Biswal *et al.*, 1995; Friston, 2011; Smith *et al.*, 2012). This coupling can be quantified by the statistical

correlation of BOLD fMRI signals between two brain regions. The pattern of correlation throughout the brain is believed to reflect neurons firing together with a common purpose (Saini *et al.*, 2004; Lewis *et al.*, 2009; Cole *et al.*, 2010) and can reveal whole-brain connectivity patterns (van den Heuvel *et al.*, 2008). Functional connectivity can vary as a function of psychological context (Friston *et al.*, 1997). Psychophysiological interaction (PPI) analysis allows us to assess whether the functional connectivity between two brain regions changes from one psychological task to another. The "physiological" aspect of PPI reveals the functional connectivity between two brain regions as reflected by their synchronous change in neural response. The "psychological" aspect of PPI explores whether this in-sync effect depends on the task. PPI analysis reveals those brain regions with more or less similar functional connectivity with a selected seed region as a function of a specific psychological contrast.

We performed PPI analysis with two different seed regions. First, we used the NAc as our seed region. We investigated the PPI functional connectivity of the NAc seed region with two separate target regions: (1) the rACC, associated with response inhibition; and (2) BLA, associated with motivational drive. We performed a second PPI analysis in which we chose the rACC as the seed region to investigate the inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA.

For PPI analysis, fMRI data are acquired while participants perform a psychological task. Our psychological task was a visual food cue task that contrasted the neural response while participants viewed images of high calorie food *vs.* low calorie food, *i.e.*, [HICAL > LOCAL]. To better understand and disambiguate these PPI results, we created additional, separate PPI maps for the more fundamental contrasts [HICAL > BASELINE] and [LOCAL > BASELINE].

PPI statistical maps

Each participant was scanned during four 5-min food cue scans. For each participant, for each 5-minute scan, we created a PPI contrast map using the general linear model (GLM). We followed the PPI analysis guidelines detailed by O'Reilly *et al.* (O'Reilly *et al.*, 2012). In brief, we created a PPI regressor from the psychological contrast of a [HICAL > LOCAL] multiplied by the BOLD fMRI signal from the seed region NAc. This resulting PPI regressor is used to identify voxels that have a stronger relationship with the seed region when viewing HICAL images compared to viewing LOCAL images. We also performed a second separate PPI analysis using the rACC as the seed region.

To reduce noise from non-grey matter activity, additional regressors of no interest were included in the GLM: mean relative motion correction distance (Power *et al.*, 2012); scrubbed motion (Power *et al.*, 2012; Yan *et al.*, 2013); mean BOLD signal from white matter (O'Reilly *et al.*, 2010); and mean BOLD signal from cerebral spinal fluid (Dagli *et al.*, 1999). We performed separate PPI analyses for the more fundamental contrasts [HICAL > BASELINE] and [LOCAL > BASELINE]. Finally, for each participant, we created a mean PPI map from the scan-specific PPI maps via fixed-effects GLM.

Measures of PPI functional connectivity

To measure response inhibition-associated PPI functional connectivity with the NAc, we used the rACC region as the target region within the PPI map created from the NAc seed region. For each participant, we computed a mean PPI *z*-score, denoted as [RACC:NAC] PPI, from the rACC region in each of the three psychological contrasts: [HICAL > LOCAL], [HICAL > BASELINE], and [LOCAL > BASELINE]. To measure motivational drive-associated PPI functional connectivity with

the NAc, we used the BLA region as the target region. For each participant, we computed a mean PPI *z*-score, denote as [BLA:NAc] PPI, from the BLA region in each of the three contrasts. To measure the inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA, we used the rACC as the seed region and BLA as the target region. We computed a mean PPI *z*-score, denote as [rACC:BLA] PPI, from the BLA region in each of the three psychological contrasts.

Statistical analyses.

Relationship of PPI functional connectivity and adiposity status (Figure 3.1 Path A)

To evaluate the relationship between response inhibition-associated and motivational drive-associated PPI functional connectivity, we computed simple linear regressions of [rACC:NAc] *vs.* [BLA:NAc] PPI via Python's `scipy.stats.linregress` (`iPython` version 3.2.0; `scipy` version 0.15.1; `statsmodels` version 0.6.1) for each weight class. We calculated the statistical difference between the regression slopes, *i.e.*, their effects, of each weight class via a *t*-test (Paternoster *et al.*, 1998). We evaluated the association of [rACC:NAc] *vs.* [BLA:NAc] PPI in each of the three separate psychological contrasts: [hiCal > loCal], [hiCal > baseline], and [loCal > baseline]. Because participants were selected by discontinuous weight classifications, we also tested the equality of means of PPI functional connectivity by weight class via a two-sample *t*-test using `scipy.stats.ttest_ind`. For those comparisons in which the PPI values were not normally distributed, we used the Mann-Whitney *U* test via `scipy.stats.mann whitneyu`. We tested for normality via the Shapiro-Wilk test using `scipy.stats.shapiro`.

Relationship of PPI functional connectivity and eating behaviors (Figure 3.1 Path B)

To evaluate the relationship between external eating, and restrained eating, with PPI functional connectivity, we computed simple linear regressions of eating behavior scores *vs.* [RACC:NAC] PPI, or [BLA:NAC] PPI, or [RACC:BLA] PPI, for each weight class via Python's `scipy.stats.linregress`. We evaluated the association of each eating behavior score *vs.* PPI from each of the three separate psychological contrasts: [HICAL > LOCAL], [HICAL > BASELINE], and [LOCAL > BASELINE].

Relationship of weight class and eating behaviors (Figure 3.1 Path C)

We assessed equality of means of external eating, and restrained eating, by weight class via a two-sample *t*-test, or Mann-Whitney *U* if one or both distributions were not normally distributed. We also tested for interactions between weight class and sex via a 2x2 ANOVA using `statsmodels.stats.anova`.

For all statistical tests, we designated an association as statistically significant if it had a *p*-value ≤ 0.05. If ($0.05 < p$-value ≤ 0.10), then we noted that the association as statistically trending toward significance.

Results.

Participants

We acquired data from 34 children (F=16; M=18; HW=17; OB=17), ages [8-12] (mean = 10.3; stdev = 1.3) years old (Table 3.1). Figure 3.5 shows the distribution of BMI *z*-score *vs.* age for all participants. There was no interaction between weight class and sex for age. There was no significant difference of main effect for sex. However, there was a trending difference of main

effect for weight class in which children who were obese were older ($p = 0.097$; $F = 2.940$). Hunger ratings prior to scanning did not differ between weight classes ($p = 0.326$; $U = 123.0$). The average time between meal completion and arrival at the scanner was 13.6 (stdev = 5.6) min. To accommodate the schedules of participants, scan times varied. Sixty-two percent of scans began between 8:00-11:00; 18% of scans began between 11:00-13:00; 15% of scans began between 15:00-16:00; and 6% of scans began between 17:00-18:00. Scans in which the participant moved more than 2 mm were omitted from analysis. All 34 participants had at least two useable fMRI food-cue scans.

***N* = 34 (F = 16 / M = 18)**	**Mean (Stdev)**	**Min, Max**
Age (yrs)	10.3 (1.3)	8.2, 12.8
Weight (lbs) (*N*=34)	103.5 (41.8)	55.0, 226.8
Healthy weight (*N*=17)	71.9 (11.4)	55.0, 90.0
Obese (*N*=17)	135.2 (36.8)	95.0, 226.8
BMI *z*-score (*N*=34)	0.960 (1.207)	-1.200, 2.591
Healthy weight (*N*=17)	-0.118 (0.722)	-1.200, 1.053
Obese (*N*=17)	2.038 (0.264)	1.710, 2.591

Table 3.1: Participant demographic summary. F: female; M: male; StDev: standard deviation; Min: minimum; Max: maximum; BMI: body mass index.

Relationship of PPI functional connectivity and adiposity status (Figure 3.1 Path A)

All associations between pairs of physiological PPI measurements, by psychological task, by adiposity status, are listed in Table 3.2.

PPI FUNCTIONAL CONNECTIVITY WITH NAC ASSOCIATED WITH CALORIC CONTENT, [HICAL > LOCAL]

When viewing [HICAL > LOCAL] images, HW showed increasing [RACC:NAC] PPI with increasing [BLA:NAC] PPI ($p = 0.010$; $R^2 = 0.368$; $r = 0.607$; Figure 3.6A). In contrast, among

OB, [RACC:NAC] PPI decreased with increasing [BLA:NAC] PPI ($p = 0.032$; $R^2 = 0.271$; $r = -0.521$). The difference of effects by adiposity status was statistically significantly different ($p = 0.001$; $t = 3.636$).

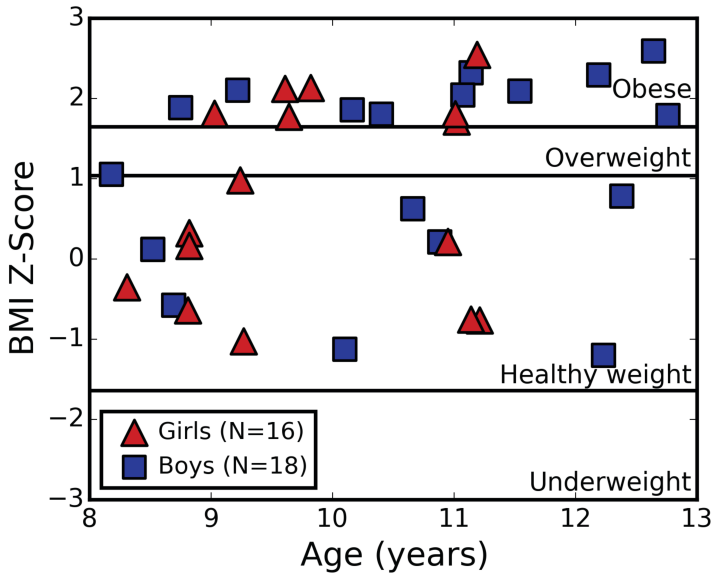

Figure 3.5: Distribution of BMI *z*-score *vs.* age. Children were classified as healthy weight for (-1.64 ≤ BMI z-scores < 1.04); and obese for (BMI z-scores ≥ 1.64).

FUNCTIONAL CONNECTIVITY WITH NAC WHEN VIEWING HIGH CALORIE FOOD, [HICAL > BASELINE]

When viewing [HICAL > BASELINE] images, among HW, there was no statistically significant association of [RACC:NAC] *vs.* [BLA:NAC] PPI functional connectivity ($p = 0.133$; $R^2 = 0.144$; $r = 0.379$) nor for OB ($p = 0.185$; $R^2 = 0.114$; $r = -0.337$). However, the difference of effects by adiposity status was trending toward statistical significance ($p = 0.052$; $t = 2.022$).

FUNCTIONAL CONNECTIVITY WITH NAC WHEN VIEWING LOW CALORIE FOOD, [LOCAL > BASELINE]

When viewing [LOCAL > BASELINE] images, among HW, there was no statistically significant association of [RACC:NAC] *vs.* [BLA:NAC] PPI functional connectivity ($p = 0.370$; $R^2 = 0.054$; $r = 0.232$) or for OB ($p = 0.622$; $R^2 = 0.018$; $r = -0.134$). The difference of effects by adiposity status was not significant ($p = 0.305$; $t = 1.043$).

PPI 1	PPI 2	Contrasted Food Images	Adiposity Class	*p*-value	R^2	*r*	ANCOVA *p* (*t*-stat)
[BLA:NAc]	[rACC:NAc]	[hiCal > loCal]	HW	0.010**	0.368	0.607	0.001 (3.636)**
			OB	0.032*	0.271	-0.521	
		[hiCal > baseline]	HW	0.133	0.144	0.379	0.052 (2.022)-
			OB	0.185	0.114	-0.337	
		[loCal > baseline]	HW	0.370	0.054	0.232	0.305 (1.043)
			OB	0.622	0.018	-0.134	
[rACC:BLA]	[BLA:NAc]	[hiCal > loCal]	HW	0.839	0.003	-0.053	0.142 (1.509)
			OB	0.037*	0.260	0.510	
		[hiCal > baseline]	HW	0.098-	0.172	0.414	0.715 (0.369)
			OB	0.231	0.094	0.307	
		[loCal > baseline]	HW	0.401	0.048	0.218	0.402 (0.850)
			OB	0.148	0.134	0.366	
[rACC:BLA]	[rACC:NAc]	[hiCal > loCal]	HW	0.021*	0.308	0.555	0.017 (2.519)*
			OB	0.257	0.085	-0.291	
		[hiCal > baseline]	HW	0.010**	0.367	0.606	0.001 (3.579)**
			OB	0.061-	0.215	-0.463	
		[loCal > baseline]	HW	0.080-	0.191	0.437	0.029 (2.291)*
			OB	0.196	0.109	-0.330	

Table 3.2: Relationships between pairs of psychophysiological interaction (PPI) functional connectivity measurements. ANCOVA tests for significant difference in effects between each adiposity class; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala; NAc: nucleus accumbens; [rACC:NAc]: response inhibition-associated PPI functional connectivity between rACC and NAc; [BLA:NAc]: motivational drive- associated PPI functional connectivity between BLA and NAc; [rACC:BLA]: inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA; hiCal: high calorie food images; loCal: low calorie food images; baseline: image of fixation cross; HW: healthy weight cohort; OB: obese cohort; Significance: -: $p \leq 0.10$; *: $p \leq 0.05$; **: $p \leq 0.01$.

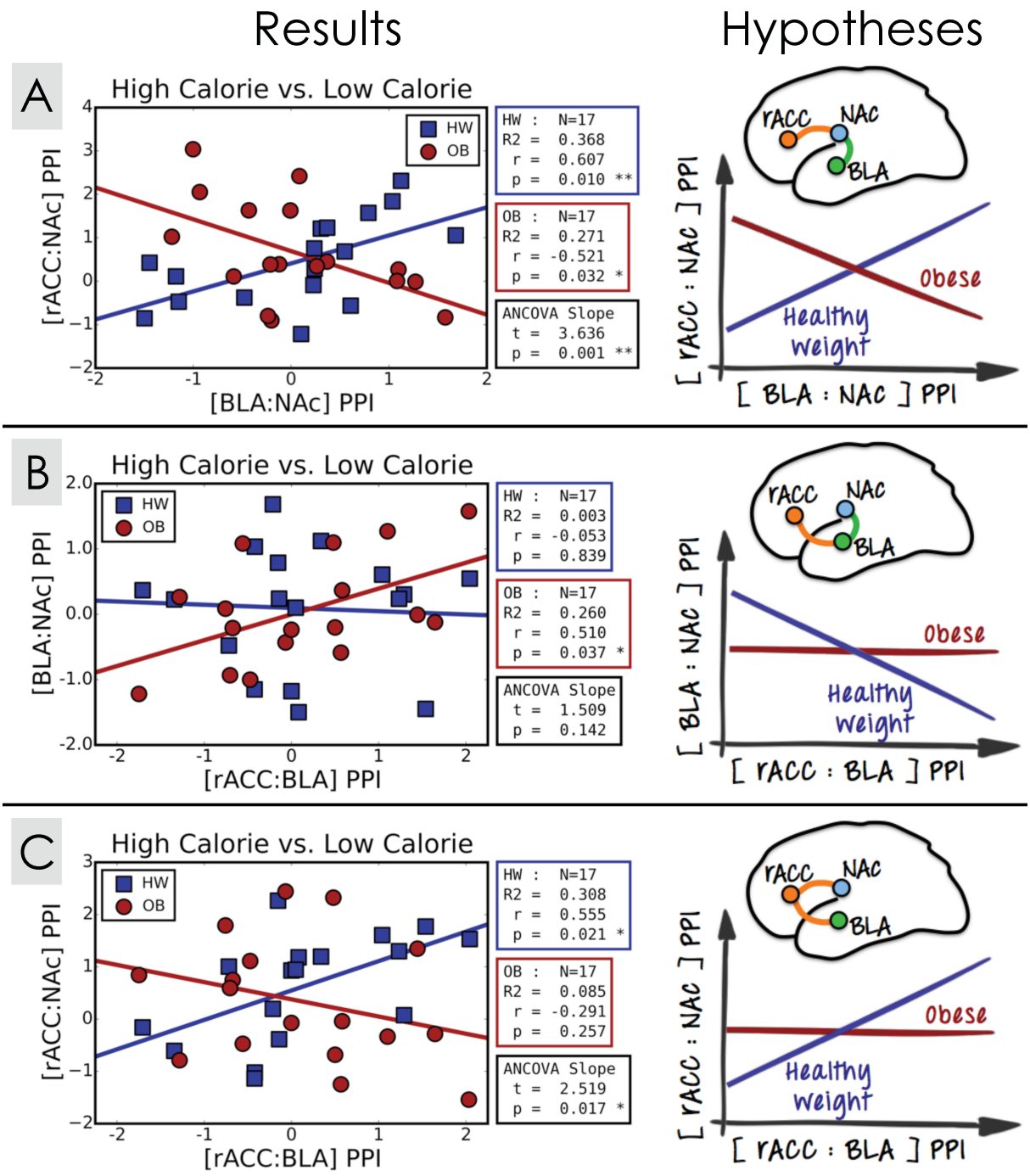

Figure 3.6: Results of PPI vs. PPI and hypotheses. Left column displays results; right column displays associated hypotheses. (A) Relationship of [rACC:NAc] *vs.* [BLA:NAc] PPI functional connectivity by adiposity status; (B) Relationship of [BLA:NAc] *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (C) Relationship of [rACC:NAc] *vs.* [rACC:BLA] PPI functional connectivity by adiposity status. PPI: psychophysiological interaction; NAc: nucleus accumbens; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala.

PPI FUNCTIONAL CONNECTIVITY BETWEEN RACC AND BLA ASSOCIATED WITH CALORIC CONTENT [HICAL > LOCAL]

When viewing [HICAL > LOCAL] images, HW showed no significant relationship between [BLA:NAC] *vs.* [RACC:BLA] PPI ($p = 0.839$; $R^2 = 0.003$; $r = -0.053$; Figure 3.6B). However, among OB, as [RACC:BLA] PPI increased, [BLA:NAC] PPI increased ($p = 0.037$; $R^2 = 0.260$; $r = 0.510$). The difference of effects by adiposity status was not statistically significantly different ($p = 0.142$; $t = 1.509$).

When viewing [HICAL > LOCAL] images, HW showed increasing [RACC:NAC] PPI with increasing [RACC:BLA] PPI ($p = 0.021$; $R^2 = 0.308$; $r = 0.555$; Figure 3.6C). However, among OB, there was no significant association between [RACC:NAC] *vs.* [RACC:BLA] PPI ($p = 0.257$; $R^2 = 0.085$; $r = -0.291$). The difference of effects by adiposity status was statistically significantly different ($p = 0.017$; $t = 2.519$).

FUNCTIONAL CONNECTIVITY BETWEEN RACC AND BLA WHEN VIEWING HIGH CALORIE FOOD, [HICAL > BASELINE]

When viewing [HICAL > BASELINE] images, among HW, increasing [RACC:NAC] PPI was associated with increasing [RACC:BLA] PPI ($p = 0.010$; $R^2 = 0.367$; $r = 0.606$). Among OB, this relationship was trending toward a negative association ($p = 0.061$; $R^2 = 0.215$; $r = -0.463$). The difference of effects by adiposity status was statistically significantly different ($p = 0.001$; $t = 3.579$).

FUNCTIONAL CONNECTIVITY BETWEEN RACC AND BLA WHEN VIEWING HIGH CALORIE FOOD, [LOCAL > BASELINE]

When viewing [LOCAL > BASELINE] images, among HW, the relationship between [RACC:BLA] *vs.* [RACC:NAC] PPI was trending toward a positive association ($p = 0.080$; $R^2 = 0.191$; $r =$

0.437). Among OB, there was no significant relationship ($p = 0.196$; $R^2 = 0.109$; $r = -0.330$). The difference of effects by adiposity status was statistically significantly different ($p = 0.029$; $t = 2.291$).

Relationship of PPI functional connectivity and external eating (Figure 3.1 Path B)

All 34 children completed the DEBQ-C. The mean external eating score was 2.07 (stdev = 0.52; range = [1, 3]). The mean restrained eating score was 1.73 (stdev = 0.54; range = [1, 3]).

RESPONSE INHIBITION-ASSOCIATED [RACC:NAC] PPI FUNCTIONAL CONNECTIVITY AND EXTERNAL EATING

All results for external eating scores *vs.* PPI functional connectivity are listed in Table 3.3. When viewing [HICAL > LOCAL] images, HW exhibited trending decreased external eating habits with increasing [RACC:NAC] PPI ($p = 0.054$; $R^2 = 0.226$; $r = -0.475$; Figure 3.7A). In contrast, OB exhibited increased external eating habits with increasing [RACC:NAC] PPI ($p = 0.035$; $R^2 = 0.264$; $r = 0.514$). The difference of effects of these relationships by adiposity status was statistically significantly different ($p = 0.005$; $t = 3.044$).

When viewing [HICAL > BASELINE] images, HW showed decreased external eating habits with increasing [RACC:NAC] PPI ($p = 0.019$; $R^2 = 0.317$; $r = -0.563$). In contrast, OB showed increased external eating habits with increasing [RACC:NAC] PPI ($p = 0.001$; $R^2 = 0.522$; $r = 0.722$). The difference of effects of these relationships by adiposity status was statistically significantly different ($p = 0.000$; $t = 4.073$).

When viewing [LOCAL > BASELINE] images, there was no significant association of external eating habits with [RACC:NAC] PPI among HW ($p = 0.604$; $R^2 = 0.018$; $r = -0.135$). However, among OB, external eating habits increased with increasing [RACC:NAC] PPI ($p = 0.050$; $R^2 =$

0.233; $r = 0.482$). The difference of effects of these relationships by adiposity status was not statistically significantly different ($p = 0.123$; $t = 1.586$).

Physiological Functional Connectivity	Contrasted Food Images	Adiposity Class	*p*-value	R^2	*r*	ANCOVA *p* (*t*-stat)
[rACC:NAc]	[hiCal > loCal]	HW	0.054-	0.226	-0.475	0.005 (3.044)**
		OB	0.035*	0.264	0.514	
	[hiCal > baseline]	HW	0.019*	0.317	-0.563	0.000 (4.073)**
		OB	0.001**	0.522	0.722	
	[loCal > baseline]	HW	0.604	0.018	-0.135	0.123 (1.586)
		OB	0.050*	0.233	0.482	
[BLA:NAc]	[hiCal > loCal]	HW	0.222	0.098	-0.312	0.588 (0.548)
		OB	0.615	0.017	-0.131	
	[hiCal > baseline]	HW	0.856	0.002	-0.048	0.623 (0.496)
		OB	0.327	0.064	-0.253	
	[loCal > baseline]	HW	0.235	0.093	0.304	0.190 (1.340)
		OB	0.594	0.019	0.235	
[rACC:BLA]	[hiCal > loCal]	HW	0.352	0.058	0.241	0.130 (1.558)
		OB	0.211	0.102	-0.320	
	[hiCal > baseline]	HW	0.786	0.005	-0.071	0.561 (0.588)
		OB	0.201	0.106	-0.326	
	[loCal > baseline]	HW	0.222	0.098	-0.313	0.530 (0.636)
		OB	0.745	0.007	-0.085	

Table 3.3: Relationships of external eating behaviors to psychophysiological interaction (PPI) functional connectivity by adiposity class. ANCOVA tests for significant difference in effects between each adiposity class; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala; NAc: nucleus accumbens; [rACC:NAc]: response inhibition-associated PPI functional connectivity between rACC and NAc; [BLA:NAc]: motivational drive- associated PPI functional connectivity between BLA and NAc; [rACC:BLA]: inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA; hiCal: high calorie food images; loCal: low calorie food images; baseline: image of fixation cross; HW: healthy weight cohort; OB: obese cohort; Significance: -: $p \leq 0.10$; *: $p \leq 0.05$; **: $p \leq 0.01$.

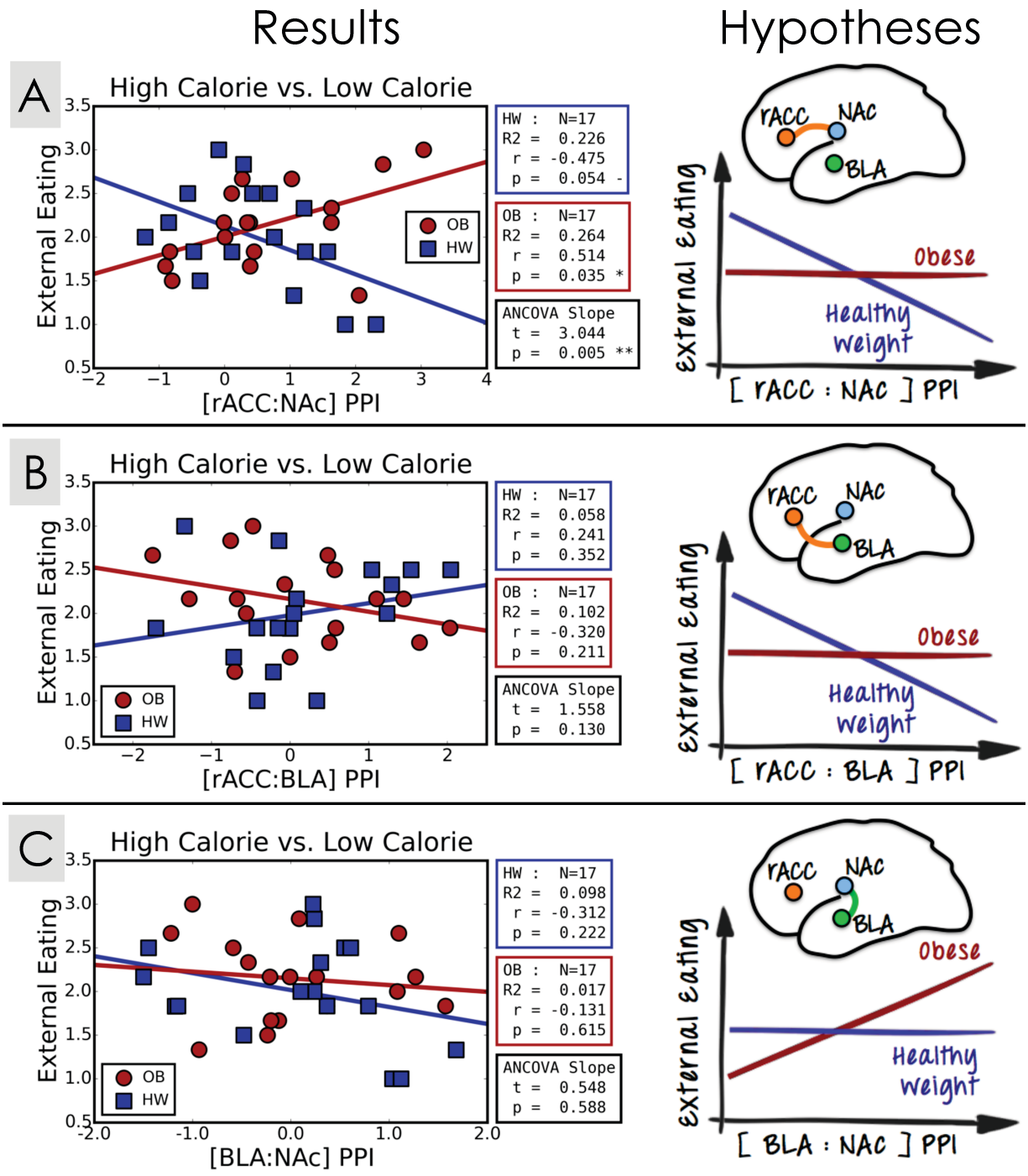

Figure 3.7: Results of external eating scores vs. PPI and hypotheses. Left column displays results; right column displays associated hypotheses. (A) Relationship of external eating scores *vs.* [rACC:NAc] PPI functional connectivity by adiposity status; (B) Relationship of external eating scores *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (C) Relationship of external eating scores *vs.* [BLA:NAc] PPI functional connectivity by adiposity status. PPI: psychophysiological interaction; NAc: nucleus accumbens; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala.

INHIBITION OF MOTIVATIONAL DRIVE-ASSOCIATED [RACC:BLA] PPI FUNCTIONAL CONNECTIVITY AND EXTERNAL EATING

There were no significant associations between external eating habits and [RACC:BLA] PPI for either adiposity class for any psychological task (Figure 3.7B). These results are listed in Table 3.3.

MOTIVATIONAL DRIVE-ASSOCIATED [BLA:NAC] PPI FUNCTIONAL CONNECTIVITY AND EXTERNAL EATING

There were no significant associations between external eating habits and [BLA:NAC] PPI for either adiposity class for any psychological task (Figure 3.7C). These results are listed in Table 3.3.

Relationship of PPI functional connectivity and restrained eating (Figure 3.1 Path B)

All results for restrained eating scores *vs.* PPI are detailed in Table 3.4. When viewing [HICAL > LOCAL] images, the association between restrained eating habits and [RACC:NAC] PPI was not statistically significant (HW: $p = 0.133$; $R^2 = 0.144$; $r = -0.379$; OB: $p = 0.103$; $R^2 = 0.168$; $r = -0.409$; Figure 3.8A). Among HW, when viewing [HICAL > LOCAL] images, restrained eating increased with increasing [RACC:BLA] PPI ($p = 0.010$; $R^2 = 0.365$; $r = 0.604$; Figure 3.8B). Among OB, when viewing [HICAL > LOCAL] images, the association between increased [BLA:NAC] PPI and increased restrained eating was trending toward statistical significance ($p = 0.084$; $R^2 = 0.185$; $r = 0.431$; Figure 3.8C).

Relationship of adiposity status and external eating (Figure 3.1 Path C)

There was no significant interaction between adiposity status and sex for external eating ($p = 0.115$; $F = 2.637$). There was no significant difference of main effects for adiposity status ($p = 0.419$; $F = 0.672$) or sex ($p = 0.728$; $F = 0.123$) with external eating.

Physiological Functional Connectivity	Contrasted Food Images	Adiposity Class	*p*-value	R^2	*r*	ANCOVA *p* (*t*-stat)
[rACC:NAc]	[hiCal > loCal]	HW	0.133	0.144	-0.379	0.860 (0.178)
		OB	0.103	0.168	-0.409	
	[hiCal > baseline]	HW	0.790	0.005	-0.070	0.829 (0.218)
		OB	0.388	0.050	-0.224	
	[loCal > baseline]	HW	0.133	0.144	0.379	0.344 (0.961)
		OB	0.713	0.009	0.096	
[BLA:NAc]	[hiCal > loCal]	HW	0.801	0.004	-0.066	0.155 (1.458)
		OB	0.084-	0.185	0.431	
	[hiCal > baseline]	HW	0.917	0.001	-0.027	0.449 (0.767)
		OB	0.322	0.065	0.256	
	[loCal > baseline]	HW	0.872	0.002	0.042	0.783 (0.277)
		OB	0.802	0.004	-0.066	
[rACC:BLA]	[hiCal > loCal]	HW	0.010*	0.365	0.604	0.223 (1.246)
		OB	0.308	0.069	0.263	
	[hiCal > baseline]	HW	0.115	0.157	0.397	0.826 (0.222)
		OB	0.150	0.133	0.365	
	[loCal > baseline]	HW	0.636	0.015	-0.124	0.346 (0.956)
		OB	0.407	0.046	0.215	

Table 3.4: Relationships of restrained eating behaviors to psychophysiological interaction (PPI) functional connectivity by adiposity class. ANCOVA tests for significant difference in effects between each adiposity class; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala; NAc: nucleus accumbens; [rACC:NAc]: response inhibition-associated PPI functional connectivity between rACC and NAc; [BLA:NAc]: motivational drive- associated PPI functional connectivity between BLA and NAc; [rACC:BLA]: inhibition of motivational drive-associated PPI functional connectivity between rACC and BLA; hiCal: high calorie food images; loCal: low calorie food images; baseline: image of fixation cross; HW: healthy weight cohort; OB: obese cohort; Significance: -: $p \leq 0.10$; *: $p \leq 0.05$; **: $p \leq 0.01$.

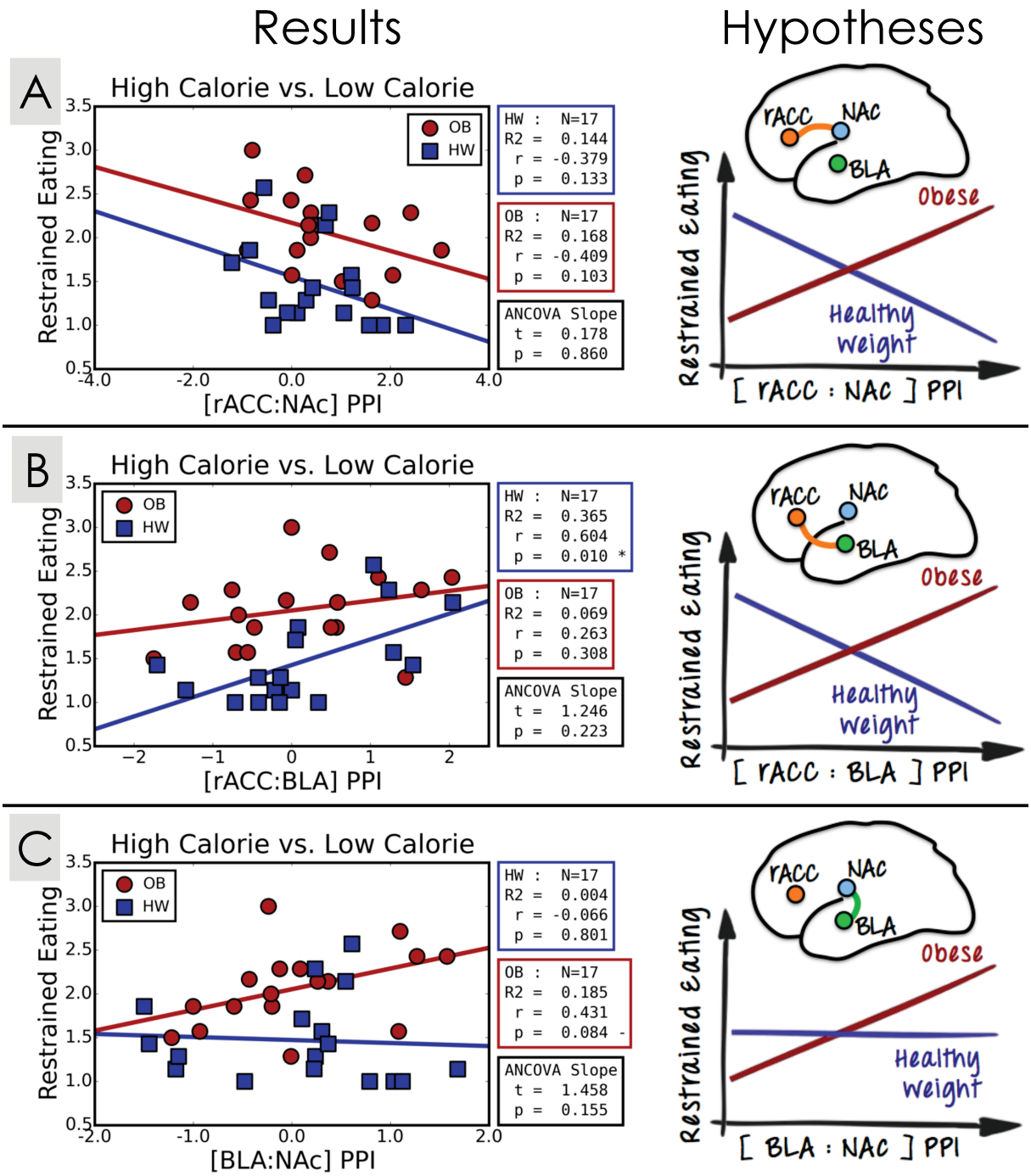

Figure 3.8: Results of restrained eating scores vs. PPI and hypotheses. Left column displays results; right column displays associated hypotheses. (A) Relationship of restrained eating scores *vs.* [rACC:NAc] PPI functional connectivity by adiposity status; (B) Relationship of restrained eating scores *vs.* [rACC:BLA] PPI functional connectivity by adiposity status; (C) Relationship of restrained eating scores *vs.* [BLA:NAc] PPI functional connectivity by adiposity status. PPI: psychophysiological interaction; NAc: nucleus accumbens; rACC: rostral anterior cingulate cortex; BLA: basolateral amygdala.

Relationship of adiposity status and restrained eating (Figure 3.1 Path C)

There was a significant difference of main effects for adiposity status with restrained eating (p = 0.001; F = 13.239), where OB had increased mean restrained eating scores compared to HW (Figure 3.9). There was no significant interaction between adiposity status and sex with restrained eating (p = 0.633; F = 0.232). There was no significant difference of main effects for sex (p = 0.348; F = 0.909) with restrained eating.

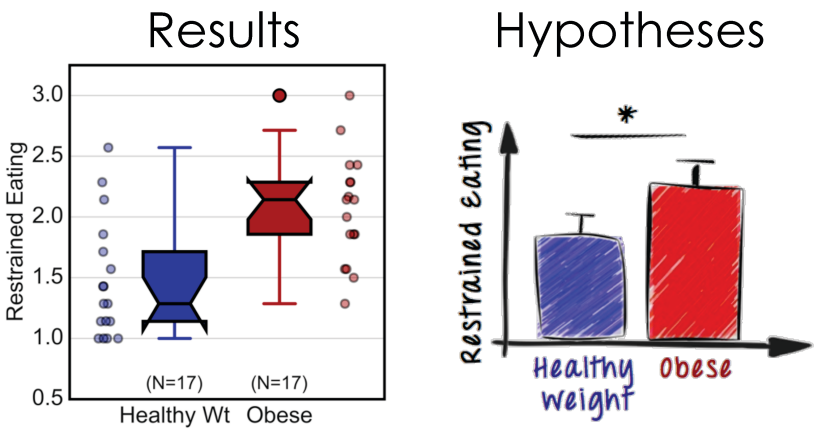

Figure 3.9: Results of restrained eating by adiposity status. Left column displays results; right column displays associated hypotheses. Relationship of restrained eating scores by adiposity status.

Discussion.

We reported novel results from a psychophysiological interaction (PPI) functional connectivity study of childhood obesity in order to elucidate brain functional connectivity in response to external food cues, ubiquitous in the lives of children in the US. To our knowledge, no previous childhood obesity PPI functional connectivity studies have been published. Our results suggest that food cue task-based analysis of neural connectivity can identify neural models that are associated with childhood obesity. Furthermore, our results suggest that ineffective response inhibition-association functional connectivity, when viewing high calorie compared to low

calorie food images, is characteristic of obesity in children, ages 8-12 year old. Additionally, response inhibition-associated functional connectivity, more so than motivational drive-associated functional connectivity, may be a key functional difference between children who are obese compared to healthy weight children. Targeting these neural networks, *e.g.*, strengthening response inhibition-associated functional connectivity, may contribute to novel obesity treatment.

We found that our most significant findings resulted from the [HICAL > LOCAL] contrast. These results were driven by high calorie food images, i.e., [HICAL > BASELINE], rather than low calorie food images [LOCAL > BASELINE]. This suggests that children as young as 8-13 years old can distinguish food by caloric content as reflected by neural response.

In agreement with our hypothesis, we found that among children who are healthy weight (HW) as motivational drive-associated [BLA:NAC] PPI functional connectivity increased, response inhibition-associated [RACC:NAC] PPI functional connectivity also increased when viewing high calorie compared to low calorie food images (Figure 3.6A), suggesting that response inhibition-associated functional connectivity keeps pace with motivational drive-associated functional connectivity. Conversely, among children who are obese (OB), response inhibition-associated functional connectivity is progressively blunted with increased motivational drive. In other words, among OB, response inhibition-associated functional connectivity fails to keep pace with motivational drive-associated functional connectivity. These results suggest that effective neural communication supports healthy weight when both response inhibition-associated functional connectivity and motivational drive-associated functional connectivity are in balance. We speculate that increased adiposity may be due to imbalanced functional connectivity in which response inhibition is ineffective, thereby biasing balance toward motivational drive.

To better understand the blunting of response inhibition-associated functional connectivity with increasing motivational drive-associated functional connectivity among OB when viewing high calorie compared to low calorie food images, we investigated the relationships of [BLA:NAc] *vs.* [rACC:BLA]PPI (Figure 3.6B). Because increased neural response in rACC is associated with decreased response in BLA, we hypothesized that, among HW, as [rACC:BLA] PPI increased, [BLA:NAc] PPI would decrease. And that, among OB, we hypothesized no significant relationships as we speculated that this functional system of checks and balances is ineffective. Our results do not support these hypotheses. Instead, among OB, as [rACC:BLA] PPI increased, [BLA:NAc] PPI paradoxically increased. One possible explanation for this increase may be that ineffective [rACC:BLA] functional connectivity results in a compensatory increase in functional connectivity. We speculate that perhaps, in spite of increased functional connectivity, the integration of rACC input to BLA is ineffective. Tuulari *et al.*, also reported increased functional connectivity in regions association with appetite control during an eating inhibition task when comparing adults who were obese with healthy weight adults (Tuulari *et al.*, 2015). The authors suggest that this increase may be compensatory due to increased neural input needed to inhibit food intake.

Additionally, among HW, increasing [rACC:BLA] PPI was not associated with decreasing [BLA:NAc] PPI (Figure 3.6B), as we hypothesized. A possible explanation for this may be better understood by considering the association between [rACC:NAc] *vs.* [rACC:BLA] PPI (Figure 3.6C) and the possible dual nature of rACC inhibition. We proposed a mechanism of direct inhibition from rACC to NAc and indirect inhibition from rACC through BLA to NAc. Our results suggest that indirect inhibition may not be as influential in maintaining healthy weight (Figure 3.6B), whereas direct inhibition is (Figure 3.6C). Taken together, these results

suggest that childhood obesity may be supported by ineffective integration of input from response inhibition-associated rACC when viewing high calorie compared to low calorie food images.

Atalayer *et al.*, reported sex-specific differences in PPI functional connectivity in response to high and low calorie food images among adults, ages 25-45 years old, who were obese (Atalayer *et al.*, 2014). To further investigate this finding, we performed *post hoc* linear regressions of [BLA:NAc] *vs.* [rACC:NAc] PPI by adiposity class by sex for [hiCal > loCal]. Because these analyses reduced our sample sizes to 10 participants or fewer, these *post hoc* analyses must be interpreted with caution. We found no significant interaction between adiposity status and sex. There was no significant difference of main effects for sex. Although these results must be revisited with larger sample sizes, a preliminary interpretation suggests that sex-specific differences in PPI functional connectivity when viewing food cues may not yet exist among children as young as 8-12 years old.

Killgore and Yurgelun-Todd reported age-related developmental changes in neural response in regions associated with executive control (Killgore and Yurgelun-Todd, 2005). They investigated neural response when viewing images of high calorie foods contrasted with low calorie foods among healthy weight girls, 9-15 years old, compared to healthy weight women, 21-28 years old. To further investigate this relationship, we performed *post hoc* regression analyses of [rACC:NAc] *vs.* [BLA:NAc] PPI functional connectivity as a function of age. To better match Killgore and Yurgelun-Todd's all-female cohort, we also included an interaction term for sex. These analyses reduced our sample sizes to 10 participants or fewer, therefore these exploratory analyses must be interpreted with caution. We found no significant relationship with age and no significant interaction between adiposity status and sex with age for any of our psychological

tasks. The lack of relationship with age may be due to the narrow age range we studied, 8-12 years old, whereas Killgore and Yurgelun-Todd compared their adolescent cohort to adults. A preliminary interpretation suggests that age-associated differences in PPI functional connectivity when viewing food cues may not yet exist among children as young as 8-12 years old.

We also investigated the relationship of external eating habits, *i.e.*, eating in response to the sight or smell of food, with PPI functional connectivity during a food cue task. Our results confirmed our hypotheses that, among HW, decreased external eating is associated with increased response inhibition-associated [RACC:NAC] PPI (Figure 3.7A). However, among OB, increased external eating is associated with increased [RACC:NAC] PPI, suggesting that integration of response inhibition-associated functional connectivity is ineffective, as characterized by compensatory increased functional connectivity. Additionally, our hypothesis that, among HW, external eating would decrease with increasing [RACC:BLA] PPI, was not supported by our results (Figure 3.7B). Rather, there was no association. This result suggests again that indirect inhibition from rACC through BLA to NAc may not be strongly influential in maintaining healthy weight. There were no significant associations between external eating and motivational drive-associated [BLA:NAC] PPI for either weight class (Figure 3.7C), suggesting a more prominent role of response inhibition-associated functional connectivity compared to motivational drive-associated functional connectivity. We speculate that ineffective response inhibition-associated functional connectivity, if it leads to ineffective behavioral inhibition, could contribute to obesity given the easy accessibility of abundant high calorie foods. Note also that PPI functional connectivity in other motivational drive-associated brain regions, in addition to the amygdala, may be associated with external eating.

Passamonti *et al.*, reported increased external eating with an increase in PPI functional connectivity between the amygdala and NAc among healthy weight adults, ages 19-39 years old, in response to viewing images of appetizing foods *vs.* bland foods (Passamonti *et al.*, 2009). To better approximate Passmonti's [APPETIZING > BLAND] task, we added a *post hoc* analysis of [FOOD > BASELINE] contrast. We found no significant relationship between external eating and [BLA:NAC] PPI when contrasting [FOOD > BASELINE] among HW or among OB, which agrees with our original [HICAL > BASELINE] results. Taken together these results suggest that motivational drive-associated [BLA:NAC] functional connectivity is not the more significant factor in external eating among children, but may develop with age.

We investigated the relationship of restrained eating habits, *i.e.*, eating less to lose or maintain weight, with PPI functional connectivity during a food cue task. Our hypothesis that among OB, increased restrained eating would also be associated with increased [RACC:NAC] PPI, indicating ineffective response inhibition, was not supported by our results (Figure 3.8A). The resulting association among OB is similar to that of HW with the caveat that the overall restrained eating scores are greater for OB, discussed below. This similarity in the associations of restrained eating *vs.* [RACC:NAC] PPI for both adiposity groups suggests that, in a practical sense, response inhibition is ultimately ineffective as some children are obese.

Neither of our hypotheses about restrained eating habits and indirect inhibition from rACC through BLA to NAc, [RACC:BLA] PPI, were supported by our results (Figure 3.8B). While there was no association among OB, there was a positive association among HW. There is continuing debate as to whether restrained eating is strictly an unhealthy eating behavior. Some researchers suggest that restrained eating is a healthy response to our obesogenic environment (Johnson *et al.*, 2012). Therefore this positive association may be indicative of a healthy

response to maintaining healthy weight. Additionally, our results supported our hypotheses that, among OB, increased restrained eating is associated with increased [BLA:NAc] PPI (Figure 3.8C), suggesting that an increase in motivational drive-associated functional connectivity is consciously paired to desire to diet among children who are obese.

We investigated the relationship of adiposity status with external and restrained eating habits. We found no difference in external eating scores comparing OB with HW, in contradiction to our hypothesis. The discriminative validity of the DEBQ external eating scale has been called into question (Jansen *et al.*, 2011); for rebuttal, see (van Strien *et al.*, 2012). While many studies reported that increased external eating scores were associated with increased adiposity, some studies reported no relationship (Snoek *et al.*, 2013; Witt *et al.*, 2014), or inverse relationships (Ledoux *et al.*, 2011). In the rebuttal by van Strien *et al.*, they concluded that the external eating score is valid when participants have "sufficiently extreme" external eating scores. We speculate that our non-significant results may be attributable to insufficiently extreme scores among our participants. However, in agreement with other studies, we found increased restrained eating scores comparing OB with HW (Figure 3.9). Our results suggest that dieting occurs among children as young as 8-12 years old.

Strengths and limitations

To our knowledge, ours is the first published study to investigate childhood obesity using PPI functional connectivity analysis. However, a limitation of PPI analysis, and that of all functional connectivity methods, is that its results cannot be used to infer directionality of effect. That is, PPI results alone cannot indicate whether, say, the rACC influences NAc or NAc influences rACC. However, our brain model incorporated circuitry derived from animal studies from which

directionality can be determined. Incorporating anatomical circuitry into our model suggests that a portion of the observed functional connectivity arises from direct anatomical connections. Another limitation is that BMI *z*-score is an indirect measure of adiposity. Using an imaging-based measure of adiposity, *e.g.*, whole-body MRI, or leptin levels from a blood draw, might provide a more accurate association between brain function and adiposity. A final point of consideration is that our *a priori* brain model includes three brain regions. Investigating a larger brain network, perhaps the whole brain, using graph theoretic methods (Alexander-Bloch *et al.*, 2013) would provide additional information, such as uncovering other regions within a functional network that support childhood obesity.

Conclusion.

Intensive lifestyle interventions in adults do not typically lead to long-lasting weight loss, and co-morbidities such as diabetes and cardiovascular disease develop over many years. Therefore an understanding of the developing neurofunctionality of childhood obesity would provide unique interventional insight. Furthermore, identification of children at risk for obesity would permit the development of novel obesity treatment and prevention efforts. As a first step, we investigated a brain model in a cohort of children who are obese and healthy weight using psychophysiological interaction (PPI) functional connectivity. Our results showed a marked difference in response inhibition- and motivational drive-associated functional connectivity among children who are obese compared to healthy weight children in response to images of food. We speculate that these differences may translate into eating related behaviors, such as external eating, that determine, in part, a child's overall adiposity.

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CHAPTER 4

Imbalance in Resting State Functional Connectivity is Associated with Eating Behaviors and Adiposity in Children

ABSTRACT

Background and Hypothesis

Over the past 30 years, childhood obesity in the US has nearly doubled, while obesity has tripled among adolescents. Non-homeostatic eating, influenced by impulsivity and inhibition, may undermine successful long-term weight loss. We hypothesized that unhealthy eating habits and adiposity among children are associated with functional connectivity between brain regions associated with response inhibition, impulsivity, and reward.

Methods

We analyzed resting state functional magnetic resonance images from 38 children, ages [8-13] years old. Using seed-based resting state functional connectivity, we quantified connectivity between brain regions associated with response inhibition (inferior parietal lobe [IPL]), impulsivity (frontal pole), and reward (nucleus accumbens [NAc]). We assessed the relationship of resting state functional connectivity with adiposity, quantified by BMI *z*-score, and eating behaviors, as measured by the Child Eating Behaviour Questionnaire (CEBQ). We computed an imbalance measure—the difference between [FRONTAL POLE:NAC] and [IPL:NAC] functional connectivity—and investigated the relationship of this imbalance with eating behaviors and adiposity.

Results

As functional connectivity imbalance is increasingly biased toward impulsivity, adiposity increases. Similarly, as impulsivity-biased imbalance increases, food approach behaviors increase and food avoidance behaviors decrease. Increased adiposity is associated with increased food approach behaviors and decreased food avoidance behaviors.

Conclusions

In the absence of any explicit food-related stimuli, the developing brain is primed toward food approach and away from food avoidance behavior with increasing adiposity. Imbalance in resting state functional connectivity that is associated with non-homeostatic eating develops during childhood, as early as 8-13 years of age. Our results indicate the importance of identifying children at risk for obesity for earlier intervention. In addition to changing eating habits and physical activity, strategies that normalize neural functional connectivity imbalance are needed to maintain healthy weight. For example, mindfulness training, associated with increased response inhibition and decreased impulsivity, may recalibrate neural functional connectivity imbalance that, in turn, may contribute to maintaining a healthy weight.

Introduction.

Long-term maintenance of weight loss among adults is poor. Fildes *et al.*, reported that the probability of adults who are obese reaching healthy weight is 0.50% for men and 0.80% for women (Fildes *et al.*, 2015). Up to 90% of dieters return to baseline weight within 3 years after weight loss (Cooper *et al.*, 2010; Butryn *et al.*, 2011).

The aim of this study was to better understand resting state functional connectivity between regions in the brain associated with non-homeostatic eating among children across a range of adiposity values. We therefore defined a neural model comprised of three *a priori*-defined regions (Figure 4.1): (1) inferior parietal lobe (IPL), associated with response inhibition; (2) frontal pole (fPole), associated with impulsivity; and (3) the nucleus accumbens (NAc), associated with reward and reward-motivated behaviors. We also investigated the associations of resting state functional connectivity with eating behaviors. Insight into these relationships will provide a better understanding of the mechanisms and potential efficacy of novel treatments for weight loss and maintenance among children.

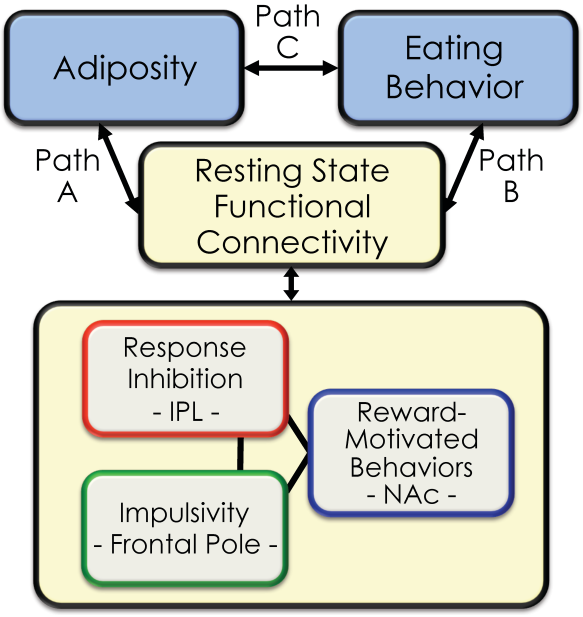


Figure 4.1: Neural model. Upper: We hypothesized that resting state functional connectivity is associated with adiposity and eating behaviors. Lower: The functional neural model is comprised of three brain regions: (1) inferior parietal lobe (IPL); (2) frontal pole; and (3) nucleus accumbens (NAc).

Response inhibition is the ability to override a planned or already initiated response (Bari *et al.*, 2011; Swick *et al.*, 2011). Neuroimaging studies show that decreased response inhibition is associated with increased BMI (Nederkoorn *et al.*, 2006; Kamijo *et al.*, 2012a; Kamijo *et al.*, 2012b; Barkin, 2013). Increased neural activity in the inferior parietal lobe (IPL) has been consistently associated with increased response inhibition (Garavan *et al.*, 2002; Swick *et al.*, 2011; Steele *et al.*, 2013; van Belle *et al.*, 2014); when comparing lean adults to obese adults (Hendrick *et al.*, 2012); and among patients with restricting type anorexia compared to patients with binge/purging eating disorders and to healthy controls (Lock *et al.*, 2011). Taken together, this evidence suggests that decreased response inhibition is associated with increased BMI, as well as decreased neural response in the IPL.

Impulsivity is a poorly conceived, risky, or inappropriate action, often resulting in undesirable consequences (Daruna and Barnes, 1993). Increased impulsivity is associated with obesity among adults and children and decreased weight loss during treatment (Nederkoorn *et al.*, 2006; Anzman and Birch, 2009; Batterink *et al.*, 2010; Kamijo *et al.*, 2012a; Fields *et al.*, 2013; Thamotharan *et al.*, 2013). The frontal pole, the most anterior part of Brodmann area 10 (BA 10), is associated with impulsivity. Decreased neural response in the frontal pole among healthy adults was associated with increased impulsivity during a delayed discounting task (Jimura *et al.*, 2013). Compared to healthy controls, adults with impulsive aggression showed decreased neural response in the frontal pole when viewing images of angry faces (Coccaro *et al.*, 2007). Decreased neural activity in the frontal pole was associated with poorer weight management in women one year after a 12-week diet (Weygandt *et al.*, 2015). Taken together, this evidence suggests that increased impulsivity is associated with increased BMI, as well as decreased neural response in the frontal pole.

The nucleus accumbens (NAc) is associated with reward, food-related reward, and reward-motivated behaviors (Delgado *et al.*, 2000; Goto and Grace, 2005; Biesdorf *et al.*, 2015) (for a comprehensive discussion of its functions, see (Floresco, 2015)). Cauda *et al.*, reported resting state functional connectivity and structure-based meta-analytic connectivity between NAc and IPL (Cauda *et al.*, 2011). Choi *et al.*, also reported resting state functional connectivity between NAc and the frontal pole (Choi *et al.*, 2012).

We will refer to response inhibition-associated resting state functional connectivity between IPL and NAc as [IPL:NAC] resting state functional connectivity (rsFC). Similarly, we will refer to impulsivity-associated resting state functional connectivity between frontal pole and NAc as [FPOLE:NAC] rsFC. And we will refer to resting state functional connectivity between frontal pole and IPL as [FPOLE:IPL] rsFC.

Given the three paths depicted in our neural model (Figure 4.1), we have a three-pronged, interdependent hypothesis. Our hypothesis is built on the premise that increased functional connectivity reflects increased functional integration (Friston *et al.*, 1997), and that decreased response inhibition and increased impulsivity are associated with increased adiposity. We hypothesized that decreased response inhibition-associated [IPL:NAC] rsFC and increased impulsivity-associated [FPOLE:NAC] rsFC will be associated with: increased adiposity (Figure 4.1, Path A); increased food approach behaviors; and decreased food avoidance behaviors (Figure 4.1, Path B). Additionally, as increased food approach behaviors and decreased food avoidance behaviors are associated with increased BMI among children ages 7-12 years (Webber *et al.*, 2009) (Figure 4.1, Path C), we hypothesized a similar association in this study. Furthermore, we hypothesized that imbalance in resting state functional connectivity mediates, in a statistical way, the relationship between eating habits and adiposity in children.

Materials and Methods.

Participants

Data were acquired from the Enhanced Nathan Kline Institute Rockland Sample (NKI-RS) (Milham, 2012) from children [8-13] years old. The NKI-RS was designed as a large dataset with broad and deep phenotypic measures and state-of-the-art neuroimaging data, in an open neuroscience model where all data are shared prospectively (Milham, 2012). A strength of the NKI-RS study is its controlled recruitment from across all of Rockland County, NY, which is representative of the US population as described by the 2010 US census (Nooner *et al.*, 2012). All participants were screened for psychiatric, neurological, and chronic medical illnesses, and for MRI safety considerations. Participants were encouraged to eat breakfast before arriving and were provided lunch. Institutional Review Board (IRB) approval was obtained at NKI and Montclair State University. Participants and their legal guardians provided written informed consent. Data were de-identified prior to receipt.

Adiposity

We used BMI *z*-scores as a proxy measure for childhood adiposity. In all statistical analyses, we used continuous BMI *z*-scores. However, when reporting summary statistics, children were classified as healthy weight for ($-1.64 \leq$ BMI *z*-scores < 1.04); overweight for ($1.04 \leq$ BMI *z*-scores < 1.64); and obese for (BMI *z*-scores ≥ 1.64) (Wang and Chen, 2012). We calculated an age- and sex-specific BMI *z*-score for each child using LMS transformation parameters *lambda*, *mu*, and *sigma* (CDC; Kuczmarski *et al.*, 2002).

Eating behaviors

The Child Eating Behaviour Questionnaire (CEBQ) is a validated 35-item questionnaire that measures 8 aspects of eating behavior (Wardle *et al.*, 2001):

1. **DD** Desire to Drink indicates frequent drinking;
2. **EF** Enjoyment of Food indicates an overall interest in food;
3. **EOE** Emotional Overeating indicates increased eating under negative emotions;
4. **EUE** Emotional Undereating indicates decreased eating under negative emotions;
5. **FF** Food Fussiness indicates rejection of both new and familiar foods;
6. **FR** Food Responsiveness assesses eating in response to food cues;
7. **SE** Slowness in Eating assesses reduced eating due to low interest and/or enjoyment of food; and
8. **SR** Satiety Responsiveness assesses how well a child controls the amount he/she eats in response to eating recently.

"Food approach" behavior is indicated by increasing DD, EF, EOE, and FR scores, whereas "food avoidance" behavior is indicated by increasing EUE, FF, SE, and SR scores (Wardle *et al.*, 2001). Food approach behaviors have been associated with increased weight among children and food avoidance behaviors have been associated with decreased weight (Carnell and Wardle, 2008; Sleddens *et al.*, 2008; Webber *et al.*, 2009; Spence *et al.*, 2011; Svensson *et al.*, 2011). The NKI-RS study was designed such that the CEBQ was administered only to children younger than 12 years old.

The NKI-RS study did not acquire food recall surveys. However, the CEBQ was developed to measure eating styles among children through parental-reporting (Wardle *et al.*, 2001). Reported behavioral measures are preferable to retrospective food recall as recalls often result in an under-estimate of food consumption due to, in part, memory bias and social expectations and pressure (Ahmed *et al.*, 2006). While a 24-hour recall may be more accurate compared to a retrospective recall, food consumption can vary greatly from day to day such that a single day may not be

representative (Block, 1982). Self-report instruments, such as the CEBQ, identify eating habits rather than actual food intake. The CEBQ has good factorial validity and external validity (van Strien *et al.*, 1986; Schlundt, 1995; Braet and van Strien, 1997; Wardle *et al.*, 2001; Carnell and Wardle, 2007).

Neural model

We defined an *a priori* model with three brain regions associated with: (1) response inhibition; (2) impulsivity; and (3) reward-motivated behaviors (Figure 4.1). The specific determination of these three regions is discussed below. Because we are interested in the functional organization of the brain, we defined regions based on functionality rather than anatomy, particularly as anatomically-defined regions may encompass functionally heterogeneous areas. To this end, we used Neurosynth `(neurosynth.org)` (Yarkoni *et al.*, 2011), which identifies functionally related brain regions via meta-analytic methods across more than 11,000 neuroimaging studies. We identified functional regions using Neurosynth's reverse inference maps. The forward inference map defines regional co-activations from a psychological term, whereas the more selective reverse inference map defines a psychological term from regional co-activations `(http://neurosynth.org/faq/#q15)`.

To investigate the possibility that our results were due to global, brain-wide phenomena, we defined a second model as a negative control. We selected *a priori* brain regions not typically associated with response inhibition or impulsivity, auditory and foot motor cortex, while retaining the same reward region, NAc.

INFERIOR PARIETAL LOBE (IPL) / RESPONSE INHIBITION

Because the IPL is associated with response inhibition, we used Neurosynth to identify an *a priori* region in the IPL via a meta-analysis using the term "response inhibition." Using the resulting reverse inference map from 176 neuroimaging studies, we identified the IPL and noted its most statistically significant voxel. We then created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]) centered on the peak *z*-score of 6.6 at (38, -54, 44) in Montreal Neurological Institute (MNI)-space (Figure 4.2).

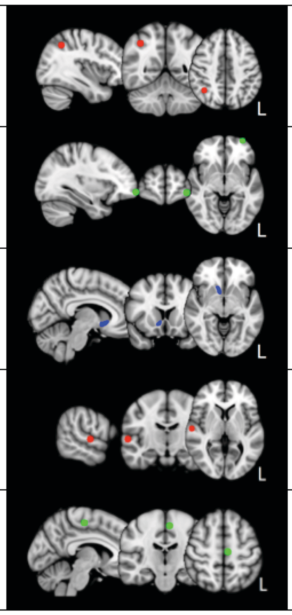
	Region	Association	Region in MNI-space	MNI coordinates / Atlas	Volume mm^3 (voxels)	
Functional Model	**Inferior parietal lobe** (IPL; right)	Response inhibition		38, -54, 44	648 (81)	
	Frontal pole (left)	Self-control		-32, 62, -6	648 (81)	
	Nucleus accumbens (NAc; right)	Reward		Harvard-Oxford subcortical atlas	472 (59)	Negative Control Model
	Auditory cortex (right)	Negative control		60, -14, 4	648 (81)	
	Foot motor cortex (left)	Negative control		-6, -20, 54	648 (81)	

Figure 4.2: Brain regions in our neural model associated with non-homeostatic eating and in our negative control model. MNI = Montreal Neurological Institute; L = left hemisphere.

FRONTAL POLE / IMPULSIVITY

Because the frontal pole is associated with impulsivity, we used Neurosynth to identify an *a priori* region in the frontal pole via a meta-analysis using the term "impulsivity." Using the resulting reverse inference map from 76 neuroimaging studies, we identified the frontal pole and

selected its most statistically significant voxel. We created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]) centered on a peak *z*-score of 5.4 at (-32, 62, -6) (Figure 4.2).

NUCLEUS ACCUMBENS (NAC) / REWARD-MOTIVATED BEHAVIORS

The NAc is associated with reward, food-related reward, and reward-motivated behavior. We used the right NAc region as defined in the Harvard-Oxford subcortical atlas (Frazier *et al.*, 2005; Desikan *et al.*, 2006; Makris *et al.*, 2006; Goldstein *et al.*, 2007) (volume = 472 mm^3 [59 voxels]) (Figure 4.2). After reviewing results using the right NAc, we examined an alternative, *post hoc* functional neural model using the left NAc. The left NAc region as defined in the Harvard-Oxford subcortical atlas has a volume of 544 mm^3 [68 voxels].

Note that animal studies are able to distinguish the NAc shell from its core. However, given the current spatial resolution of these fMRI scans performed at 3 Tesla, we were unable to resolve the NAc shell from the core in intact humans. The NAc shell is associated with reward salience, wanting, and positive reinforcement (Pecina and Berridge, 2005). The NAc core is associated with motor function related to reward (Malenka *et al.*, 2009).

NEGATIVE CONTROL NEURAL MODEL

Our negative control model was comprised of three regions: (1) auditory cortex; (2) foot motor cortex; and (3) NAc (Figure 4.2). We used Neurosynth to identify an *a priori* brain region associated with "auditory cortex." We created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]), centered on a peak *z*-score of 19.6 at (60, -14, 4) (Figure 4.2). We also identified an *a priori* brain region associated with "foot" motor cortex. We created a spherical ROI with radius 5 mm (volume = 648 mm^3 [81 voxels]), centered on a peak *z*-score of 8.3 at (-6, -20, 54) (Figure 4.2). We used the same right NAc region as defined above.

Magnetic resonance images

MRI scans were acquired on a Siemens 3T MAGNETOM TrioTim at NKI and Montclair State University. A high-resolution anatomical T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) scan with TR = 1900 ms and voxel size = $(1 \cdot 0.98 \cdot 0.98)$ mm^3 was acquired from each participant. Each subject participated in a 9.4-min blood oxygenation level-dependent (BOLD) resting-state multiband (Xu *et al.*, 2012) T2*-weighted echo planar image (EPI) scan, collected with repetition time (TR) = 1400 ms; echo time (TE) = 30 ms; flip angle = 65 degrees; multi-band acceleration factor = 4; 404 dynamics, 64 slices, and voxel size = $(2 \cdot 2 \cdot 2)$ mm^3. Children were scanned while resting quietly with eyes closed with no overt stimuli.

MRI preprocessing

We processed the MRI datasets with FMRIB Software Library (FSL) v6.00 (Jenkinson *et al.*, 2012). Preprocessing included removal of non-brain tissue (Smith, 2002); spatial smoothing using a Gaussian kernel of full-width at half maximum 3.0 mm; 4D grand-mean intensity normalization; highpass temporal filtering using Gaussian-weighted least-squares straight line fitting with sigma = 200 sec; motion correction (Jenkinson *et al.*, 2002); and linear and nonlinear spatial normalization (Jenkinson *et al.*, 2002; Andersson *et al.*, 2007; Andersson *et al.*, 2008; Greve and Fischl, 2009) to an age-appropriate MRI brain atlas for ages [7.5-13.5] years old (Fonov *et al.*, 2009; Fonov *et al.*, 2011). We discarded any scan during which a participant moved more than 2 mm.

Resting state functional connectivity

Biswal *et al.,* observed that BOLD fMRI signals from the motor cortex during quiet rest were strongly correlated with signals in other brain regions associated with motor function (Biswal *et*

al., 1995). This observation gave rise to the idea of "resting state" brain function: when the brain is not engaged in an explicit task, the low-frequency changes in neural response reflect inherent brain function along with its attendant networks (Fox and Raichle, 2007). The functional coupling between distal brain regions can be quantified by the statistical correlation of BOLD fMRI signals. The pattern of correlation throughout the brain, called functional connectivity (Biswal *et al.*, 1995; Friston, 2011; Smith *et al.*, 2012), is believed to reflect neurons firing together with a common purpose (Saini *et al.*, 2004; Lewis *et al.*, 2009; Cole *et al.*, 2010), and can reveal whole-brain functional connectivity patterns (van den Heuvel *et al.*, 2008).

For each participant, we calculated mean BOLD signals from each region in our neural model. To reduce noise from non-grey matter activity, we regressed out the following confounders: mean relative motion correction distance (Power *et al.*, 2012); mean BOLD signal from white matter (O'Reilly *et al.*, 2010); and mean BOLD signal from cerebral spinal fluid (Dagli *et al.*, 1999). We removed unwanted signal fluctuation due to respiration and heartbeat via a 0.10 Hz lowpass filter (Van Dijk *et al.*, 2010). Using partial correlation, we calculated the functional connectivity between pairs of BOLD signals from the three regions: (1) IPL and NAc, denoted as [IPL:NAC] rsFC; (2) frontal pole and NAc, denoted as [FPOLE:NAC] rsFC; and (3) IPL and frontal pole, denoted as [FPOLE:IPL] rsFC. We used partial correlation to remove common effects from the other region within the model. For example, the resulting [IPL:NAC] rsFC is the correlation between IPL and NAc over and above any correlation with the frontal pole, *i.e.*, controlling for the effects of the frontal pole.

We examined the partial correlation coefficient, often denoted as $\rho_{XY.Z}$, as it is a measure of the strength of the relationship between BOLD signals X and Y, after controlling for another BOLD signal, Z. $\rho_{XY.Z}$ is bounded by [-1, +1]. A $\rho_{XY.Z}$ approaching ±1 indicates that X and Y are

approaching a perfect linear relationship. A related, although different, measure is β, the effect (or slope) of BOLD signal *X* on BOLD signal *Y*, after controlling for BOLD signal *Z* (Eq. 1). β can be estimated via a simple general linear model (GLM):

$$Y = \alpha + \beta X + \gamma Z + \varepsilon \tag{4.1}$$

Equation 4.1. A simple general linear model.

β is unbounded and indicates the change of the expected value of *Y* for each 1-unit change in *X* after controlling for *Z*. β is also called an "effect," *i.e.*, when *X* is changed by +1 unit, the effect on *Y* is a change of β units. β and $\rho_{XY.Z}$ are related as shown in Eq. 2 (Kenney and Keeping, 1962). β and $\rho_{XY.Z}$ are equal only when `std(X)` and `std(Y)` are equal.

$$\beta = \rho_{XY.Z} \cdot \frac{std(X)}{std(Y)} \tag{4.2}$$

Equation 4.2. Relation of β and $\rho_{XY.Z}$ where `std(X)` is the standard deviation of *X* and `std(Y)` is the standard deviation of *Y*.

We chose not to investigate β as it is conceivable that the effect can be transformed via a change in neural response (*e.g.*, via a neural gain function) while the strength of the relationship remains unchanged. Changes in neural response may be altered due to different levels of CO_2 in the blood (Davis *et al.*, 1998; Cohen *et al.*, 2002); changes in vasoconstriction, *e.g.*, from caffeine use (Laurienti *et al.*, 2002; Mulderink *et al.*, 2002); or changes in metabolic demand (Ogawa *et al.*, 1990). However, the partial correlation coefficient, $\rho_{XY.Z}$, quantifies the strength of the relationship between *X* and *Y* regardless of the effect quantified by β.

We computed partial correlation coefficients via MATLAB's `partialcorr` (Release 2014a, The MathWorks, Inc., Natick, MA). We designated functional connectivity as statistically significant

if the association has a p-value ≤ 0.05. If (0.05 < p-value ≤ 0.10), then we designated functional connectivity as trending toward statistical significance.

Relative difference in brain functional connectivity

To capture in a single measure the relative difference in functional connectivity between response inhibition-associated [IPL:NAc] rsFC and impulsivity-associated [fPole:NAc] rsFC, we calculated a simple difference measure that reflects resting state functional connectivity imbalance:

$$DELTA = ([fPole: NAc]\ rsFC - [IPL: NAc]\ rsFC) \quad (4.3)$$

Equation 4.3. Difference between rsFC measures indicating imbalance in resting state functional connectivity.

DELTA values can range from [-2, +2] where positive values indicate greater impulsivity-associated [fPole:NAc] rsFC relative to response inhibition-associated [IPL:NAc] rsFC; negative values indicate greater [IPL:NAc] rsFC relative to [fPole:NAc] rsFC. For example, if the BOLD signal from the frontal pole is perfectly in-sync with the BOLD signal from the NAc, then [fPole:NAc] rsFC = +1. And if the BOLD signal from the IPL is perfectly out-of-sync with the BOLD signal from the NAc, then [IPL:NAc] rsFC = -1. In this example, then:

$$DELTA = ([fPole: NAc]\ rsFC - [IPL: NAc]\ \ rsFC) = \big(+1 - (-1)\big) = +2 \quad (4.4)$$

Equation 4.4. Example of maximal difference between rsFC measures that is biased toward impulsivity-associated [fPole:NAc] functional connectivity.

DELTA = +2 indicates that the two functional connectivity measures to the NAc are maximally different with greater "in sync" functional connectivity between the frontal pole and NAc. To clarify, DELTA is not a measure of functional connectivity. Rather, DELTA is a single measure that

indicates the relative imbalance between the two functional connectivity measures with respect to the NAc. DELTA = 0 indicates that impulsivity-associated [fPOLE:NAC] functional connectivity is in balance with response inhibition-associated [IPL:NAC] functional connectivity, regardless of the actual value of the functional connectivity measures. For example, DELTA = 0 when [fPOLE:NAC] = [IPL:NAC] = 0.80, or when [fPOLE:NAC] = [IPL:NAC] = -0.10. We computed simple linear regressions between adiposity and DELTA via Python's `scipy.stats.linregress`.

Statistical analyses

RELATIONSHIP OF ADIPOSITY WITH BRAIN FUNCTIONAL CONNECTIVITY (FIGURE 4.1, PATH A).

To evaluate the relationship between adiposity, measured by BMI *z*-score, and resting state functional connectivity, we computed simple linear regressions between BMI *z*-scores and functional connectivity values, [IPL:NAC] rsFC, [fPOLE:NAC] rsFC, and [fPOLE:IPL] rsFC, via Python's `scipy.stats.linregress` (`iPython` version 3.2.0; `scipy` version 0.15.1; `statsmodels` version 0.6.1). We also computed linear regressions between BMI *z*-score and DELTA, the relative imbalance in functional connectivity. To evaluate the effect of age on the relationships between BMI *z*-score and rsFC and DELTA, we computed ordinary least squares (OLS) linear regression via Python's `statsmodels.formula.api.ols`.

RELATIONSHIPS OF EATING BEHAVIORS WITH BRAIN FUNCTIONAL CONNECTIVITY (FIGURE 4.1, PATH B).

To evaluate the relationship between eating behavior and resting state functional connectivity, we computed simple linear regressions between CEBQ scores and each of the functional connectivity values, [IPL:NAC] rsFC, [fPOLE:NAC] rsFC, and [fPOLE:IPL] rsFC, via Python's `scipy.stats.linregress`. We also computed linear regressions between CEBQ scores and

DELTA, the relative imbalance in functional connectivity. In the initial validation of the CEBQ by Wardle *et al.*, they noted that only FF showed a sex difference, which was greater among boys ($t = 2.4$; $p \leq 0.02$) (Wardle *et al.*, 2001). We therefore performed a *post hoc* linear regression with interaction analysis of brain functional connectivity by sex with FF via Python's `statsmodels.formula.api.ols`.

RELATIONSHIP BETWEEN EATING BEHAVIORS AND ADIPOSITY (FIGURE 4.1, PATH C).

To evaluate the relationship between eating behavior and adiposity, we computed simple linear regressions between CEBQ scores and BMI *z*-scores via Python's `scipy.stats.linregress`. We also performed *post hoc* linear regression with interaction analysis of FF by sex with BMI *z*-score via Python's `statsmodels.formula.api.ols`.

BRAIN FUNCTIONAL CONNECTIVITY MEDIATION BETWEEN ADIPOSITY AND EATING BEHAVIORS.

Mediation can be assessed when statistically significant relationships are found in all three paths, A, B, and C, in Figure 4.1. We performed mediation analysis using Bootstrap Regression Analysis of Voxelwise Observations (BRAVO), v2.0 (Gianaros *et al.*, 2013). To determine the significance of the relationships of each path, we ran BRAVO's bootstrap permutation tests with 10,000 simulations.

Results.

Participants

Data from 38 children (F=17; M=21), ages [8-13] (mean=11.2; std=1.7) years old, were acquired from the NKI-RS (Table 4.1). Figure 4.3 shows the distribution of BMI *z*-score *vs.* age. There was no significant relationship of BMI *z*-score with age ($p = 0.766$; $R^2 = 0.002$; $N = 38$). Nor is there a relationship of BMI *z*-score with sex (girls: $p = 0.981$; $R^2 = 0.000$; $N = 17$; boys: $p =$

0.730; R^2 = 0.006; N = 21). Five of the 38 participants (13%) were classified as obese. This is comparable to 17% of US children who were classified as obese in 2010 (Ogden *et al.*, 2012). Six of the 38 participants (16%) were classified as overweight, which is comparable to 15% of US children classified as overweight in 2010 (Ogden *et al.*, 2012). Twenty-four of the 38 participants (63%) completed the CEBQ as the CEBQ was administered only to children younger than 12 years old (mean=10.1; sd=1.1 years old). Of these 24 children, 11 (46%) were girls and 13 (54%) were boys. Of the 24 children who were administered the CEBQ, 3 (12.5%) were classified as obese; 3 (12.5%) were classified as overweight; and 18 (75%) were classified as healthy weight. Forty-two percent of participants were scanned between 8:30-10:00 am; 18.4% were scanned between 10:00 am-noon; 39.5% of participants were scanned between noon-2:00 pm.

Adiposity is associated with resting state functional connectivity (Figure 4.1, Path A)

Table 4.2 lists the relationships between adiposity and [IPL:NAC] rsFC. Table 4.3 lists the relationships between adiposity and [FPOLE:NAC] rsFC. Table 4.4 lists the relationships between adiposity and [FPOLE:IPL] rsFC. Table 4.5 lists the relationships between adiposity and DELTA.

Increasing BMI *z*-scores trended toward significance with decreasing response inhibition-associated [IPL:NAC] rsFC (p = 0.084; R^2 = 0.080; r = -0.284; Table 4.2; Figure 4.4A). In contrast, increasing BMI *z*-scores trended toward significance with increasing impulsivity-associated [FPOLE:NAC] rsFC (p = 0.089; R^2 = 0.078; r = 0.280; Table 4.3; Figure 4.4B). There was no significant relationship between BMI *z*-scores and [FPOLE:IPL] rsFC (p = 0.525; R^2 = 0.011; r = -0.106; Table 4.4). BMI *z*-scores increased with increasing DELTA (p = 0.035; R^2 = 0.117; r = 0.342; Table 4.5; Figure 4.4C). There was no significant association of age in the relationship between BMI *z*-score and any rsFC measure or DELTA (all $p \geq 0.688$).

Sex (N=38)	**Count (%)**	
Female	17 (44.7)	
Male	21 (55.3)	
Handedness (N=36)		
Right	30 (83.3)	
Left	5 (13.9)	
Ambidextrous	1 (2.8)	
Race (N=38)		
American Indian or Native Alaskan	3 (7.89)	
Asian	2 (5.26)	
Black or African American	16 (42.11)	
Native Hawaiian or Other Pacific Islander	0 (0.00)	
White	17 (44.74)	
Other Race	0 (0.00)	
(N=38)	**Mean (sd)**	**Min, Max**
Age (yrs)	11.2 (1.7)	8.4, 13.9
Weight (kg)	44.0 (13.9)	25.9, 81.7
BMI *z*-score	0.4 (1.1)	-1.4, 2.4
BMI%	59.9 (30.4)	8.4, 99.2
Tanner stage (N=36)		
Girls (N=17)	2.3 (1.0)	1, 4
Boys (N=19)	2.2 (1.2)	1, 5

Table 4.1. Clinical and demographic summary. F: female; M: male; sd: standard deviation; BMI: body mass index.

Eating behaviors are associated with resting state functional connectivity (Figure 4.1, Path B)

FOOD APPROACH EATING BEHAVIOR ENJOYMENT OF FOOD (EF)

Table 4.2 lists the relationships between eating behaviors and [IPL:NAC] rsFC. Table 4.3 lists the relationships between eating behaviors and [FPOLE:NAC] rsFC. Table 4.4 lists the relationships between eating behaviors and [FPOLE:IPL] rsFC. Table 4.5 lists the relationships between eating behaviors and DELTA.

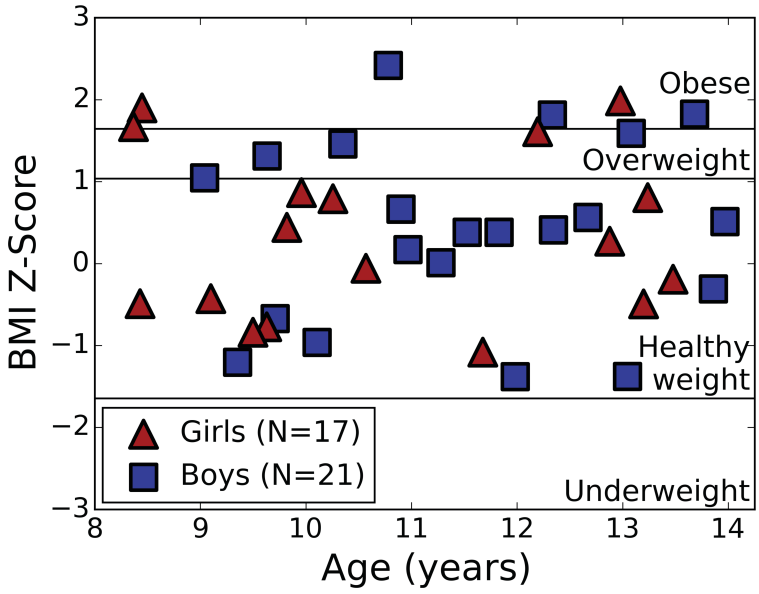


Figure 4.3: Distribution of BMI *z*-score *vs.* age. Children were classified as healthy weight for (-1.64 ≤ BMI z-scores < 1.04); overweight for (1.04 ≤ BMI z-scores < 1.64); and obese for (BMI z-scores ≥ 1.64).

Neural model: [IPL:NAc] resting state functional connectivity					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs. [IPL:NAc]	-0.284	0.080	0.084 †	
CEBQ eating behaviors vs. [IPL:NAc]		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	0.043	0.002	0.843	Food Approach
	EF: Enjoyment of Food	-0.472	0.223	0.020 *	
	EOE: Emotional Overeating	-0.371	0.138	0.074 †	
	FR: Food Responsiveness	-0.427	0.182	0.037 *	
N = 24	EUE: Emotional Under-Eating	0.047	0.002	0.827	Food Avoidance
	FF: Food Fussiness	0.224	0.050	0.294	
	SE: Slowness in Eating	0.345	0.119	0.098 †	
	SR: Satiety Responsiveness	0.352	0.124	0.092 †	

Table 4.2. Relationships of adiposity *vs.* [IPL:NAc] rsFC, and eating behaviors *vs.* [IPL:NAc] rsFC. rsFC: resting state functional connectivity; IPL: inferior parietal lobe; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Neural model: [fPole:NAc] resting state functional connectivity					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. [fPole:NAc]	0.280	0.078	0.089 †	
CEBQ eating behaviors vs. [fPole:NAc]		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	-0.208	0.043	0.330	Food Approach
	EF: Enjoyment of Food	0.361	0.130	0.083 †	
	EOE: Emotional Overeating	0.296	0.088	0.160	
	FR: Food Responsiveness	0.256	0.066	0.227	
N = 24	EUE: Emotional Under-Eating	-0.216	0.047	0.311	Food Avoidance
	FF: Food Fussiness	-0.474	0.224	0.019 *	
	SE: Slowness in Eating	-0.416	0.173	0.043 *	
	SR: Satiety Responsiveness	-0.425	0.181	0.038 *	

Table 4.3. Relationships of adiposity *vs.* [fPole:NAc] rsFC, and eating behaviors *vs.* [fPole:NAc] rsFC. rsFC: resting state functional connectivity; fPole: frontal pole; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Neural model: [fPole:IPL] resting state functional connectivity					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. [fPole:IPL]	-0.106	0.011	0.525	
CEBQ eating behaviors vs. [fPole:IPL]		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	-0.110	0.012	0.608	Food Approach
	EF: Enjoyment of Food	-0.234	0.055	0.271	
	EOE: Emotional Overeating	-0.070	0.005	0.746	
	FR: Food Responsiveness	0.002	0.000	0.992	
N = 24	EUE: Emotional Under-Eating	-0.337	0.114	0.107	Food Avoidance
	FF: Food Fussiness	0.071	0.005	0.743	
	SE: Slowness in Eating	0.067	0.005	0.754	
	SR: Satiety Responsiveness	0.035	0.001	0.871	

Table 4.4. Relationships of adiposity *vs.* [fPole:IPL] rsFC, and eating behaviors *vs.* [fPole:IPL] rsFC. rsFC: resting state functional connectivity; fPole: frontal pole; IPL: inferior parietal lobe; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Neural model: DELTA = [fPOLE :NAC] – [IPL:NAC]					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. DELTA	0.342	0.117	0.035 *	
CEBQ eating behaviors vs. DELTA		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	-0.153	0.023	0.477	Food Approach
	EF: Enjoyment of Food	0.482	0.232	0.017 *	
	EOE: Emotional Overeating	0.386	0.149	0.062 †	
	FR: Food Responsiveness	0.392	0.154	0.058 †	
N = 24	EUE: Emotional Under-Eating	-0.160	0.026	0.455	Food Avoidance
	FF: Food Fussiness	-0.417	0.174	0.043 *	
	SE: Slowness in Eating	-0.447	0.200	0.028 *	
	SR: Satiety Responsiveness	-0.456	0.208	0.025 *	

Table 4.5. Relationships of adiposity *vs.* DELTA, and eating behaviors *vs.* DELTA. DELTA: difference in resting state functional connectivity measures; fPole: frontal pole; IPL: inferior parietal lobe; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

EF scores increased with decreasing response inhibition-associated [IPL:NAC] rsFC ($p = 0.020$; $R^2 = 0.223$; $r = -0.472$; Figure 4.4D; Table 4.2). In contrast, the relationship between EF scores and impulsivity-associated [FPOLE:NAC] rsFC trended toward a positive increase ($p = 0.083$; $R^2 = 0.130$; $r = 0.361$; Figure 4.4E; Table 4.3). There was no significant relationship between EF and [FPOLE:IPL] rsFC ($p = 0.271$; $R^2 = 0.055$; $r = -0.234$; Table 4.4). EF scores increased with increasing DELTA ($p = 0.017$; $R^2 = 0.232$; $r = 0.482$; Figure 4.4F; Table 4.5).

FOOD AVOIDANCE EATING BEHAVIOR SATIETY RESPONSIVENESS (SR)

Increasing SR scores trended toward significance with increasing [IPL:NAC] rsFC ($p = 0.092$; $R^2 = 0.124$; $r = 0.352$; Figure 4.4G; Table 4.2). In contrast, SR scores decreased with increasing [FPOLE:NAC] rsFC ($p = 0.038$; $R^2 = 0.181$; $r = -0.425$; Figure 4.4H; Table 4.3). There was no significant relationship between SR and [FPOLE:IPL] rsFC ($p = 0.871$; $R^2 = 0.001$ $r = 0.035$;

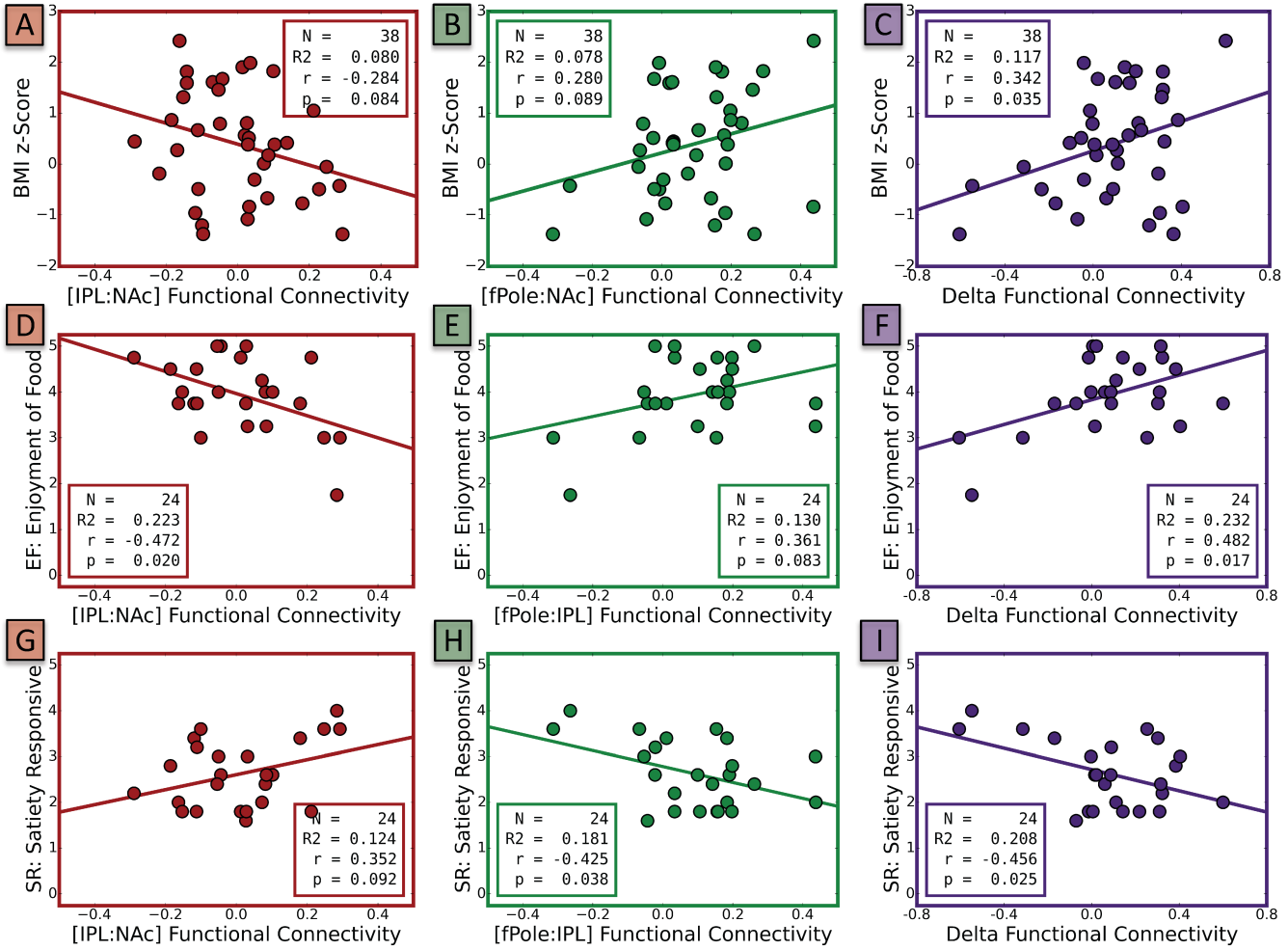


Figure 4.4: Relationships of adiposity and eating habits with neural model associated with non-homeostatic eating. (A) Association of BMI *z*-score *vs.* [IPL:NAC] resting state functional connectivity (rsFC). (B) Association of BMI *z*-score *vs.* [FPOLE:NAC] rsFC. (C) Association of BMI *z*-score *vs.* DELTA, a measure of impulsivity-biased imbalance in rsFC. (D) Food approach eating behavior Enjoyment of Food (EF) *vs.* [IPL:NAC] rsFC. (E) EF *vs.* [FPOLE:NAC] rsFC. (F) EF *vs.* DELTA. (G) Food avoidance eating behavior Satiety Responsiveness (SR) *vs.* [IPL:NAC] rsFC. (H) SR *vs.* [FPOLE:NAC] rsFC. (I) SR *vs.* DELTA. [IPL:NAC] rsFC: resting state functional connectivity between inferior parietal lobe (IPL) and nucleus accumbens (NAc); [FPOLE:NAC] rsFC: resting state functional connectivity between frontal pole (FPOLE) and NAc; DELTA Functional Connectivity: The difference in resting state functional connectivity measurements ([FPOLE:NAC] - [IPL:NAC]); EF: Enjoyment of Food; SR: Satiety Responsiveness.

Table 4.4). SR scores decreased with increasing DELTA ($p = 0.025$; $R^2 = 0.208$; $r = -0.456$; Figure 4.4I; Table 4.5). Our *post hoc* analysis of FF scores with functional connectivity by sex showed no statistically significant main effects for functional connectivity or for sex (all $p \geq 0.388$).

Adiposity is associated with eating behaviors (Figure 4.1, Path C)

Table 4.6 lists the relationships between BMI *z*-score and each CEBQ score. BMI *z*-scores increased with increasing food approach behaviors in a statistically significant way (all $p \leq 0.002$), with the exception of DD ($p = 0.917$). Concomitantly, BMI *z*-scores decreased with increasing food avoidance behaviors SE ($p = 0.002$; $R^2 = 0.353$; $r = -0.594$) and SR ($p = 0.005$; $R^2 = 0.311$; $r = -0.558$). EUE and FF show no statistically significant relationships (EUE: $p = 0.176$; FF: $p = 0.103$). Our *post hoc* analysis of BMI *z*-scores as a function of FF by sex showed no statistically significant main effects for FF or for sex (FF: $p = 0.427$; $t = -0.811$; sex: $p = 0.835$; $t = -0.211$) and no significant interaction between FF and sex ($p = 0.758$; $t = -0.312$).

BMI *z*-score *vs.* CEBQ eating behaviors					
		r	***R²***	***p***	
N = 24	DD: Desire to Drink	-0.022	0.001	0.917	Food Approach
	EF: Enjoyment of Food	0.591	0.349	0.002 **	
	EOE: Emotional Overeating	0.623	0.388	0.001 **	
	FR: Food Responsiveness	0.698	0.487	0.000 **	
N = 24	EUE: Emotional Under-Eating	0.286	0.082	0.176	Food Avoidance
	FF: Food Fussiness	-0.341	0.116	0.103	
	SE: Slowness in Eating	-0.594	0.353	0.002 **	
	SR: Satiety Responsiveness	-0.558	0.311	0.005 **	

Table 4.6. Relationships between adiposity and eating behaviors. CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Brain functional connectivity mediation between adiposity and eating behaviors

After regressing out the effects of the third measurement via partial regression, we found no relationships in all paths, A, B, and C, which were eligible for mediation analysis.

Post hoc neural model with left nucleus accumbens

We performed a *post hoc* analysis of an alternative model with the left NAc. With one exception, there were no statistically significant associations between functional connectivity measures with BMI *z*-scores or with eating behaviors (Tables 4.7, 4.8, 4.9, and 4.10). We found a negatively trending relationship between EUE and [fPOLE:IPL] rsFC ($p = 0.063$; $R^2 = 0.149$; $r = -0.386$; Table 4.9).

Left NAc | Neural model: [IPL:NAC] functional connectivity					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs [IPL:NAC]	0.000	0.000	0.998	
CEBQ eating behaviors vs. [IPL:NAC]		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	0.123	0.015	0.568	Food Approach
	EF: Enjoyment of Food	-0.341	0.116	0.103	
	EOE: Emotional Overeating	0.023	0.001	0.915	
	FR: Food Responsiveness	0.076	0.006	0.725	
N = 24	EUE: Emotional Under-Eating	0.072	0.005	0.740	Food Avoidance
	FF: Food Fussiness	0.123	0.015	0.568	
	SE: Slowness in Eating	0.202	0.041	0.344	
	SR: Satiety Responsiveness	0.140	0.020	0.514	

Table 4.7. Relationships of adiposity *vs.* [IPL:NAC] rsFC, and eating behaviors *vs.* [IPL:NAC] rsFC, with left nucleus accumbens. This *ad hoc* neural model includes the left nucleus accumbens (NAc). rsFC: resting state functional connectivity; IPL: inferior parietal lobe; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Left NAc | Neural model: [fPOLE:NAC] functional connectivity					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. [fPOLE:NAC]	-0.001	0.000	0.997	
CEBQ eating behaviors vs. [fPOLE:NAC]		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	-0.038	0.001	0.860	Food Approach
	EF: Enjoyment of Food	-0.272	0.074	0.198	
	EOE: Emotional Overeating	-0.328	0.108	0.117	
	FR: Food Responsiveness	-0.352	0.124	0.091	
N = 24	EUE: Emotional Under-Eating	0.030	0.001	0.890	Food Avoidance
	FF: Food Fussiness	-0.093	0.009	0.666	
	SE: Slowness in Eating	-0.025	0.001	0.908	
	SR: Satiety Responsiveness	-0.016	0.000	0.943	

Table 4.8. Relationships of adiposity *vs.* [fPOLE:NAC] rsFC, and eating behaviors *vs.* [fPOLE:NAC] rsFC, with left nucleus accumbens. This *ad hoc* neural model includes the left nucleus accumbens (NAc). rsFC: resting state functional connectivity; fPole: frontal pole; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Left NAc | Neural model: [fPOLE:IPL] functional connectivity					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. [fPOLE:IPL]	-0.068	0.005	0.683	
CEBQ eating behaviors vs. [fPOLE:IPL]		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	-0.149	0.022	0.487	Food Approach
	EF: Enjoyment of Food	-0.212	0.045	0.320	
	EOE: Emotional Overeating	-0.076	0.006	0.726	
	FR: Food Responsiveness	-0.013	0.000	0.952	
N = 24	EUE: Emotional Under-Eating	-0.386	0.149	0.063	Food Avoidance
	FF: Food Fussiness	0.050	0.002	0.817	
	SE: Slowness in Eating	0.040	0.002	0.853	
	SR: Satiety Responsiveness	0.013	0.000	0.951	

Table 4.9. Relationships of adiposity *vs.* [fPOLE:IPL] rsFC, and eating behaviors *vs.* [fPOLE:IPL] rsFC, with left nucleus accumbens. This *ad hoc* neural model includes the left nucleus accumbens (NAc), which was controlled for during partial correlation. rsFC: resting state functional connectivity; fPole: frontal pole; IPL: inferior parietal lobe; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Left NAc | Neural model: DELTA = [fPOLE :NAC] – [IPL:NAC]					
		r	***R^2***	***p***	
N = 38	BMI *z*-score vs. DELTA	0.0001	0.000	0.997	
CEBQ eating behaviors vs. DELTA		***r***	***R^2***	***p***	
N = 24	DD: Desire to Drink	0.113	0.013	0.600	Food Approach
	EF: Enjoyment of Food	-0.076	0.006	0.726	
	EOE: Emotional Overeating	0.223	0.050	0.294	
	FR: Food Responsiveness	0.277	0.077	0.190	
N = 24	EUE: Emotional Under-Eating	0.033	0.001	0.878	Food Avoidance
	FF: Food Fussiness	0.147	0.022	0.492	
	SE: Slowness in Eating	0.162	0.026	0.449	
	SR: Satiety Responsiveness	0.111	0.012	0.605	

Table 4.10. Relationships of adiposity *vs.* DELTA, and eating behaviors *vs.* DELTA, with left nucleus accumbens. This *ad hoc* neural model includes the left nucleus accumbens (NAc). DELTA: difference in resting state functional connectivity measures; fPole: frontal pole; IPL: inferior parietal lobe; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Negative control neural model

To investigate whether our results were due to global, brain-wide phenomena, we defined a second neural model as a negative control. This functional neural network included auditory and foot motor cortex regions and the right NAc. With one exception, we found no associations between adiposity and functional connectivity, or between eating habits and functional connectivity (Tables 4.11, 4.12, 4.13, and 4.14). EF scores increased with increasing [FOOT:NAC] functional connectivity ($p = 0.028$; $R^2 = 0.201$; $r = 0.448$; Table 4.11).

Negative control model: [FOOT:NAc] functional connectivity					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs. [FOOT:NAc]	0.101	0.010	0.548	
CEBQ eating behaviors vs. [FOOT:NAc]		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	-0.346	0.120	0.098	Food Approach
	EF: Enjoyment of Food	0.448	0.201	0.028 *	
	EOE: Emotional Overeating	0.202	0.041	0.345	
	FR: Food Responsiveness	0.033	0.001	0.879	
N = 24	EUE: Emotional Under-Eating	0.042	0.002	0.844	Food Avoidance
	FF: Food Fussiness	-0.246	0.061	0.246	
	SE: Slowness in Eating	-0.229	0.053	0.281	
	SR: Satiety Responsiveness	-0.362	0.131	0.082	

Table 4.11. Relationships of adiposity *vs.* [FOOT:NAc] rsFC, and eating behaviors *vs.* [FOOT:NAc] rsFC, with right nucleus accumbens. rsFC: resting state functional connectivity; Foot: foot motor cortex; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Negative control model: [AUDITORY:NAc] functional connectivity					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs. [AUDITORY:NAc]	0.280	0.079	0.088	
CEBQ eating behaviors vs. [AUDITORY:NAc]		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	0.026	0.001	0.905	Food Approach
	EF: Enjoyment of Food	0.207	0.043	0.332	
	EOE: Emotional Overeating	0.193	0.037	0.367	
	FR: Food Responsiveness	0.237	0.056	0.264	
N = 24	EUE: Emotional Under-Eating	-0.033	0.001	0.879	Food Avoidance
	FF: Food Fussiness	-0.074	0.005	0.731	
	SE: Slowness in Eating	-0.130	0.017	0.545	
	SR: Satiety Responsiveness	0.013	0.000	0.953	

Table 4.12. Relationships of adiposity *vs.* [AUDITORY:NAc] rsFC, and eating behaviors *vs.* [AUDITORY:NAc] rsFC, with right nucleus accumbens. rsFC: resting state functional connectivity; Auditory: auditory cortex; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Negative control model: [FOOT:AUDITORY] functional connectivity					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs. [FOOT:AUDITORY]	-0.078	0.006	0.642	
CEBQ eating behaviors vs. [FOOT:AUDITORY]		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	-0.059	0.004	0.783	Food Approach
	EF: Enjoyment of Food	0.022	0.000	0.918	
	EOE: Emotional Overeating	-0.241	0.058	0.257	
	FR: Food Responsiveness	-0.243	0.059	0.252	
N = 24	EUE: Emotional Under-Eating	0.100	0.010	0.642	Food Avoidance
	FF: Food Fussiness	-0.064	0.004	0.767	
	SE: Slowness in Eating	0.315	0.099	0.134	
	SR: Satiety Responsiveness	0.117	0.014	0.587	

Table 4.13. Relationships of adiposity *vs.* [FOOT:AUDITORY] rsFC, and eating behaviors *vs.* [FOOT:AUDITORY] rsFC, with right nucleus accumbens. rsFC: resting state functional connectivity; Foot: foot motor cortex; Auditory: auditory cortex; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Negative control model: DELTA = [FOOT:NAc] – [AUDITORY:NAc]					
		r	***R²***	***p***	
N = 38	BMI *z*-score vs. DELTA	-0.134	0.018	0.424	
CEBQ eating behaviors vs. DELTA		***r***	***R²***	***p***	
N = 24	DD: Desire to Drink	-0.235	0.055	0.268	Food Approach
	EF: Enjoyment of Food	0.145	0.021	0.500	
	EOE: Emotional Overeating	-0.001	0.000	0.995	
	FR: Food Responsiveness	-0.138	0.019	0.521	
N = 24	EUE: Emotional Under-Eating	0.049	0.002	0.822	Food Avoidance
	FF: Food Fussiness	-0.106	0.011	0.622	
	SE: Slowness in Eating	-0.058	0.003	0.788	
	SR: Satiety Responsiveness	-0.237	0.056	0.265	

Table 4.14. Relationships of adiposity *vs.* DELTA, and eating behaviors *vs.* DELTA, with right nucleus accumbens. DELTA: difference in resting state functional connectivity measures; Foot: foot motor cortex; Auditory: auditory cortex; NAc: nucleus accumbens; CEBQ: Child Eating Behaviour Questionnaire; indicators for statistical significance: **: $p \leq 0.01$; *: $p \leq 0.05$; †: $p \leq 0.10$.

Discussion.

As a means of better understanding the organization and communication of the young obese brain, we investigated a neural model in a cohort of children using *a priori*-defined, seed-based resting state functional connectivity. We focused on the relationships of resting state functional connectivity with adiposity and with eating behaviors. We investigated functional connectivity between regions associated with response inhibition (inferior parietal lobe [IPL]), impulsivity (frontal pole), and reward (nucleus accumbens [NAc]). Our results suggest the following key findings.

Finding 1. Eating behaviors and adiposity

In agreement with other childhood obesity studies, increasing food approach behavioral scores – enjoyment of food (EF), food responsiveness (FR), and emotional overeating (EOE) – and decreasing food avoidance behavioral scores – slowness in eating (SE) and satiety responsiveness (SR) – are associated with increasing adiposity.

Finding 2: Resting state functional connectivity and adiposity

Adiposity is associated with resting state functional connectivity within our neural model among children ages 8-13 years old. As response inhibition-associated functional connectivity increases, adiposity decreases in a statistically trending relationship. As impulsivity-associated functional connectivity increases, adiposity increases in a statistically trending relationship. As the difference between these two functional connectivity measures – between response inhibition-associated and impulsivity-associated resting state functional connectivity with the NAc – is biased toward impulsivity, adiposity increases in a statistically significant manner.

Finding 3: Resting state functional connectivity and eating behaviors

Eating behaviors are associated with resting state functional connectivity within our neural model among children ages 8-13 years old. As response inhibition-associated functional connectivity increases, food approach behaviors EF and FR decrease, while food avoidance behaviors SE and SR trend toward an increasing relationship. As impulsivity-associated resting state functional connectivity increases, food approach behavior EF trends toward an increasing relationship, while food avoidance eating behaviors food fussiness (FF), SE, and SR decrease. As the difference between these two functional connectivity measures is biased toward impulsivity, food approach behaviors increase while food avoidance behaviors decrease in a statistically significant manner.

Finding 4: Resting state functional connectivity relationships are not a global, brain-wide phenomenon

The relationships of resting state functional connectivity with adiposity and with eating behaviors are not a global, brain-wide phenomenon, with the exception of enjoyment of food.

Taken together, these results suggest that, in the absence of any explicit food-related stimuli, the developing brain is primed toward food approach and away from food avoidance behavior with increasing adiposity. While this bias is advantageous in an evolutionary sense, it is detrimental in today's environment of easy accessibility to high-energy dense food, as indicated by an associated increase in adiposity and unhealthy eating habits among children. Our results suggest a persistent relationship between resting state functional connectivity and enjoyment of food. We speculate that this is indicative of the importance of enjoying food to survival.

Also of note, resting state functional connectivity imbalance associated with adiposity and eating habits develops during childhood, as early as 8-13 years of age. This early development indicates the importance of identifying children at risk for obesity for earlier intervention.

Our results indicate that associations with increased adiposity and unhealthy eating behaviors are driven not solely by decreased response inhibition-associated resting state functional connectivity and not solely by increased impulsivity-associated resting state functional connectivity. Rather, increased adiposity and unhealthy eating behaviors are most strongly associated with the *imbalance* between response inhibition- and impulsivity-associated functional connectivity.

This neural imbalance suggests that mindfulness may help treat and/or prevent childhood obesity. Mindfulness is described as paying attention *on purpose* and being in the present moment with acceptance and without judgment (Kabat-Zinn, 2003). Mindfulness is associated with increased response inhibition (Sahdra *et al.*, 2011; Friese *et al.*, 2012) and decreased impulsivity (Lattimore *et al.*, 2011; Peters *et al.*, 2011; Teper and Inzlicht, 2013). As brain regions associated with response inhibition, impulsivity, and reward are recognized as potent modulators of non-homeostatic eating habits, mindfulness may recalibrate an imbalance in neural systems associated with childhood obesity. The use of mindfulness for weight loss and weight control among adults has produced mixed results (Katterman *et al.*, 2014; Olson and Emery, 2015). This may indicate the extreme tenaciousness of adult obesity, perhaps reflecting a relative lack of "plasticity" in the adult brain, further arguing for the importance of early identification and treatment of children at risk for increased adiposity. While mindfulness is readily translatable to children, and encourages them to respond to everyday adversity in healthy ways (Greenberg and Harris, 2012; Godsey, 2013), few studies report mindfulness for weight loss, weight maintenance, or eating healthfully among children.

Among food approach behaviors, DD was not associated with brain network imbalance (p = 0.495), nor with BMI z-score (p = 0.917). Some studies have reported no relationship between DD and weight (Sweetman *et al.*, 2008), while others have reported associations with the consumption of *sweetened* drinks with weight (Malik *et al.*, 2013). Given these mixed results, we advocate the view put forth by Sweetman *et al*., that the type of drink consumed influences this relationship (Sweetman *et al.*, 2008). Among food avoidance behaviors, EUE was not associated with brain network imbalance ($p < 0.450$), nor with BMI z-score ($p < 0.176$). While developing the CEBQ, Wardle *et al*., noted that EUE decreased with increasing age. Therefore our results may be attributable to the older ages of the children in this study.

While FF was associated with brain network imbalance (p = 0.046), it was not quite trending toward statistical significance with BMI z-score (p = 0.103). In the initial validation of the CEBQ, Wardle *et al.*, noted that only FF showed a sex difference, in which boys had higher FF scores. Our *post hoc* analyses showed no significant interactions of FF association by sex. We conclude that in our cohort FF is not dependent on the sex of the child. Food fussiness is characterized by restricted eating in both the amount and types of food eaten, along with an unwillingness to try new food (Jacobi *et al.*, 2008). Food fussiness is typically associated with low weight. However, it has also been associated with increased weight (Rydell *et al.*, 1995; Antoniou *et al.*, 2015) as fussy eaters often restrict the consumption of fruits and vegetables. Decreased consumption of fruits and vegetables is associated with increased consumption of fats (Dennison *et al.*, 1998), contributing to increased adiposity. Our lack of negative association between BMI z-scores and FF may be due to restricted eating that includes an increase in high energy dense food.

Given the relationships among the three principal outcomes in our study – functional connectivity, adiposity, and eating behaviors – a logical, subsequent hypothesis is that resting state functional connectivity mediates the relationship between adiposity and eating behavior. Mediation analysis helps explain *how* or *why* a relationship exists between two variables and is an obvious next analytical step. Robust mediation analysis requires larger sample sizes than ours (Fritz and MacKinnon, 2007). However, in advance of additional data, we investigated our mediation hypothesis in an exploratory analysis. To identify candidate relationships for mediation analysis, the relationships between pairs of measurements must be adjusted for the third measurement (Taylor and MacKinnon, 2012; Valeri and VanderWeele, 2013). We found no model in which all three relationships were significant after adjustment. Potential reasons for this outcome are: low power due to a small sample size; mediation is associated with other brain regions; or resting state functional connectivity is not a mediator.

We found no statistically significant associations between eating behaviors and brain network imbalance in an alternative model in which the NAc was located in the left hemisphere. These results may be due to hemispheric laterality. There are two predominant hypotheses of laterality:

(1) The left hemisphere is associated with reward / approach; and
    The right hemisphere is associated with punishment / avoidance.
(2) The left hemisphere is associated with emotions with positive valence; and
    The right hemisphere is associated with emotions with negative valence.

However, numerous studies support or contradict either hypothesis (Wager *et al.*, 2003; Balconi *et al.*, 2015; Behan *et al.*, 2015; Lindquist *et al.*, 2015). Miller *et al.*, hypothesize that laterality may change across temporal and spatial domains, depending upon circumstances (Miller *et al.*, 2013). In light of this hypothesis, future work is needed to investigate resting state functional connectivity in relation to hemispheric laterality.

Strengths and limitations

Our overarching hypothesis is that disrupted resting state functional connectivity within a neural model related to non-homeostatic eating is associated with increased adiposity and unhealthy eating behaviors among children. Previously published resting state functional connectivity studies in childhood obesity compared categorical weight classes: children who were severely obese with healthy weight children. Here we examined children across a continuous range of adiposity values to better understand functional connectivity and its imbalance as a function of adiposity.

Future work will consider larger brain networks using graph-based analyses and machine learning-based connectivity classification. A limitation of all functional connectivity analyses is that correlation does not imply causality. It is therefore important not to over-interpret functional connectivity results. Nonetheless, functional connectivity can be used to distinguish disease states (Craddock *et al.*, 2009) and as a summary of neuronal activity (Friston, 2011).

Our mediation hypothesis raises the question: Are differences in functional connectivity integrity present at birth or do they develop throughout childhood? Longitudinal studies are needed to better understand whether functional connectivity imbalance is present at birth or if imbalance develops during childhood. Longitudinal studies, beginning during very early childhood, are necessary to identify children who are at risk for developing obesity, to follow the development and integrity of resting state functional connectivity, and to develop and assess obesity intervene-tions. Of note, Figure 4.4C shows a cluster of four children who have lower BMI *z*-scores but higher impulsivity-biased imbalance. Following these participants over time would reveal whether these children are at risk for developing obesity.

Conclusions.

Our results establish the interplay among resting state functional connectivity, adiposity, and eating behaviors during childhood. We reported novel results from a resting state functional connectivity study of childhood obesity in which we examined children across a range of adiposity values. To our knowledge, no previous childhood obesity resting state functional connectivity studies have examined adiposity as a continuous measure. Our results suggest that resting state functional connectivity can identify neural models that are associated with adiposity and with eating habits. Furthermore, the identification of an imbalance in resting state functional connectivity that is associated with adiposity and unhealthy eating habits contributes to our knowledge of non-homeostatic factors involved in childhood obesity. Long-lasting weight loss maintenance may be elusive because, in addition to changing eating habits and physical activities, one must also change brain function.

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CHAPTER 5

Discussion

The alarming increase in the prevalence of obesity worldwide requires a better understanding of the development, pathophysiology, early recognition, treatment, and prevention of obesity. The poor efficacy of long-term weight loss among adults suggests that obesity, at least for the vast majority of individuals, may be irreversible. Therefore the best strategy to control the societal and economic costs of obesity is prevention. We must therefore examine children to better understand the early trajectory of the development of obesity and identify "targets" for prevention. Furthermore, given the almost certain weight regain after loss, the notion of simple energy balance, *i.e.*, "energy consumed *vs.* energy expended" as a model for healthy weight, is insufficient and incomplete. Because the brain plays a central role in homeostatic and non-homeostatic eating, our hypotheses stem from the overarching position that healthy weight maintenance involves not only a change in eating habits and physical activity, but also a change in brain function. To generate inroads into this overarching hypothesis, we therefore investigated the role brain functional connectivity plays in childhood obesity. Functional connectivity identifies patterns in neural activity that indicates the integration and communication of brain regions.

We posit that brain networks of children who are overweight or obese are biased toward increased drive to eat and away from control. As such, we hypothesize that the functional connectivity associated with the drive to eat, *e.g.*, motivation and impulsivity, is greater than the functional connectivity associated with cognitive control, *e.g.*, response inhibition.

Summary of results.

Because food is ubiquitous in the US, we first examined changes in functional connectivity when children were exposed to food-related stimuli using food cue task-based psychophysiological interaction (PPI) functional connectivity analysis. The results from our PPI analysis suggest that ineffective response inhibition-associated functional connectivity, when viewing high calorie compared to low calorie food images, is characteristic of obesity in children, ages 8-12 year old. Next, to understand the overall disposition of the childhood brain with respect to adiposity, in the absence of overt food-related stimuli, we evaluated brain functional connectivity when children were at quiet rest using resting state functional connectivity analysis. The results from our resting state functional connectivity (rsFC) analysis were consistent with our PPI results suggesting that as rsFC imbalance is increasingly biased toward impulsivity and away from inhibition, adiposity increases. Additionally, as impulsivity-biased imbalance increases, food approach behaviors increase and food avoidance behaviors decrease. Our analyses of independent food cue and resting state paradigms allowed us to probe network integrity under two different, but common-place and complementary, conditions, revealing consistent results.

Overall contribution of neuroimaging to the understanding of obesity.

Neuroimaging studies comparing adults who are overweight or obese with healthy weight adults have identified differences in brain regions associated with reward (*e.g.*, nucleus accumbens), emotional drive (*e.g.*, amygdala), and cognitive control (*e.g.*, prefrontal cortex [PFC] and anterior cingulate cortex [ACC]). Furthermore, adult obesity has been associated with differences in functional connectivity, white matter integrity (Kullmann *et al.*, 2015), and grey matter morphology (Kurth *et al.*, 2013). While there are fewer studies examining childhood obesity, as

this area of research is at an early stage, childhood obesity studies have also identified differences in brain regions associated with reward, drive, and cognitive control.

Foundational childhood obesity neuroimaging studies have begun to lay the groundwork for understanding the developing neurobiology of obesity. While the results from childhood obesity studies are not as mixed as that for adult studies, these results have not yet coalesced into actionable insight into the development, early recognition, and prevention of obesity. This lack of actionable insight is likely due, in part, to methodological differences and the varied hypotheses examined. Furthermore, more robust methods have developed since the implementation of early, proof-of-concept childhood obesity studies.

Improvements in analysis methodology.

In our research, we addressed some of the methodological weaknesses seen in early childhood obesity neuroimaging studies. For example, the earliest childhood obesity studies included only healthy weight children. More recent studies have compared children who are obese and healthy weight. However, most studies dichotomized their participants into "obese" and "healthy weight" groups, as we have done with our psychophysiological interaction (PPI) functional connectivity study. With our resting state functional connectivity study, however, we used a continuous measure of adiposity thereby allowing for greater statistical power in our analyses. Additionally, many studies quantified adiposity via BMI, which is not appropriate for children, or BMI percentiles, which is suboptimal for statistical analysis. We used BMI *z*-scores, which are appropriate for children and appropriate for statistical analysis.

Some of the earliest childhood obesity studies included only girls. Later studies, as well as ours, included both sexes. Many previous studies included participants across a large age range,

spanning childhood and adolescence, or examined adolescents only. Evidence suggests a relationship between childhood obesity and the onset of puberty (Ahmed *et al.*, 2009), therefore the effects of puberty should be considered. In our studies, we included participants in a narrow age range, 8-13 years old. In our PPI study, girls who had reached menarche were excluded. Tanner stages, a scale that describes the progression of pubertal changes, were acquired from the participants in our resting state functional connectivity study. The average Tanner stage was 2, indicating minimal progression of sexual characteristics.

Many childhood obesity studies examined children in a fasted state, either overnight or after 4+ hours. Due to the importance of eating for survival, fasting influences numerous regulatory processes such as signaling pathways and hormonal and neurotransmitter levels (Woods *et al.*, 1998; Schwartz *et al.*, 2000). Given the wide-ranging effects of fasting on the central nervous system, acquiring brain scans from individuals who are fasted may result in a loss of difference in effects or produce extreme effects. In our PPI study, children had just eaten. In our resting state functional connectivity study, children followed a naturalistic eating schedule.

We wish to broach a final methodological concern that pertains to most neuroimaging studies, beyond those investigating childhood obesity: the effects of collinearity in the design matrix for the general linear model (GLM). When analyzing subject-specific brain data using GLM, one creates a model that describes the expected neural response given the experimental paradigm. (For more detail about GLM analysis, see *Appendix A: Brief Overview of GLM Analysis* in FSL's User Guide: `http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FEAT/UserGuide#Appendix_A:_Brief_Overview_of_GLM_Analysis`.) For example, if the experimental paradigm includes stimuli, such as flashes of light, at 10, 30, and 50 sec from the beginning of the scan, then the design matrix should include a variable of interest that describes an expected neural response at approximately

15, 35, and 55 sec, where the ~5 sec lag is due to the delay in hemodynamic response of the brain. Other variables of interest, called explanatory variables (EVs), are often included in the design matrix. Some EVs isolate effects of no interest such as noise in the data. One such EV describes subject motion during the scan. Subject motion can be estimated from the subject's scan and is typically described via three translational and three rotational parameters, each with respect to the *x*-, *y*-, and *z*-axes. These six parameters are typically included in the design matrix as six separate EVs to describe subject motion. There are two methodological weaknesses to this.

First, the six motion parameters should not be considered separately. The motion parameters must be considered in total, which includes order. For example, if given a set of directions, the final destination of [turning right, driving 3 miles, turning left, driving 5 miles] is different from [turning left, driving 5 miles, turning right, driving 3 miles]. The separate motion parameters are convenient but lack crucial ordering information. (The motion parameters are accurately described by a 4·4 homogeneous transformation matrix, which cannot be used, as is, as an EV.)

Second, and most concerning, the six motion parameters are typically highly collinear. This weakness becomes apparent when estimating the βs in the GLM model:

$$Y = \beta X + \varepsilon \qquad \text{(Eq. 5.1)}$$

where Y is the acquired signal from the brain, X is the design matrix, β is the effect of the EVs defined in X, and ε is the error in the model fit. To estimate the βs, both sides of Eq. 5.1 are multiplied by the inverse of X, X^{1}:

$$YX^{-1} = \hat{\beta} \qquad \text{(Eq. 5.2)}$$

If the columns within X are strongly collinear, then design matrix X cannot be inverted and the

GLM fails. However, if the columns within X are collinear but not strongly enough to fail, the inverse of X, X^{-1}, may be incorrect and the GLM will estimate inaccurate βs. In other words, consider yourself fortunate if your GLM fails rather than proceeding with inaccurate β estimates. (To assess collinearity of a design matrix, see Matthijs Vink's Design Magic: `http://www.ni-utrecht.nl/downloads/d_magic`.)

To avoid both weaknesses, we used a single parameter to describe subject motion, the mean relative displacement, calculated by FSL's motion correction tool, `MCFLIRT`. For details, see FMRIB Technical Report TR99MJ1, *Measuring Transformation Error by RMS Deviation*, `http://www.fmrib.ox.ac.uk/analysis/techrep/tr99mj1/tr99mj1.pdf`.

Comparison with childhood obesity fMRI activation studies.

Traditional activation studies, which report differences in discrete brain regions, while foundational, may provide incomplete evidence about the role the brain plays in childhood obesity. Using activation analysis, Bruce *et al.*, compared differences in neural response between children who are obese with children who are healthy weight before and after eating (Bruce *et al.*, 2010). They hypothesized increased neural response in limbic regions, *e.g.*, amygdala and hippocampus, and regions within the PFC, *e.g.*, ACC, among children who were obese, in both the fasted and sated states, when viewing images of food. They reported that children who are obese do not show decreased neural response in the amygdala and ACC to visual food cues after eating as healthy weight children do. We used a similar visual food cue paradigm and investigated psychophysiological interaction (PPI) functional connectivity providing complementary insight. We examined a neural model consisting of similar brain regions, the basolateral amygdala (BLA) and rostral anterior cingulate cortex (rACC), and their PPI functional connectivity with the

nucleus accumbens (NAc), after our participants ate a standardized meal. The BLA is associated with motivation; rACC is associated with response inhibition; and the NAc is associated with reward. We reported that, among children who are obese, [rACC:BLA] PPI functional connectivity increased as [BLA:NAc] PPI functional connectivity increased. We suggested that the increasing relationship of [rACC:BLA] *vs.* [BLA:NAc] PPI is indicative of ineffective [rACC:BLA] functional connectivity resulting in a compensatory increase in functional connectivity between [rACC:BLA]. Our interpretation may explain the Bruce *et al.*, result of increased neural activity (or, as Bruce *et al.*, reported, lack of decrease neural response) in a region associated with response inhibition in support of childhood obesity. A conventional interpretation of their result is that increased neural activity in a brain region associated with response inhibition reflects increased response inhibition, which is incompatible with obesity among their young participants. Our interpretation of a compensatory increase of ineffective response inhibition-associated functional connectivity may explain Bruce's increased neural activity in a response inhibition-associated region in support of obesity.

Comparison with other childhood obesity functional connectivity studies.

To the best of our knowledge, there are no other published childhood obesity PPI functional connectivity studies. See below for a comparison with adult PPI obesity studies. To our knowledge, there are three published childhood obesity resting state functional connectivity studies. Because the image modalities, hypotheses, and regions examined vary from study to study, it is difficult to compare results.

Black *et al.*, compared the resting state functional connectivity of children who are obese with that of healthy weight children using bilateral middle frontal gyri as seed regions (Black *et al.*,

2014). They hypothesized increased rsFC between regions associated with self-control and response inhibition, *i.e.*, middle frontal gyrus, and regions associated with reward valuation, *i.e.*, orbitofrontal cortex (OFC) and ventromedial prefrontal cortex (vmPFC). They reported increased rsFC among children who are obese between the left middle frontal gyrus and left vmPFC. They also reported increased rsFC between the left middle frontal gyrus and left OFC. They reported no increased rsFC among healthy weight children compared to children who are obese. Black *et al*. speculated that among children who are obese, regions associated with cognitive control may receive greater input from reward motivation regions thereby perhaps resulting in less self-control and increased valuation of food-related stimuli.

Of note, as of January 1, 2016, we noted a few errors in the Black *et al*. publication. As per personal correspondence with Dr. W. Kyle Simmons, December 2, 2015, the correct coordinates in Talairach space for the left vmPFC are (-7, 21, -14) with a cluster size = 35,462 mm^3. The correct coordinates for the left lateral OFC are (-31, 33, -4) with a cluster size = 4074 mm^3.

One difference compared to Black *et al.*, is the seed regions. Another significant difference is that Black *et al.*, analyzed categorical group differences, *i.e.*, they compared two discontiguous adiposity groups, whereas we analyzed adiposity as a continuous variable. Nonetheless, conceptually we are in agreement that greater functional connectivity with regions associated with reward is associated with adiposity. Our conclusions suggest a more nuanced interpretation in that response inhibition, rather than the broader notion of self-control, is impaired. Additionally, our results suggest that *relative* functional connectivity, *i.e.*, the balance of functional connectivity between pairs of regions, is associated with adiposity amount.

Zhang *et al.*, compared rsFC among children with Prader-Willi syndrome (PWS) who are obese with that of their healthy weight siblings (Zhang *et al.*, 2013). They defined regions of interest based on differences in the amplitude of low-frequency fluctuations (ALFF) between the two groups. ALFF analysis quantifies the relative magnitude of resting state neural fluctuations (Yu-Feng *et al.*, 2015). They reported decreased rsFC among children with PWS, compared to their healthy siblings between various pairs of regions.

It is difficult to directly compare the results from Zhang *et al.*, with our resting state functional connectivity results as we investigated different regions. They also examined categorical group differences, where one group was severely obese with a genetic disorder, whereas we used adiposity as a continuous variable. Although Zhang *et al.*, investigated the rsFC with the IPL, as we did, our regions are sufficiently different as the IPL is not a small brain region, covering a volume of ~11,000 mm^3 (11 ml) in the right hemisphere (Maldjian *et al.*, 2003). Zhang *et al.*, investigated MNI coordinate (51, -39, 54) in the supramarginal gyrus in the IPL (Figure 5.1(A)), which, according to a meta-analysis performed via Neurosynth (`neurosynth.com`) (Yarkoni *et al.*, 2011), is strongly associated with nociception (Obermann *et al.*, 2009; Hohmeister *et al.*, 2010; Uematsu *et al.*, 2011; Sprenger *et al.*, 2015). We investigated (38, -54, 44) in the angular gyrus in the IPL (Figure 5.1(B)), associated with response inhibition. Another important difference is that we measured the rsFC with respect to a common region, the NAc, whereas Zhang *et al.*, investigated rsFC between regions within a single resting state network. However, to approximate the work of Zhang *et al.*, we performed an *ad hoc* analysis of rsFC between our frontal pole and IPL regions, [FPOLE:IPL] and made conceptual inferences.

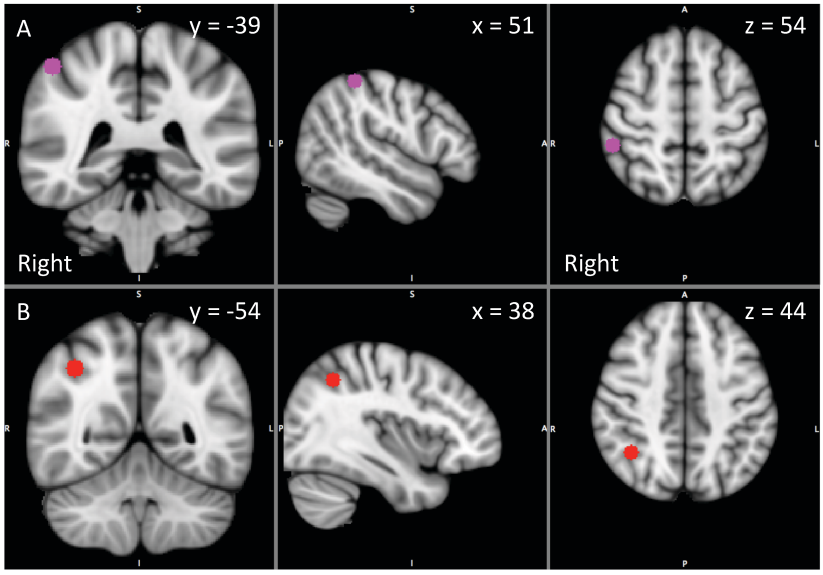

Figure 5.1: Locations within inferior parietal lobe. (A) Location of supramarginal gyrus in the inferior parietal lobe (IPL), at (51, -39, 54), associated with nociception. (B) Location of angular gyrus in IPL, at (38, -54, 44), associated with response inhibition.

Zhang *et al.*'s dlPFC location (-38, 49, 31) is associated with response inhibition (Roth *et al.*, 2007; Steele *et al.*, 2013), as is our frontal pole region at (-32, 62, -6). Zhang *et al.*'s OFC, at (-34, 62, 5), is associated with impulsivity (Torregrossa *et al.*, 2008), and is very close to our frontal pole, at (-32, 62, -6), also associated with impulsivity. Zhang *et al.* reported decreased rsFC between the dlPFC and OFC among children with PWS, who are obese, compared to their healthy weight siblings. We reported no significant relationship in our *ad hoc* analysis between BMI *z*-score and rsFC [FPOLE:IPL] ($p = 0.525$; $R^2 = 0.011$; $r = -0.106$; $N = 38$). However, we also reported decreased [IPL:NAC] rsFC with increased BMI *z*-score; increased [FPOLE:NAC] rsFC with increased BMI *z*-score; and increased relative difference, DELTA, with increased BMI *z*-score, where a positive DELTA indicates a bias toward impulsivity-associated frontal pole rsFC:

$$DELTA = ([fPole{:}NAc]\ rsFC - [IPL{:}NAc]\ rsFC) \qquad \text{(Eq. 5.3)}$$

The Zhang *et al.* result suggesting that decreased [DLPFC:OFC] rsFC indicates decreased

response inhibition relative to impulsivity is conceptually similar to our [FPOLE:IPL] DELTA analysis indicating the same.

Olde Dubbelink, *et al.*, examined resting state functional connectivity in girls, ages 9-12 years, using magnetoencephalography (MEG) (Olde Dubbelink *et al.*, 2008). They reported increased synchronization in the delta and beta frequency bands among girls who were severely obese compared to healthy weight girls. MEG has superior temporal resolution compared to fMRI, therefore discussing results in terms of frequency bands is common with MEG studies but rare with fMRI studies. MEG has poorer spatial resolution compared to fMRI, therefore discussing results in terms of anything but broad brain regions is common with MEG. Given the differences in temporal and spatial resolutions, it is difficult to compare results.

Comparison with adult obesity PPI functional connectivity studies.

Although to date there have been no PPI functional connectivity studies comparing children who are obese with healthy weight children, there are PPI studies among adults (Nummenmaa *et al.*, 2012; Atalayer *et al.*, 2014; Carnell *et al.*, 2014; Opel *et al.*, 2015; Tuulari *et al.*, 2015). It is difficult to compare results between adult PPI studies and our study as the seed and target regions differ and the psychological contrasts are different.

For example, our PPI study is similar to a study by Passamonti *et al*., except they did not include weight status as a variable of interest among their adult participants (Passamonti *et al.*, 2009). Their 21 participants had a mean age of 25.3 years (range [19-39] years) and mean BMI of 24 (std 4.6) kg/m^2. The participants completed the Dutch Eating Behavior Questionnaire (DEBQ) (van Strien *et al.*, 1986) and, while in the MRI scanner, they viewed appetizing and bland foods. Passamonti *et al*., assessed the association between PPI functional connectivity and external

eating as reported by the DEBQ, as we did. Their psychological contrast compared appetizing foods to bland foods. Using the NAc as the source region, as we did, Passamonti *et al.*, assessed the PPI functional connectivity with the basolateral amygdala (BLA), associated with motivational drive, and with the anterior cingulate cortex (ACC), associated with response inhibition. These regions are similar to those we used. They reported that when viewing [APPETIZING > BLAND] food images, their participants exhibited increasing external eating habits with increasing [BLA:NAC] PPI ($p = 0.001$; $R^2 = 0.62$; $r = 0.79$; $n = 21$). In our analysis of the comparison of viewing [HICAL > LOCAL] food images, we reported no association between external eating habits and [BLA:NAC] PPI for children who were obese or healthy weight (both $p > 0.22$; both $R^2 < 0.10$; $n = 17$).

The difference in results may be explained by lack of stated weight status, age differences, difference in psychological contrasts, and/or other methodological differences. In the Passamonti *et al*., study, the mean BMI is 24 kg/m^2 (std = 4.6 kg/m^2). The definition of overweight for adults is BMI range [25-30) kg/m^2. Given the standard deviation of Passamonti's reported BMI values, and assuming the distribution of their BMIs is approximately normal, it is probable that half the participants in the Passamonti *et al*., study are at least overweight. Nevertheless, when we combined our participants into a single weight group to approximate Passamonti's study, we still found no association between external eating habits and [BLA:NAC] PPI. Passamonti *et al*., reported no association between external eating habits and BMI. In agreement, we also report no such association ($p = 0.419$).

Passamonti *et al*., also reported increased external eating habits with decreasing response inhibition-associated [ACC:NAC] PPI ($p < 0.001$; $R^2 = 0.61$; $r = -0.78$; $n = 21$). We reported the same: increased external eating habits with decreasing [RACC:NAC] PPI, but only among

children who were healthy weight ($p = 0.054$; $R^2 = 0.226$; $r = -0.475$; $n = 17$). We found the opposite relationship among children who were obese: increased external eating habits were associated with increasing [ACC:NAc] PPI ($p = 0.035$; $R^2 = 0.264$; $r = 0.514$; $n = 17$). Without the weight status of the participants in the Passamonti *et al*., study, further interpretation would be conjecture.

Insights into the developing neurobiology of obesity.

To date, childhood obesity neuroimaging studies largely mimic adult obesity studies, identifying differences between obese and healthy weight groups. This is true of our PPI functional connectivity study. However, our resting state functional connectivity study moves beyond conventional group analysis by identifying the relationship of continuous adiposity levels with functional connectivity imbalance. Our results indicate that adiposity-associated differences in neural function fall along a continuum and may not require decades of unhealthy eating. Furthermore, this neural difference is not triggered only by food-related stimuli, but exists in the absence of food-related stimuli. Because the majority of childhood obesity neuroimaging studies are cross-sectional, we cannot yet speculate whether neural differences develop during childhood or if these differences are inherent.

Future work.

Longitudinal studies

Longitudinal neuroimaging studies, beginning at an early age, are needed to track the neural development of obesity. Longitudinal studies might also identify causal relationships between obesity and neural function. Figure 5.2 shows the relationship between adiposity and functional connectivity imbalance from our resting state functional connectivity study. Note the participants

with larger impulsivity-biased imbalance but lower adiposity. Following these participants would reveal whether they are at risk for developing obesity. Longitudinal studies might also identify novel therapeutic or interventional targets. As discussed in Chapter 4, we suggest that mindfulness might help treat and/or prevent childhood obesity by recalibrating this neural imbalance.

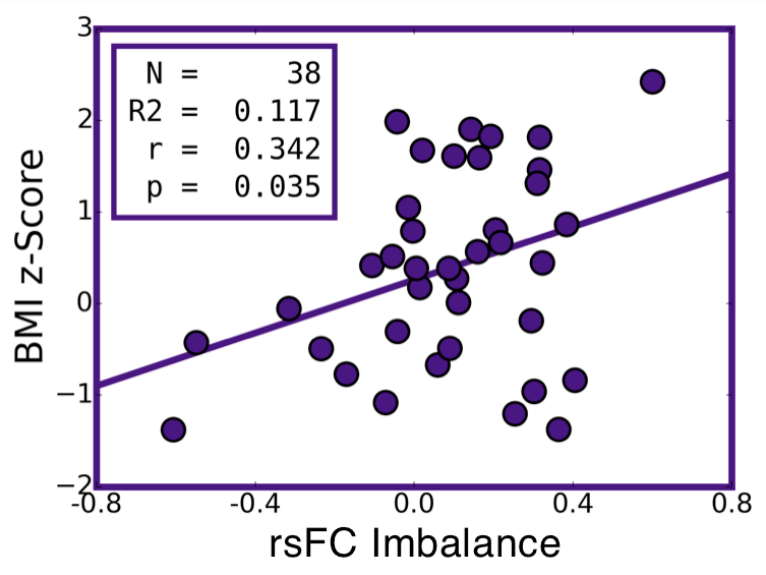

Figure 5.2: Adiposity *vs.* resting state functional connectivity imbalance. Relationship between adiposity, quantified by BMI *z*-score, and resting state functional connectivity (rsFC) imbalance where positive values indicate an impulsivity-associated bias.

True network analysis

Our resting state functional connectivity analysis examined only three brain regions; our PPI analysis also examined three brain regions. The brain is complex and comprised of multiple networks. Future work will use analysis techniques that take into account this complexity. Graph-based analysis can describe whole-brain networks and assess interactions within and across networks. Graph-based analysis can also describe networks at varying spatial and temporal resolutions. These are properties that lend themselves to a more realistic representation of the brain.

One of the reasons we examined a single three-node network was to avoid the penalties incurred when correcting for multiple comparisons. In future work, we will use machine learning techniques to categorize brain regions into representative classes which will allow for a more accurately represented network while restricting it to a manageable size. Additionally, we will use multivariate pattern analysis to identify behavioral and/or phenotypic data that are associated with these representative brain classes. With these more comprehensive and potentially more sensitive analysis tools, we might identify new therapeutic or interventional targets.

Conclusions.

Adult obesity has reached epidemic proportions where, in the US, being an overweight or obese adult is the rule rather than the exception. The increased prevalence of childhood obesity ensures that the obesity epidemic in the US will continue. Given the serious health consequences of obesity, the obesity epidemic must be halted and reversed. Efforts to treat obesity, primarily via behavioral changes such as eating more healthfully and increasing exercise, have been largely unsuccessful. New targets for treatment are needed. We suggest that a "balanced" brain with respect to food, in concert with balanced eating and exercise, may be a viable treatment.

Neuroimaging, particularly functional magnetic resonance imaging, which is non-invasive and does not use ionizing radiation, can help us identify and track neural "balance." Additionally, fMRI may allow us to objectively determine the trajectory and effectiveness of obesity treatments. Note that we are not proposing fMRI scans as treatment. Rather, we suggest that neuroimaging can be an important part of efficient clinical trials.

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