Examining Behavioral Phenotypes of Overeating and Obesity: Environmental, Psychological, and Neurobiological Influences on Food Motivation and Palatable Food Consumption

by

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ABSTRACT

Obesity is a substantial problem in the U.S., with growing rates particularly at early developmental stages (e.g., childhood, adolescents). Several factors may contribute to the development of overeating and obesity, including elevated craving in response to food-related cues, individual susceptibility to food-related cues, and neural changes associated with behavioral phenotypes implicated in obesity. The current dissertation aims to shed light on these contributing factors, in an effort to better understand obesity risk and contribute to the development of effective interventions.

Study 1 aimed to test the incentive-sensitization theory of addiction by examining food motivation, hunger, and consumption in a cue-rich compared to neutral environment. Participants (n = 126) were randomized to either a naturalistic fast-food laboratory or a neutral laboratory, where they provided self-reported ratings of "wanting," "liking," and hunger, and engaged in a task assessing food motivation and food consumption. Study 1 found that "wanting," hunger, and consumption were greater in the cue-rich compared to neutral laboratory, while "liking" did not differ between conditions. This study provides support for the incentive-sensitization theory as applied to eating behavior.

Study 2 developed and tested a novel paradigm for identifying two phenotypes of cue-responsivity, sign-tracking and goal-tracking. Children aged 5-7 (n = 64) engaged in a Pavlovian conditioning task designed to assess propensity to engage with a cue (sign-tracking) versus the location of a reward (goal-tracking). Children then engaged in tasks assessing food motivation

and inhibitory control. Contrary to hypotheses, Study 1 did not find a distinct goal-tracking phenotype, and did not find sign-tracking behavior to be associated with either food motivation or inhibitory control. Considerations for how to examine these phenotypes in future research are discussed.

Study 3 examined how resting state functional connectivity (rsFC) relates to obesity, food consumption, food motivation, and inhibitory control in adolescents (n = 164) aged 13-16 who ranged from lean to obese. Participants completed tasks assessing food motivation and inhibitory control, then on a second visit underwent a resting-state scan and then completed a food consumption task in a cue-rich environment. Obesity and elevated food motivation were found to be marked by altered connectivity in areas in the salience network (e.g., caudate, NAcc, OFC) and the default mode network (e.g., PCC, hippocampus). However, obesity was not found to be associated with behavioral outcomes, thus these behaviors were not found to mediate associations between obesity and rsFC patterns. These findings provide suggestions as to effective prevention and intervention targets.

The current dissertation provides evidence for a strong role of elevated food motivation (especially in the context of food cues) in the overconsumption of palatable foods. Clinical implications and suggestions for intervention are discussed.

CHAPTER I

Introduction to Three-paper Project

Obesity is a growing problem in the United States, where nearly 35% of adults and nearly 17% of children and adolescents are obese (Ogden, Carroll, Kit, & Flegal, 2014). The high rate of obesity in children is especially concerning, as those who become obese early in life are likely to remain obese in adulthood (Dietz, 1998). With obesity also comes risk for developing physical health problems, such as heart disease and diabetes (Dixon, 2010; Must et al., 1999; Reilly et al., 2003), and obesity and its ensuing issues contribute to higher healthcare costs (Allison, Zannolli, & Venkat Narayan, 1999). Despite the negative public health consequences of obesity, current treatment approaches show limited long-term effectiveness (Dombrowski, Knittle, Avenell, Araujo-Soares, & Sniehotta, 2014; Ho et al., 2012; Kelly et al., 2013), illustrating the need for improved intervention efforts.

Research into the mechanisms underlying behaviors associated with obesity may assist in the development of targeted interventions, as it is possible that different behavioral phenotypes of obesity may be more responsive to different treatments. For example, one intervention may be most effective for those who have difficulty controlling the urge to eat when presented with palatable food, while another intervention may be most effective for those who are highly motivated to seek out food. Recent research has suggested that overeating and obesity may share biological and psychological underpinnings with addiction (Avena, Rada, & Hoebel, 2008;

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Berridge, Ho, Richard, & DiFeliceantonio, 2010; Volkow, Wang, Fowler, & Telang, 2008), suggesting that intervention approaches effective in the treatment of addiction (e.g., targeting craving or ability to resist the impulse to use) may also assist in the treatment of obesity. In order to develop interventions targeted to specific behavioral phenotypes, it is first necessary to understand various developmental, behavioral and environmental influences on different profiles of risk for obesity. This will assist in the identification of those at particular risk for developing obesity, and the development and selection of appropriately targeted interventions.

Incentive Salience: Enhanced "Wanting" for Food

Overconsumption of palatable foods can be influenced by excessive "wanting," or enhanced motivation to obtain a reward (Berridge, 2009). Under the incentive-sensitization (IS) theory of addiction, "wanting," mediated by neural dopamine circuitry, is a primary driver of compulsive behavior (Berridge & Robinson, 2016; T. E. Robinson & Berridge, 2000). "Wanting" is thought to be unconscious and is distinct from "liking" (i.e., hedonic pleasure derived from a reward) (Peciña, Caginard, Berridge, Aldridge, & Zhuang, 2003). "Wanting" is also thought to occur primarily in the context of relevant cues (Berridge & Robinson, 2016). For example, upon repeated intake of highly palatable foods, cues related to such foods become particularly attractive, and can develop the power to trigger craving and overeating (Berridge et al., 2010). Upon repeated exposure, these cues are imbued with increased incentive salience (i.e., are more likely to draw and hold attention). Thus, individuals who are attuned or responsive to cues may be at increased risk for compulsive consumption of a substance such as food. In obesity, this phenomenon is particularly important to consider, as cues (e.g., advertisements, fast food restaurants, vending machines, etc.) for highly palatable foods are omnipresent in the Western food environment. In an environment rich in food cues, it may be difficult to resist the

urge to overeat, even for individuals who are relatively healthy. These cues may also prompt overconsumption in individuals displaying high motivation to consume palatable foods.

"Wanting" may manifest as craving, or an increased motivational drive to obtain and consume a substance such as food (Epstein, Leddy, Temple, & Faith, 2007) (T. E. Robinson & Berridge, 2000). One potential index of motivational drive for food is the Relative Reinforcing Value (RRV) task (Epstein & Leddy, 2006; Saelens & Epstein, 1996). This task assesses how much effort participants are willing to expend, by making a progressively increasing number of button presses, for a reward such as food. The RRV task has been used with both child and adult samples, and appears to be related to elevated BMI and elevated consumption across age ranges (Gearhardt et al., 2017; Rollins, Loken, Savage, & Birch, 2014; Temple, 2014; Temple, Legierski, Giacomelli, Salvy, & Epstein, 2008). Further, the reinforcing value of food as measured by the RRV task has been shown to assess a separate construct from the hedonic value of food, just as wanting is theorized to be separable from liking (Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003). Another potential measure of wanting may be feelings of hunger. Food-related cues have been shown to be associated with both self-report of hunger and an increase in hunger-related gut peptides (e.g., ghrelin, orexin), even when an individual is in a state of satiety (Cohen, 2008; Cornell, Rodin, & Weingarten, 1989; Volkow, Wang, & Baler, 2011). It is possible that cue-triggered feelings of hunger may represent increased wanting, and thus may contribute to excess consumption.

To test IS in human eating behavior, Study 1 of this dissertation employed a cue-rich, naturalistic, fast-food restaurant laboratory to examine the impact of cues on enhanced motivation, or "wanting," as measured by both self-report and RRV performance. The study also investigated the effect of cues on feelings of hunger, which may also represent wanting. We also

tested whether the cue-rich environment has an effect on liking, as according to IS theory liking should be separable from wanting. Finally, Study 1 measured caloric consumption, in order to determine the impact that cue-triggered wanting may have on food intake.

Individual Differences in the Attribution of Incentive Salience to Cues

While environmental food cues may increase risk for developing obesity, not every individual exposed to cues will become obese. Behavioral and biological differences may help explain why some individuals are at risk compared to others. Animal models have identified different conditioned responses to food cues that may promote understanding of different pathways to obesity. When trained to associate a cue (e.g., a light or sound) with the delivery of food, animals such as rats can display a tendency to interact with the cue itself (sign-tracking) or with the area where the food is to be delivered (goal-tracking) (Boakes, 1977; Brown & Jenkins, 1968). For sign-trackers (STs), the cue appears to take on incentive salience, while for goal-trackers (GTs), it does not (Flagel, Akil, & Robinson, 2009). In animal models, STs appear to be most prone to displaying reward-seeking behavior in response to discrete, localizable cues, while GTs may be more responsive to cue-rich contexts associated with use (T. E. Robinson, Yager, Cogan, & Saunders, 2014; Saunders, O'Donnell, Aurbach, & Robinson, 2014). However, to our knowledge, very few studies have attempted to identify these phenotypes in humans (Garofalo & di Pellegrino, 2015; Versace, Kypriotakis, Basen-Engquist, & Schembre, 2016). Understanding whether and how these phenotypes manifest in human eating behavior may help in the development of more effective interventions to manage one's response to food cues, for example by training one's ability to inhibit response to distinct cues, or by teaching coping skills for use when one is in a cue-rich context.

Individuals who have difficulty implementing executive function abilities in response to enhance cue-triggered wanting may also be more likely to develop obesity. Executive function (EF), or processes allowing for the cognitive control of behavior, can encompass several different facets, including working memory, cognitive flexibility, and inhibitory control (Diamond, 2013). Inhibitory control, or the ability to resist temptation or urges to act impulsively (Diamond, 2013), is particularly relevant with regard to food intake. When presented with a highly palatable food such as a cookie or pizza, or even with a cue pertaining to the food, inhibitory control is required to prevent excess consumption of the food. The Go-No-go task (Batterink, Yokum, & Stice, 2010; Grammer, Carrasco, Gehring, & Morrison, 2014; Kawashima et al., 1996), which requires one to inhibit a pre-potent response to certain stimuli, may be a useful measure to assess inhibitory control difficulties that might increase ones vulnerability to overeating in the presence of cues.

For some individuals, additional factors might contribute to increasing the difficulty of controlling the impulse to consume food. For example, those who find palatable foods highly rewarding or "wanted" may have more difficulty inhibiting the desire to consume the food. In fact obesity appears to be associated with neural circuitry involved in both increased reward sensitivity and impaired inhibitory control (Volkow et al., 2011). In rats, a sign-tracking phenotype is also related to lower attentional and inhibitory control ability and a greater propensity for impulsive action, suggesting that STs may be at even further risk of excessive consumption in the presence of salient food cues (Lovic, Saunders, Yager, & Robinson, 2011; Paolone, Angelakos, Meyer, Robinson, & Sarter, 2013). Thus, factors such as motivational drive for food and an inability to inhibit a response could make some individuals especially vulnerable to overconsumption in response to cues. However, there is some evidence that training one to

inhibit a prepotent response may be an effective method to reduce the risk of overeating (Houben & Jansen, 2011; Lawrence et al., 2015). As these phenotypes may be indicative of future risk for developing obesity, identifying them early in development (i.e., childhood) would provide greater opportunity to implement obesity prevention efforts.

To investigate how individual differences in the attribution of incentive-salience to cues may impact eating behavior, Study 2 of this dissertation developed and tested the feasibility of a novel paradigm to identify sign-tracking and goal-tracking phenotypes in human children. This study also examined how food motivation and inhibitory control are associated with each phenotype by investigating the association between sign-tracking and goal-tracking behavior and performance on the RRV and Go-No-go tasks.

Developmental and Biological Influences on Eating Behavior

Across development, individuals may be more vulnerable to overeating and obesity due to factors prominent at different points in development. For example, children in general appear to be highly motivated to access and consume food, and children experience higher craving than adults (Rollins et al., 2014; Silvers et al., 2014). Upon reaching adolescence, parental control over behavior is diminished and adolescents typically experience an increase in access to disposable income and personal decision-making (Sanders, 2013). These aspects of adolescence can lead to increased opportunity to access and consume unhealthy foods. However, at this stage individuals may still be more influenced by bottom-up control (e.g., rewarding and motivating properties of food) rather than by top-down inhibitory control (Casey, Jones, & Hare, 2008; Sanders, 2013). This increased independence continues in young adulthood, particularly in a college setting, when individuals are making even more independent decisions about food consumption (Cluskey & Grobe, 2009; Nelson, Kocos, Lytle, & Perry, 2009).

The relative differences in developmental rate of certain areas of the brain may also increase the likelihood of overeating at different ages. In children, the prefrontal cortex (PFC), which is responsible for successful executive function capabilities, is not yet fully developed (Casey, Giedd, & Thomas, 2000). Thus, children are not as adept at decision-making about foods or inhibiting the response to consume foods as adults may be. In adolescence, not only is the PFC still developing, but neural regions implicated in reward are relatively more developed (Casey et al., 2000; Casey et al., 2008). This leaves adolescents especially prone to engaging in risky behaviors, including excess consumption of highly palatable foods. As children and adolescents are at high risk for developing obesity, it is important to understand developmental influences on food consumption. Seventeen percent of children in the United States are obese (Ogden et al., 2014), and research suggests that once an individual is obese, they are more likely to remain so than to return to a healthy weight (Dietz, 1998; Kelly et al., 2013; Reilly, 2005). Thus, identifying factors that influence overconsumption at different stages of development and targeting these factors in prevention efforts may help reduce the risk of obesity in children and adolescents.

Biological differences may also be present in individuals who are more prone to overeating and obesity. Neuroimaging studies have found that when presented with images of palatable foods, individuals who are obese compared to lean show increased activity in regions implicated in reward processing (Bruce et al., 2010; Rothemund et al., 2007). In addition to differing patterns of activation in response to food-related tasks, preliminary research suggests that obesity may be marked by underlying differences in patterns of resting state functional connectivity (rsFC) (Garcia-Garcia et al., 2013; Kullmann et al., 2012; Lips et al., 2014). rsFC patterns reflect areas of the brain that are more likely to become activated or deactivated

together, allowing for the identification of neural networks consisting of regions involved in related functions (Greicius, 2008; van den Heuvel & Hulshoff Pol, 2010). Research on healthy adults has identified intrinsic neural networks involved in functions such as salience processing, executive control, vision, and attention (Barrett & Sapute, 2013). Task-based neuroimaging research has found altered activation in regions implicated in reward processing and executive function to be associated with obesity (Carnell, Gibson, Benson, Ochner, & Geliebter, 2012). As regions of the brain function together to facilitate psychological and behavioral processes (Barrett & Sapute, 2013), rsFC analysis allows for better understanding of how neural networks contribute to behaviors of interest. Examining rsFC in individuals who are obese compared to lean will allow us to understand how intrinsic neural networks might be dysregulated in obesity.

Previous research has found obesity to be associated with alterations in canonical intrinsic networks including the salience network (SN) and default mode network (DMN) (Garcia-Garcia et al., 2013; Kullmann et al., 2012). However, studies on rsFC and obesity to date have focused primarily on adults, have employed relatively small sample sizes, and have not examined the relationship between rsFC and behavioral phenotypes of obesity. Study 3 of this dissertation aimed to address these gaps by testing for associations between rsFC patterns and weight status (i.e., lean, overweight, or obese) and performance on tasks assessing food motivation (RRV) and inhibitory control (Go/No-go task). Relating rsFC patterns to behavioral task performance will provide insight into how any dysregulation in rsFC networks might be associated with facets that may contribute to overeating behavior. A greater understanding of these relationships can assist in the development of improved targeted obesity interventions.

Clinical Implications and Summary

The factors contributing to obesity risk in varying situations and at varying stages of development could imply that specific treatment targets may be more effective for certain populations and in certain situations. In childhood, the primary focus may be on prevention. There is evidence that children's brains are highly plastic in response to behavioral intervention (E. A. Hayes, Warrier, Nicol, Zecker, & Kraus, 2003), thus childhood may be a prime time period to train skills such as inhibitory control. Thus, identifying risky profiles such as sign-tracking could provide the opportunity for early intervention focused on training children to inhibit their automatic response to cues signaling food delivery, such that the likelihood of excess consumption and development of obesity is reduced.

As neural regions related to reward processing become more powerful in adolescence, it may be important to incorporate intervention strategies aimed at coping with elevated craving, or motivation to consume rewarding foods. One example of such a strategy may be to use mindfulness skills such as "urge surfing" (Bowen & Marlatt, 2009; K. T. Jenkins & Tapper, 2014), to enhance one's ability to wait out a craving without giving in to the urge to consume. Research suggests that behavioral interventions can actually lead to connectivity changes in the brain (Kuhn, Gleich, Lorenz, Lindenberger, & Gallinat, 2014; R. Li et al., 2014; Voss et al., 2010), so a fuller understanding of resting state network dysregulation and related behavioral phenotypes of obesity will provide insight into appropriate behaviors and networks to target.

In young adulthood, as individuals leave the parental home and begin living independently, interventions focusing on stimulus control may become increasingly important. Individuals entering a new environment such as college may be faced with increased access to unhealthy foods and increased exposure to related cues. As they begin to make an increasing number of independent decisions about the food they consume with less influence from their

parents, young adults may be particularly vulnerable to the effects of food cues. For example, while one may be able to inhibit the urge to consume unhealthy foods in a cue-rich environment (e.g., dining hall) one day, daily exposure to the same environment or exposure under times of stress could make it more difficult to resist that urge. Therefore, an effective intervention at this stage may involve learning to identify and anticipate risky environments or situations and limit one's exposure.

In sum, the three studies comprising this dissertation aimed to provide insight into factors related to increased likelihood of obesity, thus aiding in the identification of effective intervention targets. Study 1 examined how enhanced food motivation and elevated consumption are impacted by environmental cues for palatable foods. Study 2 attempted to identify conditioned responses to food cues and test their relationship to food motivation and inhibitory control to better understand how they may increase risk of overeating. Study 3 investigated relationships between rsFC patterns and weight status, food motivation, inhibitory control, and consumption. Taken together, the results from these three studies should provide insight into how different environmental, behavioral, and psychological influences on overeating may manifest at different developmental stages. It is hoped that this dissertation will contribute to an improved understanding of factors influencing overeating and behavioral phenotypes of obesity, thus informing the development of targeted interventions and improving efforts at the prevention and treatment of obesity.

CHAPTER II

The Impact of a Cue-Rich Context on Eating Behavior

Similar mechanisms may contribute to both overeating and addictive behaviors. For example, consumption of both drugs of abuse and calorie-dense, nutrient-poor foods (e.g., cookies, cake) activates neuronal circuitry implicated in reward and motivation (Berridge, 2009; Volkow et al., 2008). While there are important differences between food and drugs of abuse, namely that food is necessary for survival, overeating and substance use are associated with similar behavioral consequences, such as craving, withdrawal, and binging (Avena et al., 2008; P. M. Johnson & Kenny, 2010). The incentive sensitization (IS) theory outlines potential shared mechanisms, proposing that compulsive consummatory behaviors are driven by "wanting" (i.e., a strong motivation to obtain and consume a substance; typically manifesting as craving or strong desire to use), rather than by "liking" (i.e., the hedonic pleasure derived from a substance) (T. E. Robinson & Berridge, 2000). Initially, the target substance is often both wanted and liked (M. J. Robinson, Fischer, Ahuja, Lesser, & Maniates, 2015), however with continued exposure, "wanting" can occur even after "liking" is diminished (Berridge, 2009). With repeated consumption, the user becomes sensitized to substance-related cues (T. E. Robinson & Berridge, 2000), which begin to trigger dopaminergic release and increased "wanting" (Volkow et al., 2011). In fact, T. E. Robinson and Berridge (2000) emphasize that evidence of IS is only

detectable in the context of associated cues. Thus, it is important to test the predictions outlined by the IS theory regarding eating behavior in a cue-rich context.

Cues may also affect one's motivational drive to consume food by increasing feelings of hunger. While hunger is usually interpreted as a homeostatic signal indicating caloric need, food is often consumed for other reasons, such as hedonic pleasure (Lowe & Butryn, 2007). Environmental cues can lead people to feel hungry even if they are in a state of satiety (Cohen, 2008; A. W. Johnson, 2013). For example, when exposed to pizza or ice cream, individuals who previously indicated they were full expressed increased desire to eat (Cornell et al., 1989). While these feelings of hunger may be interpreted as caloric need, they may actually be occurring in response to environmental cues and may reflect increased motivational drive to acquire food. Further, biological systems implicated in reward communicate with homeostatic systems involved in the experience of hunger (Volkow et al., 2011). When individuals are exposed to palatable food cues in a state of satiety, this can lead to increased levels of gut peptides that are implicated in the experience of hunger (i.e., orexin and ghrelin) (Malik, McGlone, Bedrossian, & Dagher, 2008; Volkow et al., 2011). Thus, feelings of hunger (which are often interpreted as caloric need) can actually signal increased cue-induced "wanting" or motivation to consume food. Understanding how feelings of hunger are affected by the presence of cues may help people better interpret and respond to such feelings in cue-rich contexts. Further, hunger state may also moderate the effect of cues. Generally, being in a state of hunger amplifies a cue's incentive salience (Berridge et al., 2010). In a state of satiety, these effects may be dampened, with cues holding less motivational value. Thus, examining the interaction between hunger and the presence of cues will provide a better understanding of how hunger relates to food wanting.

Studies examining the effect of cues on eating behavior typically involve the sight and/or smell of the cued food (Boswell & Kober, 2016). Cue exposure in these paradigms has been associated with greater craving, consumption, hunger, and desire to eat (Fedoroff, Polivy, & Herman, 2003; Ferriday & Brunstrom, 2011; Tetley, Brunstrom, & Griffiths, 2010). However, little research has examined the effect of cues on both food wanting and food liking in the same study, which is necessary to thoroughly test IS. In studies that have, evidence for the separability of wanting and liking in human eating behavior has been mixed (Finlayson & Dalton, 2012; Havermans, 2011, 2012). One reason may be that the cue paradigms used in these studies are not sufficient to trigger the intense wanting postulated by the IS theory. In a recent review of liking and wanting measurement (Pool, Sennwald, Delplanque, Brosch, & Sander, 2016), 76% of the studies reviewed used a photo of the food as the cue. However, some studies employing the sight or smell of a cued food have been unable to find a main effect of cue exposure on eating behavior (Coelho, Jansen, Roefs, & Nederkoorn, 2009; Zoon, He, de Wijk, de Graaf, & Boesveldt, 2014). While an image of the target food may be a sufficient cue to trigger wanting for some individuals, cues that trigger food wanting in everyday life likely include a combination of many elements in the environment where one consumes food. In a restaurant, for example, cues might include ambient music, the experience of being served by wait staff, and the furnishings of the dining room in addition to images, smells, and presentation of the food itself. Presenting a photo or the smell of the food alone does not address these contextual factors that may serve as additional cues. It is possible that a more naturalistic, cue-rich environment, which includes contextual factors in addition to food presentation, is necessary to observe the separability of liking and wanting in laboratory studies.

Research on alcohol use has set a precedent for the study of appetitive behaviors in a cue-rich environment. Simulated bar laboratories examine drinking behavior in an environment mimicking the setting in which people are likely to consume alcohol in their day-to-day life (Wall, Hinson, McKee, & Goldstein, 2001). Participants in these naturalistic settings report greater pleasurable subjective effects (e.g., greater stimulation, pleasurable disinhibition, sociability) from drinking compared to those in neutral settings (Wall et al., 2001), suggesting that the bar lab environment captures a more thorough range of factors that may contribute to problematic use. While bar labs are an important setting in alcohol research, an equivalent in food research has not been employed to evaluate differences in liking and wanting, limiting our understanding of the ability of food-related environmental cues to trigger food-seeking behavior or a pleasurable hedonic experience. Further, there have been no tests in either alcohol or food research of how liking and wanting may be differentially related to patterns of consumption in a cue-rich relative to neutral environment. Given that cues are central to triggering wanting, and that current cue paradigms (i.e., images, smells) have had limited success in observing a distinction between liking and wanting, examining eating behavior in a cue-rich, naturalistic environment may be a more thorough way to test the tenets of IS theory.

The current study employed a cue-rich, simulated fast-food restaurant laboratory to test an IS model of eating behavior, examining how environmental cues impact food wanting, food liking, hunger, and consumption. Understanding the influence of cues in the environment on eating behavior may inform intervention and policy efforts to reduce obesity.

Specific Aims

1. Investigate the separability of wanting and liking proposed by IS in the fast-food laboratory (cue-rich environment) relative to a neutral environment.

2. Investigate how self-reported hunger differs in the cue-rich environment relative to the neutral environment. Given that hunger state may affect the incentive salience of cues, we will also conduct exploratory analyses investigating whether hunger at baseline moderates the relationship between laboratory environment and food wanting, liking, and consumption.

3. Investigate differences in caloric consumption between the two conditions, and test mechanisms contributing to overeating by testing variables that are found to significantly differ by environment as mediators in the relationship between laboratory environment and caloric consumption.

Methods

Participants

Participants were undergraduate students who received course credit as compensation. Participants were ineligible if they had food allergies or dietary restrictions that prohibited them from consuming the foods used in the protocol. One-hundred twenty-six participants completed the study. Two participants were excluded because of dietary restrictions they had not reported before participating in the study. Seven participants were excluded due to failing a validity check regarding their understanding of protocol instructions by answering one or more of three brief questions about study instructions incorrectly. Five participants were excluded for having outlying data (> 2 *SD* above the mean) in variables of interest, leaving a final sample size of 112. Participants' mean age was 18.98 (*SD* = 1.24), and mean body mass index (BMI) was 23.66 (*SD* = 4.03). Weight status distribution was as follows; 4 (3.6%) participants were underweight, 74 (66.1%) were normal-weight, 22 (19.6%) were overweight, and 10 (8.9%) were obese. Sixty-four (57.1%) participants were female, 47 (42.0%) were male, and one participant did not report

gender. The racial breakdown was as follows: 84 (75.0%) White, 22 (19.6%) Asian, 4 (3.6%)

Hispanic, 2 (1.8%) Black, 1 (0.9%) American Indian, and 2 (1.8%) other or more than one race.

Participant demographic variables are presented in Table II.1.

Table II.1

Demographic and Baseline Characteristics of the Sample

	Total	Cue-rich	Neutral	F or X^2	P	$\eta^{2\text{ or}}\varphi$
Male	47 (42.%)	26 (43.3%)	21 (40.4%)			
Female	64 (57.1%)	33 (55.0%)	31 (59.6%)			
Gender				0.15	.70	-.04
White	84 (75.0%)	43 (71.7%)	41 (78.8%)			
Asian	22 (19.6%)	11 (18.3%)	11 (21.2%)			
Hispanic	4 (3.6%)	3 (5.0%)	1 (1.9%)			
Black	2 (1.8%)	2 (3.3%)	0			
American Indian	1 (0.9%)	1 (1.7%)	0			
Other/More than one race	2 (1.8%)	1 (1.7%)	1 (1.9%)			
Race				3.88	.57	.19
Age	18.98 (1.24)	19.02 (1.20)	18.94 (1.31)	0.10	.75	.00
BMI	23.66 (4.03)	23.94 (4.23)	23.35 (3.81)	0.60	.44	.01
Underweight	4 (3.6%)	1 (1.7%)	3 (5.8%)			
Normal-weight	74 (66.1%)	39 (65.0%)	35 (67.3%)			
Overweight	22 (19.6%)	12 (20.0%)	10 (19.2%)			
Obese	10 (8.9%)	6 (10.0%)	4 (7.7%)			
Weight status				1.48	.69	.12
Baseline food wanting	2.92 (1.12)	3.02 (1.19)	2.81 (1.03)	0.98	.33	.01
Baseline game wanting	3.20 (1.38)	3.13 (1.43)	3.27 (1.33)	0.27	.61	.00
Baseline hunger	36.21 (19.44)	39.13 (19.16)	32.83 (19.40)	2.98	.09	.03
YFAS symptom count	1.72 (1.28)	1.80 (1.23)	1.61 (1.34)	0.59	.45	.01
TFEQ Restraint	12.85 (3.33)	12.82 (3.03)	12.88 (3.68)	0.01	.93	.00

Procedure

The study was approved by the University of Michigan Health and Behavioral Sciences

Institutional Review Board. Written informed consent was obtained from all participants.

Participants were randomly assigned to either a naturalistic fast-food laboratory (cue-rich

environment) or a neutral laboratory (neutral environment; see Table II.1 for demographic and

baseline characteristics of each group). To standardize hunger, participants were instructed to eat whatever constituted a typical lunch for them at least one hour prior to arriving for the study. Study sessions were all conducted between lunchtime and dinnertime. Upon participants' arrival in the lab, baseline ratings for self-reported food wanting and hunger were collected. Next, participants were taken to the randomly assigned environment. There, they engaged in the Relative Reinforcing Value (RRV; see Methods below for full description) task in order to earn tokens to be redeemed for foods typically available at a fast-food restaurant (i.e., cheeseburger, French fries, milkshake, non-diet soda) and/or for time to participate in an alternate activity (i.e., playing video games on a tablet). After completing the RRV task, participants again provided ratings for self-reported food wanting and hunger (post-RRV wanting and hunger). Next, participants redeemed their tokens for both fast food and time to play games (RRV food consumption period; calorie and weight information shown in Table II.2. There is evidence that visually stimulating tasks such as playing video games can reduce food cravings (Skorka-Brown, Andrade, & May, 2014). To reduce this effect, participants were not allowed to consume food and play games simultaneously. Instead, participants redeemed tokens and chose to either consume food or play games first, then began the second activity once they finish engaging in the first. Participants were given the choice of which activity to engage in first.

Table II.2
Serving Sizes and Calorie Counts of Foods Available during RRV Consumption Period

Food	Weight (small)	Calories (small)	Weight (large)	Calories (large)
Cheeseburger	152.54g	390.50	305.08g	781.00
French Fries	84.27g	158.43	133.53g	251.04
Non-diet soda	364.58g	138.54	516.16g	196.14
Milkshake	296.48g	490.67	435.12g	720.12

Following the consumption periods, participants received ad libitum access to snack foods (i.e., Lay's potato chips, Cheez-its, M&Ms, Skittles) and games for 10 minutes, in order to assess the amount eaten when consumption is not limited by RRV performance (ad libitum consumption period; calorie and weight information shown in Table II.3). Snack foods were provided instead of the fast food items used in the RRV consumption period to minimize any effect of sensory-specific satiety (i.e., declining satisfaction due to intake of the same type or flavor of food) on consumption (Rolls, 1986). Next, participants were taken to a separate room where they completed a survey including self-reported liking for the foods consumed during the study and demographics. Finally, height and weight measurements were collected in order to calculate body mass index (BMI; kg/m^2). These measurements were taken at the conclusion of the study in order to prevent any influence they might have on eating behavior. A flowchart illustrating the entire study procedure is shown in Figure II.1.

Table II.3
Serving Sizes and Calorie Counts of Foods Available during Ad Libitum Consumption Period

Food	Weight	Calories
Lay's potato chips	60g	342.6
Cheez-its	100g	500
Skittles	125g	500
M&Ms	125g	500

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                    ┌─────────────────────┐
                    │    Baseline self-   │
                    │  report measures:   │
                    │   Baseline hunger   │
                    │   Baseline wanting  │
                    └──────────┬──────────┘
                               ↓
                    ┌─────────────────────┐
                    │ Entry into laboratory│
                    │    environment      │
                    └─────────────────────┘
              ↙                                ↘
┌──────────────────────┐          ┌──────────────────────┐
│  Cue-rich simulated  │          │  Neutral laboratory  │
│  fast food laboratory│          │                      │
└──────────┬───────────┘          └──────────┬───────────┘
           ↓                                  ↓
┌─────────────────────────────────────────────────────────┐
│   Relative Reinforcing Value (RRV) of food and games task│
└──────────┬──────────────────────────────────┬───────────┘
           ↓                                  ↓
┌─────────────────────────────────────────────────────────┐
│                   Post-RRV measures:                    │
│                                                         │
│                  Post-RRV wanting                       │
│                  Post-RRV hunger                        │
└──────────┬──────────────────────────────────┬───────────┘
           ↓                                  ↓
┌─────────────────────────────────────────────────────────┐
│                 RRV consumption period                  │
│  Available foods: Cheeseburger, milkshake, French fries,│
│                     Non-diet cola                        │
└──────────┬──────────────────────────────────┬───────────┘
           ↓                                  ↓
┌─────────────────────────────────────────────────────────┐
│              Ad libitum consumption period              │
│  Available foods: Skittles, M&Ms, Lay's potato chips,   │
│                      Cheez-its                           │
└────────────────┬─────────────────────┬──────────────────┘
                 ↘                     ↙
              ┌─────────────────────┐
              │  Leave laboratory   │
              │    environment      │
              └──────────┬──────────┘
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              ┌─────────────────────┐
              │ Completion of post- │
              │ study self-report   │
              │    measures:        │
              │      Liking         │
              │   Food Addiction    │
              │     Restraint       │
              └──────────┬──────────┘
                         ↓
              ┌─────────────────────┐
              │ Height and weight   │
              │  measured in lab    │
              └─────────────────────┘
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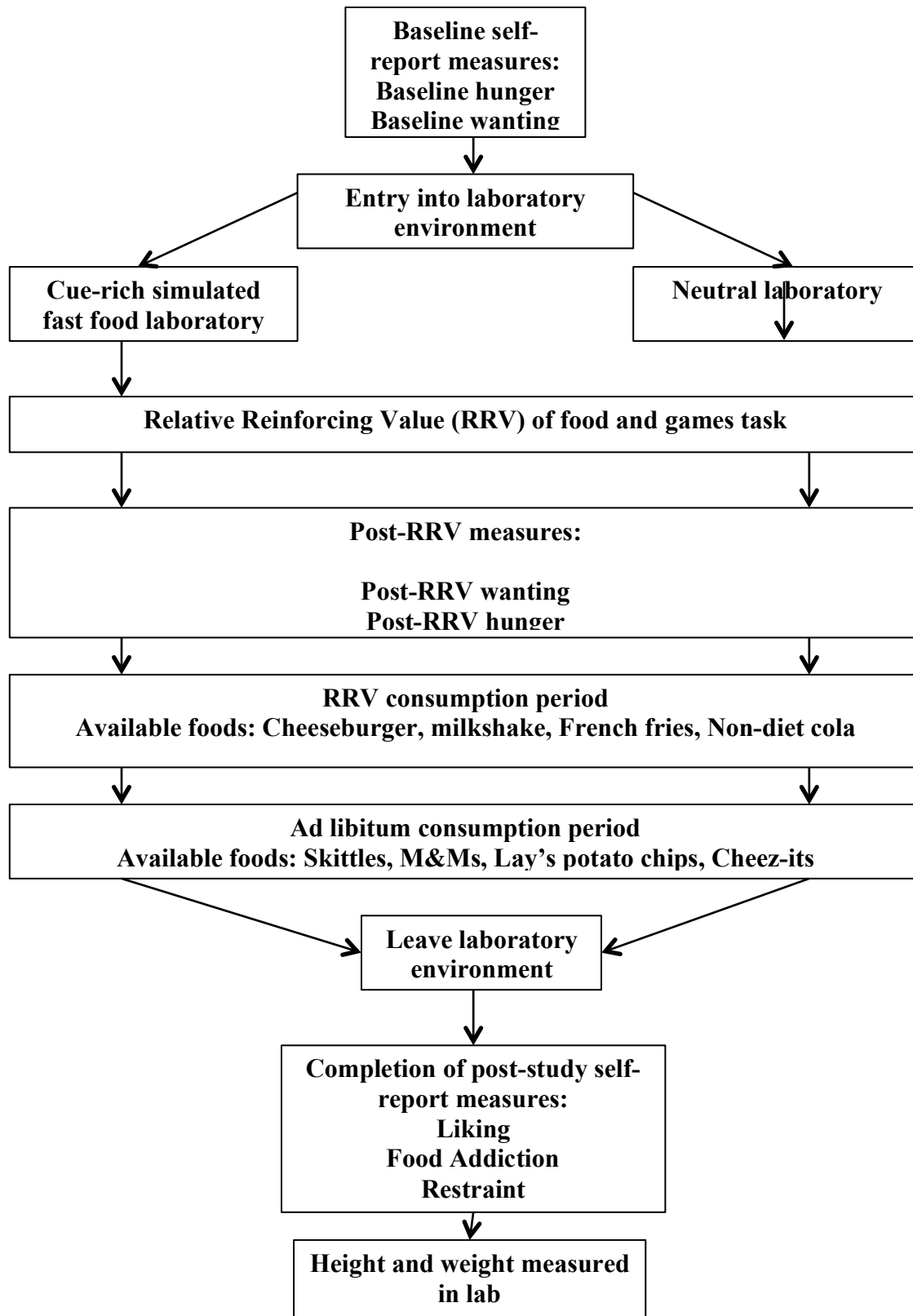
Figure II.1. Flowchart illustrating procedure timeline.

Laboratory environment. The cue-rich environment was designed to simulate the experience of being in a fast-food restaurant. This environment included condiment and napkin holders, tables and accompanying chairs and booths, and had low background music playing. Menu boards with images of each food or game were projected on large television screens (see Figures II.2 and II.3). Participants ordered from a kitchen window through which industrial restaurant-style food storage and preparation appliances were visible. Research assistants who took orders from and served participants wore aprons and hats similar to those worn by fast-food employees. French fries were cooked in the kitchen immediately before participants arrived in the lab in order to simulate olfactory aspects of the fast-food experience. In the cue-rich environment, participants were served food on red plastic trays, and serving implements (e.g., paper sleeves for burgers and French fries, cups for soda and milkshakes) were chosen to resemble those seen in fast-food restaurants.
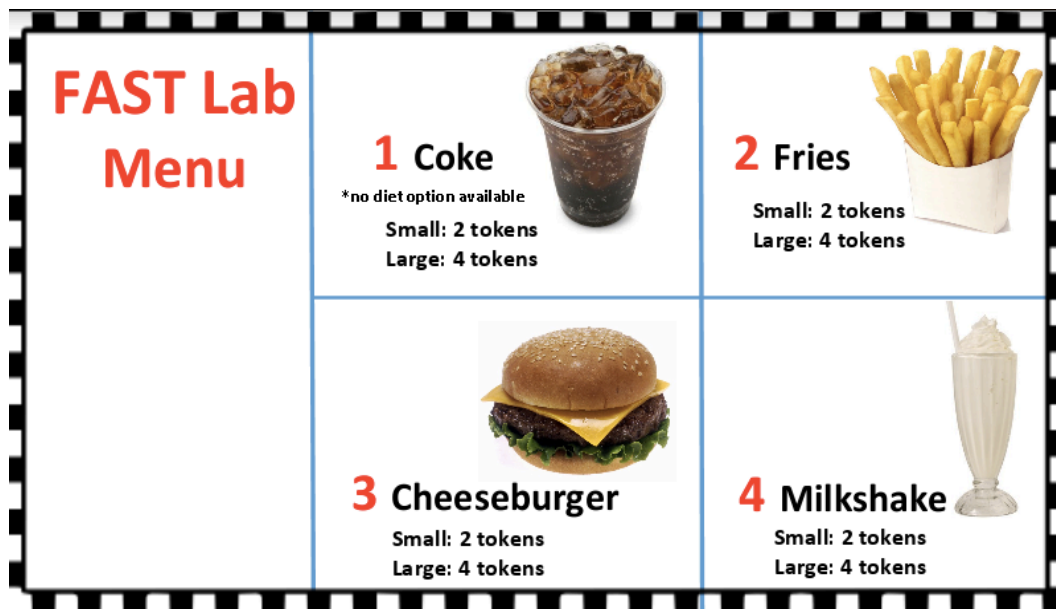

Figure II.2. Food menu board

Figure II.3. Game menu board

The neutral environment was an office space in the research laboratory. In this environment, text-only menu boards were printed on laminated paper and hung on the wall. A research assistant dressed in street clothes came in to take participants' orders, and participants did not have a view of food preparation. No music was playing, and an air filter was used to ensure a neutral scent. Participants in the neutral environment were served food on clear plastic trays, and serving implements (e.g., paper plates and cups) were chosen to resemble those that one might buy for use at home.

Measures

Relative reinforcing value of food and games (food RRV, game RRV). As a behavioral measure of wanting, we used the relative reinforcing value (RRV) task (Epstein et al., 2007; Saelens & Epstein, 1996; Temple, 2014). In this task, participants respond with a number of button presses on a computer to earn points that can be used to obtain fast foods (i.e., cheeseburger, French fries, milkshake, and soda), and time playing video games on a tablet (i.e.,

Angry Birds, Temple Run, Solitaire, and Bejeweled). Games served as an alternate reinforcer to ensure that food RRV reflected motivation to work for food, and that participants were not working for food out of boredom or lack of other options. Participants were allowed to move back and forth between stations as they wish, and could continue the task to earn as many points as they wished. The task ended when the participant chose to stop playing for points.

The RRV computer task consisted of a screen showing three different shapes, and each time the participant pressed the mouse button the shapes would change. When all three shapes matched, participants earned one point. For every 5 points earned in the current study, participants received one token that could be used toward the relevant reinforcer once they were finished with the RRV task. Points were earned on a fixed ratio reinforcement schedule beginning at 50 button presses (FR50), and doubling every 5 points (i.e., FR100, FR200, FR400, FR800, FR1600, FR3200, FR6400, FR12800). Food and game RRV were determined by the highest fixed ratio schedule completed for each reinforcer. Upon completion of the RRV task, participants were given the opportunity to redeem their tokens for food and games.

Self-reported food and game wanting. In order to assess wanting for food and games, participants responded to the questions "How much do you WANT to eat food right now?" and "How much do you WANT to play games right now?" on a 1-6 scale ranging from "Not at all" to "Very much." This measurement was collected at three timepoints: at baseline, post-RRV, and post-study.

Food and game liking. During the post-study measures, participants rated how much they liked the taste of each food they ate during the study on a 1-6 scale ranging from "Not at all" to "Very much." This rating was averaged across all the foods eaten by the participant to obtain an overall liking rating. Participants also rated on the same scale how much they enjoyed

the food they ate during the study, to capture hedonic pleasure aspects non-specific to taste. Eleven participants chose not to order any food during the RRV consumption period, and thus are not included in liking analyses. Finally, participants rated on the same scale how much they enjoyed playing the games during the study.

Hunger. To assess feelings of hunger, participants used a visual analog scale (VAS) to rate their hunger on a 0-100 scale ranging from "I am not hungry at all" to "I have never been more hungry."

Food consumption. During the RRV food consumption period, participants traded their tokens obtained during the task for small or large portions of the fast foods of their choice (serving sizes and calorie information shown in Table II.2). They also had access to packets of condiments commonly found in fast food restaurants (i.e., mustard, ketchup, mayonnaise, salt, pepper). After participants ordered food following the RRV task, researchers prepared the food and weighed the food in grams before serving. Once the participant was finished eating, researchers weighed any remaining food. The post-weight was subtracted from the pre-weight to calculate the weight consumed. The weight of any condiments consumed was estimated based on the number of condiment packets used by the participant and the standard weight of each condiment packet.

During the ad libitum consumption period, all participants had access to bowls containing standardized amounts (see Table II.3) of four snack foods for ten minutes. After this period, the bowls were weighed and the post-weights were subtracted from the pre-weights to calculate the weight of each snack food consumed. For both consumption periods, calories consumed were calculated based on the weight consumed and the calories per gram of each food item, obtained using the labeled nutrition facts of each food item. Calorie consumption was calculated based on

the amount of food consumed following the RRV task and ad libitum consumption separately. Total calories consumed were calculated by adding together the RRV and ad libitum calories consumed by each participant.

Data analytic plan

All analyses were conducted using IBM SPSS 22 (IBM, 2013). We first used frequencies to examine the distributions of all variables of interest and to check for missing data. Two outliers (>2 SD above the mean) were found in the total calorie consumption variable, two outliers were found in the ad libitum calorie consumption variable, and one outlier was found in the game RRV variable. These cases were removed to normalize the distribution of these variables. We also found some individuals to be missing data for specific variables due to reasons such as errors in the survey program preventing that data from being saved properly or not completing portions of the study protocol[1]. These participants were excluded only from analyses involving those variables for which they were missing data. To assess for potential covariates, we conducted correlational analyses and one-way analysis of variance (ANOVA) to examine relationships between demographic variables (i.e.. race, age, gender, BMI) and the dependent variables (e.g., food RRV, post-RRV food wanting, post-consumption food liking, post-RRV hunger, RRV food consumption, and ad libitum food consumption). No significant associations were found (all p's >.05), thus these variables were not included as covariates. We conducted a one-way ANOVA and chi-square analyses to determine the success of random distribution of demographic variables into each condition. Demographic variables, baseline food

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[1] Five participants were missing data from the post-RRV survey measures (i.e., food and game wanting, hunger) due to errors saving the data. The ad libitum protocol described in this article was added to the study after 12 participants had already taken part, thus ad libitum and total consumption data is only included for those who took part after the ad libitum protocol was added to the study.

and game wanting, and baseline hunger did not differ significantly by condition (all p's > .05; see Table II.1 for group means).

To test Aim 1, we conducted a one-way ANOVA to examine whether food wanting and food liking differed between experimental conditions (i.e., cue-rich or neutral laboratory environment). In order to ensure that any differences in wanting and liking were specific to food, we also tested whether game wanting and game liking differed by condition.

To test Aim 2, we conducted a one-way ANOVA to test whether self-reported hunger differed between experimental conditions. We also examined interaction terms in separate multiple regression analyses to test whether baseline hunger moderated the relationship between laboratory environment and wanting, liking, and consumption.

To test Aim 3, we first conducted a one-way ANOVA to test whether food consumption (i.e., RRV, ad libitum, and total calories consumed) differed between conditions. Then we conducted mediation analyses using the PROCESS macro developed by A. F. Hayes (2012). Since participants' consumption during the RRV period was directly tied to their RRV performance (food RRV), we focused our mediational analyses on post-RRV self-report measures (i.e., food wanting, hunger) and used total consumption as the outcome. Variables that did not significantly differ by condition were not included in mediation models. To test the hypothesized mediation models (e.g., laboratory environment → post-RRV self-reported food wanting → total food consumption), we employed the bootstrapping method with 10000 samples described by Preacher and Hayes (2008), which yields a 95% confidence interval. The completely standardized indirect effect (ab_{cs}) (Preacher & Kelley, 2011) was used to compare the effect sizes of statistically significant indirect effects. Effect sizes can be interpreted as small (.01), medium (.09), or large (.25) (Kenny, 2014).

Power analyses. Pilot food RRV data showed a mean difference between groups of 265.98 and a standard deviation of 462.26. We applied power estimation procedures based on these values and assuming 2-tailed alpha of .05. This analysis yielded an estimate of 49 participants per group required to attain a power of .80, and 65 participants needed per group to reach a attain of .90 to detect differences in RRV performance between our two groups.

Hypotheses

1. We predicted that participants in the cue-rich environment would display greater food wanting (as shown by self-report and food RRV) than those in the neutral environment. We predicted that game wanting and food liking would not differ by condition.

2. We predicted that participants in the cue-rich environment would report greater hunger than those in the neutral environment. As baseline hunger did not differ significantly by condition, we predicted that it would not significantly moderate the relationship between laboratory environment and wanting, liking, and consumption.

3. We predicted that participants in the cue-rich environment would consume a greater number of calories (RRV, ad libitum, and total) than those in the neutral environment. We also predicted that food wanting and hunger would mediate the relationship between laboratory environment and total food consumption.

Results

Aim 1

Relative Reinforcing Value of food and games. Group means for all dependent variables are presented in Table II.4. Participants in the cue-rich environment demonstrated higher food RRV ($F = 5.13$, $p = .03$, $\eta^2 = .05$) compared to those in the neutral environment.

Participants in each environment did not differ significantly in game RRV ($F = .68$, $p = .41$, $\eta^2 = .01$).

Self-reported food and game wanting. Participants in the cue-rich environment reported significantly higher post-RRV food wanting rating ($F = 6.45$, $p = .01$, $\eta^2 = .06$) than those in the neutral environment. Participants in each environment did not differ significantly in their post-RRV ratings for game wanting ($F = 0.14$, $p = .71$, $\eta^2 = .00$)

Table II.4
Means and Standard Deviations of Variables of Interest

	Cue-rich M (SD)	Neutral M (SD)	F	p	η^2
RRV calories consumed	740.26 (464.06)	533.80 (388.15)	6.70	.03*	.06
RRV weight consumed (g)	454.19 (247.16)	350.92 (214.82)	5.45	.02*	.05
Ad libitum calories consumed	69.35 (104.70)	77.48 (77.84)	0.11	.74	.00
Ad libitum weight consumed (g)	14.63 (21.79)	15.44 (15.72)	0.05	.83	.00
Total calories consumed	832.49 (467.82)	612.52 (402.28)	6.23	.01*	.06
Total weight consumed (g)	477.89 (247.58)	369.49 (219.41)	5.27	.02*	.05
Food RRV	698.33 (722.40)	432.35 (462.26)	5.13	.03*	.05
Game RRV	946.55 (1047.84)	803.92 (697.13)	0.68	.41	.01
Post-RRV food wanting	3.72 (1.40)	3.10 (1.05)	6.45	.01*	.06
Post-RRV game wanting	3.16 (1.24)	3.24 (1.23)	0.14	.71	.00
Post-RRV hunger	52.47 (20.75)	41.84 (18.90)	7.51	.01**	.07
Food liking of taste	3.43 (0.97)	3.39 (1.18)	0.05	.82	.00
Food enjoyment	2.98 (1.04)	2.91 (1.09)	0.11	.75	.00
Game enjoyment	3.86 (1.24)	3.62 (1.19)	1.16	.28	.01

Note. *p<.05, **p<.01

Self-reported food and game liking. Participants in each environment did not differ significantly in their self-reported liking for the taste the foods consumed ($F = 0.05$, $p = .82$, $\eta^2 = $

.00), enjoyment of eating the foods ($F = 0.07$, $p = .79$, $\eta^2 = .00$), or enjoyment of playing the games ($F = 1.16$, $p = .28$, $\eta^2 = .01$).

Aim 2

Main effect of laboratory environment on post-RRV hunger. Participants in the cue-rich environment reported significantly higher post-RRV hunger ratings ($F = 7.51$, $p = .01$, $\eta^2 = .07$) than those in the neutral environment.

Baseline hunger interactions. Baseline hunger did not significantly interact with laboratory environment to predict food wanting, liking, or consumption (all p's > .05). There was a non-significant trend-level interaction between baseline hunger and laboratory environment to predict food RRV ($F(3, 107) = 3.34$, $\beta = .25$, $R^2 = .11$, $p = .07$). For participants in the cue-rich environment, there was a significant, positive correlation between baseline hunger and food RRV ($r(60) = .30$, $p = .02$), while for participants in the neutral environment there was no significant correlation ($r(51) = .02$, $p = .89$). All other interaction p-values were .27 or greater.

Aim 3

Food consumption. Participants in the cue-rich environment compared to the neutral environment consumed significantly more calories during the RRV consumption period ($F = 6.70$, $p = .01$, $\eta^2 = .06$). Participants in each environment did not differ significantly in the number of calories consumed during the ad libitum consumption period ($F = 0.11$, $p = .74$, $\eta^2 = .00$). This difference remained nonsignificant after controlling for RRV consumption. ($F = 0.02$, $p = .88$). Participants in the cue-rich compared to neutral environment consumed a greater number of total calories ($F = 6.23$, $p = .01$, $\eta^2 = .06$).

Mediation models. Neither liking the taste of the foods nor enjoyment of eating the foods significantly differed by environment, thus food liking failed to meet the requirements to

be tested as a mediator and neither variable was included in the mediation models. Post-RRV food wanting ($B = 91.25$, $SE = 48.31$, 95% CI = 4.23 – 196.73, $ab_{cs} = .10$) and post-RRV hunger ($B = 107.89$, $SE = 49.77$, 95% CI = 20.39 – 219.38, $ab_{cs} = .12$) were significant mediators in the relationship between environment and total food consumption.

Discussion

The current study tested IS theory by examining food wanting and liking in both a cue-rich simulated fast-food laboratory and a neutral laboratory environment. Our first aim tested whether wanting and liking were separable in a cue-rich context, as posited by the IS theory (T. E. Robinson & Berridge, 1993). Our second aim investigated whether self-reported hunger differed in a cue-rich compared to neutral context, as hunger has been shown to be affected by environmental cues (Cohen, 2008; A. W. Johnson, 2013), as well as whether baseline hunger moderated the association between laboratory environment and food wanting, liking, and consumption. Our third aim tested whether food consumption differed in a cue-rich compared to neutral context, and investigated mechanisms by testing self-reported food wanting and hunger as mediators in the relationship between laboratory environment (i.e., cue-rich or neutral) and food consumption.

Under IS theory, food-related cues play a central role in triggering food wanting, but a less important role influencing food liking. The current study supported this theory. Both food RRV and self-reported food wanting were greater in the cue-rich compared to neutral environment, suggesting that food cues are an important influence on food wanting. However, neither liking for the taste of foods nor enjoyment of eating the foods differed between the two conditions, suggesting that cues are not as important an influence on food liking. Previous studies have had mixed results in illustrating the separability of wanting and liking (Finlayson &

Dalton, 2012; Havermans, 2011, 2012). Given that this dissociation is a central tenet of IS theory (T. E. Robinson & Berridge, 1993), the current study's demonstration that wanting and liking are separable in a cue-rich context provides important evidence in support of IS in human eating behavior.

Feelings of hunger are shown to be elevated in the presence of food-related cues (Cohen, 2008; A. W. Johnson, 2013), suggesting that the experience of hunger can be influenced by the environment as well as by homeostatic need. In the current study, baseline hunger ratings taken before entering either laboratory environment did not differ between conditions. However, after being exposed to their respective laboratory environments, participants in the cue-rich environment reported experiencing greater hunger than those in the neutral environment. The finding that hunger only increased in the presence of cues suggests that the feelings of hunger were not fully driven by homeostatic need. As this experience was still reported by participants as hunger, it is possible that individuals have difficulty distinguishing homeostatic and cue-driven hunger. This difficulty could contribute to excess consumption in cue-rich environments, as people may begin to feel hungry even when satiated. Thus, feelings of hunger could be a mechanism by which a cue-rich environment contributes to increased food consumption. While baseline hunger did not significantly interact with condition to predict the dependent variables, there was a non-significant trend-level interaction between baseline hunger and environment to predict food RRV. In the cue-rich environment, those who were hungrier at baseline found food even more reinforcing. This suggests that hunger may have marginally amplified participants' response to cues; however this effect was only present with regard to food RRV. While research suggests that homeostatic hunger has the ability to moderate one's wanting and liking in response to cues (Berridge et al., 2010), it is possible that non-homeostatic hunger does not

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interact with cues in the same way. As our self-report measure of hunger did not distinguish between caloric need and non-homeostatic feelings of hunger, future research should do so to further examine how each may differ in response to cues.

Consistent with prior research that people are more prone to eat when cued (Boswell & Kober, 2016; Ferriday & Brunstrom, 2011), participants in the cue-rich compared to neutral environment consumed more calories both in total and during the RRV consumption period. Specifically, participants in the cue-rich environment consumed an average of 219.97 additional calories compared to those in the neutral environment. Consumption of only 148 additional calories per day can lead to a gain of 15 pounds per year (Wellman & Friedberg, 2002). Thus, exposure to the ubiquitous food cues in the American food environment could, over time, lead to weight gain through accumulation of small daily increases in consumption. Further, college students such as those in our sample are in a developmental stage during which they are making increasingly independent choices about food intake and their food preferences are still being set (Cluskey & Grobe, 2009; Nelson et al., 2009; Pliner, 1982). As they get older and their metabolism slows (Rowe & Kahn, 1987), the same intake may contribute to more weight gain and obesity. Based on the current results, this possibility may be amplified by exposure to food-related cues. Therefore, although the current sample consisted of individuals currently displaying healthy BMI and few pathological eating symptoms, continued exposure to food cues could put them at risk for weight gain and obesity later in life.

While the current study observed the ability of food cues to influence excess consumption, this effect did not apply to all foods. Participants in the cue-rich and neutral environments did not significantly differ in their consumption during the ad libitum portion of the protocol. This suggests that there may be some specificity to the impact of food cues on

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consumption. The foods available during the ad libitum period (e.g., M&Ms, Cheez-its) are not foods typically consumed in a fast food restaurant, thus the fast-food cues may not have impacted consumption of these foods as strongly. We used these non-fast food related snack foods in order to minimize any effect of sensory-specific satiety for the fast food items served earlier in the study. However, it is possible that by using foods incongruent with the context we reduced our ability to induce greater consumption in response to cues. It may be that in order to trigger increased wanting and consumption, cues must be consistent with the available foods. If this is the case, this knowledge could be used to develop interventions employing the use of congruent or incongruent cues. For example, limiting cues to those for healthy foods (e.g., pictures of fruits and vegetables) in areas such as college dining halls could influence people to consume more healthy and fewer unhealthy foods in that setting. Further research is needed to better understand the effect of cues on wanting for and consumption of foods congruent with the environmental context versus foods incongruent with the environmental context.

The association between cue-rich environment and greater total caloric consumption was mediated by both self-reported food wanting and feelings of hunger. Since food liking did not differ by condition, it does not appear to be a mechanism through which a cue-rich environment is related to greater consumption. Findings from these mediation analyses support IS theory, suggesting that wanting more than liking contributes to elevated consumption in the context of cues. These findings also support a role for feelings of hunger in addition to wanting in increasing food consumption. The current Western food environment is rich with cues for calorie-dense, nutrient-poor foods (e.g., advertisements, vending machines). Given these findings, food wanting and feelings of hunger may be effective targets for interventions aimed at helping people to successfully navigate their exposure to food cues.

The current study has some limitations that should be addressed through future research. Our sample exhibited a restricted BMI range, thus we did not find any significant relationships between BMI and our variables of interest. A sample with a wider BMI range will be better able to demonstrate how cues influence eating behavior in individuals who are obese. Prior research has found an association between obesity and cue reactivity (Sobik, Hutchison, & Craighead, 2005; Tetley, Brunstrom, & Griffiths, 2009), thus perhaps the effects of our cue-rich context would be even more pronounced in individuals with obesity. Our sample was also relatively healthy, limiting generalizability to more clinical samples. As IS theory was developed in relation to addictive disorders, we may expect cue-triggered wanting, hunger, and consumption to be amplified in individuals meeting criteria for food addiction. Future studies with a greater proportion of individuals with clinically significant food addiction would have greater power to thoroughly examine this effect.

Due to the structure of the RRV paradigm, food RRV was inherently linked with total consumption, preventing us from testing food RRV as a mediator in the relationship between laboratory environment and consumption. In order to test food RRV as a mechanism, future studies may be designed such that this variable is not linked to the outcome of interest, for example, by providing unlimited access to the RRV foods rather than restricting access based on points earned. Additionally, as self-reported wanting and hunger ratings in the laboratory environment were obtained after the RRV task, it is possible that these ratings were influenced by task performance (e.g., individuals reported being hungrier because they had just worked hard for food) due to cognitive dissonance in which attitudes are shifted to reflect prior behaviors (Brehm, Back, & Bogdonoff, 1964). We believe that our findings of increased self-reported wanting and hunger in the cue-rich environment, in combination with our finding of increased

food RRV in the cue-rich environment, provides strong evidence for the ability of cues to trigger increased food motivation. However, future studies would do well to measure self-reported wanting and hunger in the presence of cues before any behavioral task to ensure that any increase seen is due to cues. Food liking measurements were taken at the end of the study and outside of the laboratory environment. It is possible that liking ratings would have been higher if obtained in the laboratory environment, and that measuring liking outside the environment may have reduced the effect of cues. Future studies should assess liking while in cue-rich versus neutral environments to ensure all effects are fully captured. Finally, though we made efforts to standardize hunger, it is possible that results may have been weakened by participants achieving satiety, particularly with regard to ad libitum food consumption. Future studies may assess eating behavior when participants are in a fasted state to gain a fuller understanding of the impact of cues on eating behavior.

The current study builds upon prior research on the role of cues in consummatory behaviors, examining food wanting and liking in a simulated fast-food laboratory. Unlike prior studies, which used food images or smells alone as cues, we observed a strong distinction between food wanting and liking in our cue-rich environmental context. These results have important implications for efforts to reduce overeating and obesity. Unhealthy food cues are ubiquitous in the Western food environment, possibly leading to greater wanting and experiences of hunger, which may be difficult to resist and result in overeating, even for healthy individuals. In those with obesity or eating-related pathology, cue reactivity could be even more pronounced, although future research is needed. The current study's findings on the impact of cues suggest that modifying one's exposure and response to these cues could be an effective target for interventions targeting overeating. As food-related cues appear to be powerful influences on

overeating even in healthy individuals, it may be helpful for people to identify triggering settings where they may be exposed to unhealthy food cues (e.g., fast-food restaurant) and take steps to either limit their exposure to these settings or mitigate their response. For example, people may choose to take their meal to go, rather than dining in a fast food restaurant, so they are less affected by the presence of cues during their meal.

Given the mediating role of wanting and hunger, treatments aimed at responding to feelings of wanting and hunger may also be effective. For example, mindfulness techniques such as "urge surfing," or learning to ride out a craving without giving in to it, have shown effectiveness in treatment of substance use disorders (Bowen & Marlatt, 2009). Recognizing these feelings and learning strategies to respond to them more effectively help people feel better equipped to resist the strong, cue-triggered urge to consume unhealthy food. In addition to interventions at the individual level, strong evidence that excessive consumption of unhealthy foods is impacted by environmental cues supports the important of population-level interventions, for example policy approaches reducing the ubiquity of some types of cues (e.g., restrictions on food advertising). While additional research is needed to determine the effect of cues across populations and situations, the current study demonstrates that IS principles appear to be at play in eating behaviors and justifies further study.

CHAPTER III

Developing a Paradigm for Identifying Pavlovian Conditioned Responses (Sign-Tracking and Goal-Tracking) To a Food Cue in Human Children

As discussed in Chapter II, food-related cues appear to contribute to enhanced wanting and elevated consumption. However, there may also be individual differences in susceptibility to these cues. Pavlovian conditioning provides one framework for understanding individual differences in the attribution of incentive-salience to cues. In a basic Pavlovian conditioning paradigm, an unconditioned stimulus (US), which provokes an unconditioned response (UR) is associated with a conditioned stimulus (CS), which eventually provokes a conditioned response (CR) (Rescorla, 1988). To put Pavlovian conditioning into the context of eating behavior, a palatable food may serve as the US, provoking the UR of reaching for and consuming the food (see Figure III.1). If an individual is conditioned to associate a cue with the delivery of the food, that cue becomes the CS, and
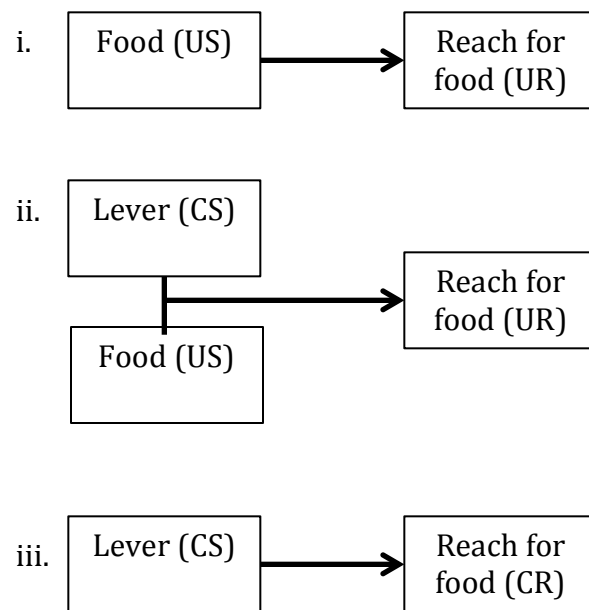
Figure III.1. Pavlovian conditioning model. The US of food leads to the UR of reaching for the food (i). After the US of food is paired with the CS of a lever (ii), the CS of lever eventually begins to lead to the CR of reaching for the food (iii).

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eventually may provoke the CR of reaching for the food when the cue is presented. For some, this cue may develop incentive salience on its own, attracting attentional bias and approach behavior, becoming a conditioned reinforcer (i.e., causing individuals to work for access to them), and eliciting enhanced motivation or wanting for the CS (Boakes, 1977; Flagel et al., 2009; Hearst & Jenkins, 1974; T. E. Robinson et al., 2014). As discussed in Chapter II, food-related cues may increase motivation to obtain and consumption of palatable foods in young adult humans. Thus, it is important to understand individual differences in the attribution of incentive salience to these cues.

Studies using animal models have identified two profiles representing differing attribution of incentive salience to cues using a Pavlovian conditioning paradigm. Individuals who assign incentive-salience to the cue itself are identified as sign-trackers (STs). When trained to associate a discrete, localizable cue (e.g., lever, light) with the delivery of a food reward, STs will interact with the cue itself, often even if it interferes with their ability to obtain the actual reward (Boakes, Poli, Lockwood, & Goodall, 1978; Hearst & Jenkins, 1974). Sign-tracking is associated with elevated impulsivity and difficulty exerting inhibitory control (Beckmann, Marusich, Gipson, & Bardo, 2011; Flagel et al., 2011; Lovic et al., 2011; T. E. Robinson et al., 2014). In the context of addiction, STs appear to be susceptible to reinstatement of reward-seeking behavior through increased impulsivity and enhanced motivation in the presence of reward-related cues (T. E. Robinson et al., 2014).

Other individuals trained to associate a discrete, localizable cue with reward delivery do not assign incentive salience to the cue, but will instead approach the reward itself. These goal-trackers (GTs), when presented with the cue, will approach or orient towards the location where they expect the reward to be delivered, interacting minimally or not at all with the cue itself

(Flagel et al., 2009; T. E. Robinson & Flagel, 2009; T. E. Robinson et al., 2014). Until recently, it was thought that STs were at greater risk for addiction than GTs, due to the association of sign-tracking with impulsivity and novelty-seeking (Beckmann et al., 2011; Lovic et al., 2011). However, recent research suggests that goal-tracking may represent an alternate pathway to addictive behaviors. While STs are susceptible to displaying enhanced motivation and reward-seeking behavior in response to a previously associated discrete cue, GTs appear to show enhanced motivation in response to a cue-rich context (Fraser & Holland, 2019; Pitchers, Phillips, Jones, Robinson, & Sarter, 2017; Saunders et al., 2014). The impact of contextual cues appears to be mediated by neural dopamine transmission in the accumbens, putting the GT individual in a motivated state (T. E. Robinson et al., 2014; Saunders et al., 2014). Generally, sign-tracking and goal-tracking responses are underscored by different patterns of dopamine release in the nucleus accumbens, with STs displaying CS-evoked release while GTs do not (Flagel et al., 2011; T. E. Robinson et al., 2014).

Sign-tracking and goal-tracking responses illustrate the dissociation of predictive versus incentive properties of reward-related cues. While both STs and GTs learn that the cue signals reward delivery (predictive value), only STs attribute incentive salience to the cue itself. Examining these responses then allows the dissociation of associated underlying neurobiological and psychological processes. This provides a useful framework for modeling risk for engaging in compulsive consummatory behaviors, identifying individuals that may be susceptible to overconsumption in response to cues.

While animal models have provided a great conceptual understanding of sign-tracking and goal-tracking, less research has examined these profiles in humans. A study examining cue-induced craving in fifteen adult smokers found that a subset of individuals experienced stronger

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craving in response to both food and smoking cues, suggesting a "cue-reactive" phenotype is present in humans (Mahler & de Wit, 2010). Focused specifically on sign-tracking and goal-tracking, one study on adults with and without obesity measured individuals' neural response to images of palatable foods and other rewards, classifying those with relatively greater response to food images as STs and those with relatively greater response to other images as GTs (Versace et al., 2016). However, the study by Versace and colleagues did not include a Pavlovian conditioning paradigm, and without evidence of a learned association between the cue and reward delivery, it is difficult to conclude that the design validly identifies ST and GT profiles.

Another study by Garofalo and di Pellegrino (2015) employed a Pavlovian conditioning task to identify sign-tracking and goal-tracking behavior in human adults. In this study, participants engaged in a computer task during which they were trained to associate a visual cue with a monetary reward, and were categorized as STs or GTs based on visual attention to the cue and the visual representation of the reward. Individuals identified as STs using this paradigm also scored higher on a self-report measure of impulsivity. This study provided preliminary evidence that sign-tracking and goal-tracking behavior may be identifiable in human adults, and illustrated a relationship between sign-tracking and impulsivity, similar to findings in animal models. We hope to expand on these findings by testing the association of sign-tracking and goal-tracking phenotypes with behavioral measures of traits implicated in overeating and obesity, namely low inhibitory control and high food motivation (Batterink et al., 2010; Temple et al., 2008). This will help us further understand how these phenotypes manifest behaviorally, which will in turn provide insight into the types of behaviors that may be effective to target in treatments aimed at preventing overeating and obesity.

Additionally, there is not yet any research to our knowledge examining sign-tracking and goal-tracking in humans at earlier developmental stages. As children are at particular risk for developing obesity, and children who become obese are likely to remain so, identifying phenotypes in childhood that may contribute to excess consumption could facilitate early intervention, minimizing the risk for developing obesity. Additionally, no study on humans to our knowledge has used a paradigm pairing the CS with an immediate, consumable food or drug reward, as has been shown in the animal models. Given the usefulness of sign-tracking and goal-tracking as potential phenotypes of addictive behavior, it is important to understand how these behaviors manifest in humans when presented with a consumable reward. Sign-tracking and goal-tracking may represent different pathways to compulsive consummatory behavior, thus identifying these profiles in humans and examining their relationships to known behavioral phenotypes of obesity could allow for improved prevention and intervention efforts.

The current study aims to develop and test a novel paradigm to identify sign-tracking and goal-tracking behavior in humans. This chapter will detail the choices made throughout the development and piloting of a novel paradigm, including the selection of a subject population, the design of a Pavlovian conditioning task and apparatus, and the determination of the most useful variables to identify sign-tracking and goal-tracking phenotypes. Developing an effective paradigm for identifying these phenotypes in humans will provide information on behavioral characteristics that can inform targeted interventions for overeating and obesity.

Specific Aims

1. Develop a Pavlovian conditioning task to reliably and validly identify sign-tracking and goal-tracking phenotypes in humans in the context of eating behavior and test the feasibility of this paradigm with a sample of at least 48 participants.

2. Investigate the association of each phenotype with food motivation and inhibitory control.

Study Development

Our goal was to develop a Pavlovian conditioning paradigm similar enough to that used in animal models to allow valid identification of sign-tracking and goal-tracking phenotypes. Methods were pre-registered with AsPredicted.org on January 15, 2019; see Appendix A for pre-registration text. We used the paradigm described by Flagel, Watson, Akil, and Robinson (2008) as a basis for the current study. Similar to the animal models, we decided to use a lever as the CS, and a bite-sized food item for the US. Participants would be trained to associate the CS presentation with the delivery of the US over the course of a number of trials. Upon learning this association, participants would be classified as STs or GTs based on their interaction with each stimulus.

Selection of Participant Sample

We chose to recruit young children as the sample population for several reasons. First, the prefrontal cortex (PFC), responsible for decision-making and other higher-order executive functions, is not fully developed until late adolescence or young adulthood (Casey et al., 2000). For this reason, children may be less likely than adults to attempt to engage in deep cognitive processing of the task, and therefore may be more likely than adults to display the phenotypes (i.e., sign-tracking and goal-tracking) that have been observed in animal models. Second, children display greater food craving compared to adults, are highly prone to engage in behaviors influenced by enhanced motivational drive, such as excessive consumption of palatable foods (Rollins et al., 2014; Silvers et al., 2014). Thus, children are an important age group in which to understand individual differences in attribution of incentive salience to food-signaling stimuli.

Finally, given the high prevalence and numerous health consequences of childhood obesity (Dietz, 1998; Ogden et al., 2014), examining the presentation of risky phenotypes early in childhood can provide the opportunity for early intervention and prevention efforts. We ultimately decided to recruit an age range of 5-7, because these children are young enough that the PFC is still at an early developmental stage, and old enough that they are able to understand simple verbal instructions from research staff and to consume small food portions with low risk of choking. We chose to exclude participants who have been diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD) or any pervasive developmental delay disorder (e.g., Autism Spectrum Disorder [ASD]), as these conditions may affect attentional control, potentially biasing measurement of attention to each stimulus.

Development of Pavlovian Conditioning Apparatus and Paradigm.

In order to replicate the animal model in our human sample, we first designed and built an apparatus capable of running the paradigm (see Figure III.2). Our apparatus consisted of two solid-colored boxes, designed to look appealing to children without being too attractive or rewarding on their own. The boxes were spaced approximately ten inches apart to reduce the ability to simultaneously visually attend to both boxes, and were located on
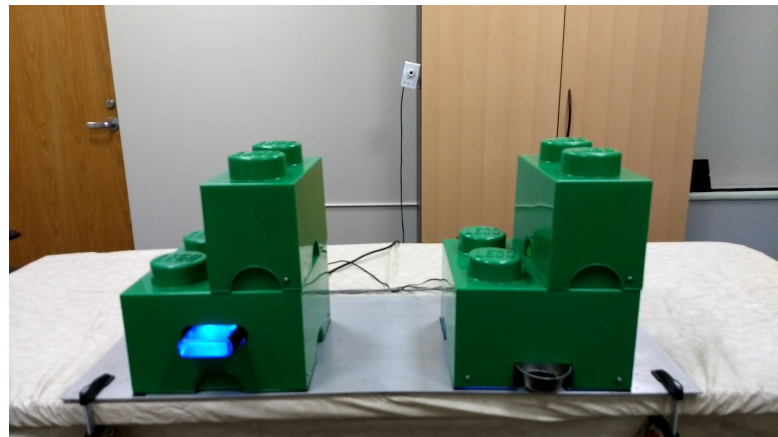

Figure III.2. Pavlovian conditioning apparatus

two separate platforms so the side of the room on which each stimulus was presented could be counterbalanced to minimize bias. The CS box contained a lever, which illuminated and

extended from the box. The US box contained a small metal tray into which a small food portion could be dispensed. The actions of the apparatus were powered using an Arduino board (Arduino, 2015). The Arduino was controlled by a researcher using a program in MATLAB (MathWorks, 2016).

When determining the food item to use as the US, we took several factors into consideration. The food needed to be rewarding to the study population, so we chose to use some type of candy or sweet treat. The food needed to be a small enough portion to allow the number of trials necessary for learning to occur without causing satiety. The shape of each portion needed to be consistent in order to be dispensed properly from the machine. For the above reasons, we chose to use M&Ms as the US. Participants were thus excluded if they had dietary restrictions preventing them from consuming M&Ms.

The basic structure of the Pavlovian conditioning paradigm described by Flagel and colleagues (2008) consists of a number of trials. On each trial, the lever (CS) is illuminated and extended for eight seconds, then retracted. Immediately following CS retraction, one food portion is delivered. Following each trial is an inter-trial interval (ITI), after which the next trial commences. In Flagel and colleagues' (2008) animal paradigm, the conditioning sessions occur over a number of days, with each training session consisting of 25 trials and lasting 35-40 minutes. For our population of young children, we wanted to condense this to a single session, to avoid participant burden of having to come in to the lab for multiple sessions. We also wanted the training session to be as short as possible, to minimize participant fatigue or inattention while still allowing enough trials to for learning of the association to occur. For initial pilot tests, we conducted three blocks of ten trials each. The ITI period was programmed to last for a randomly selected time ranging from 10-30s. Each block was followed by a "wiggle break" lasting up to

45 seconds, during which the child was given the opportunity to rest and shake out before the next trial.

In order to capture number of contacts to each stimulus and latency to touch each stimulus, the MATLAB program controlling the apparatus recorded number and timing of contacts to the CS lever and US candy tray. To obtain additional behavioral data, the task was also videotaped from an overhead angle and a head-on angle, to capture body and head orientation.

Power Analysis and Participants

We conducted a power analysis to estimate sample size based on the index of Pavlovian conditioned behavior found in an existing study of sign-tracking and goal-tracking in humans (Garofalo & di Pellegrino, 2015). This study found this index to show mean difference of .10 between STs and GTs, with a standard deviation of .12. We applied power estimation procedures based on these values and assuming 2-tailed alpha of .05. This analysis yielded an estimate of 24 individuals per group needed to achieve a power of .80, or 32 individuals per group to achieve a power of .90. Thus, we planned to recruit at least 48 participants in effort to allow sufficient power to detect differences in conditioned response between STs and GTs.

Sixty-four total children aged 5-7 (*M*=5.9, *SD*=0.8) took part in the study. Thirty-three (51.6%) children were female and 31 (48.4%) were male. Children participated with one biological parent, of whom 62 (96.9%) were mothers and two (3.1%) were fathers. The racial breakdown of children was as follows: 36 (56.3%) white, two (3.1%) black, 1 (1.6%) Asian/Pacific Islander, one (1.6%) American Indian / Alaska Native, six (9.4%) Hispanic/Latino, two (3.1%) other, and 16 (25%) more than one race. Participants were recruited via flyers posted in the community and online advertisements, with the most successful recruitment method (31%

of participants) being Facebook advertisements. Participants were compensated $20 for their time. Initially, several interested individuals declined to participate due to high cost of travel to the study location, thus we added an additional $20 travel compensation for participants who were traveling from greater than 30 miles away from the laboratory.

Modifications Based on Initial Pilot Testing

After the first nine participants completed the protocol, we examined initial data to determine what modifications might be needed. Data from these nine participants are considered initial pilot data. Initial pilot testing yielded information leading to several changes in the paradigm. Upon examination of the apparatus data output of number and timing of contacts, it was difficult to identify goal-tracking behavior. Most children were contacting the US exactly once per trial, to obtain the candy once it was dispensed. The data did show variation in number of contacts to the CS, indicating varying levels of sign-tracking. However, this highlighted the need to analyze additional data in order to be confident that we could identify goal-tracking, rather than just the absence of sign-tracking. We determined that video data would be crucial to assess approach and orientation behavior that did not include stimulus contact. To facilitate easier division into increments for video coding of behavior, we modified the timing of the paradigm, changing the ITI duration to be randomly selected from 8, 16, 24, or 32s. Finally, as an additional measure of non-contact behavior, we added behavioral observation by a researcher sitting behind the participant, recording proximity and head orientation toward each stimulus. During the initial stages of data collection, these behavioral observations were collected by a researcher familiar with the hypotheses, in order to allow that researcher to develop and test an optimal coding scheme. However, once the full research team was adequately trained in this behavioral coding, these observations were obtained by researchers blind to the hypotheses.

Observations made by researchers who were blind versus not blind to the hypotheses were compared to ensure that bias due to researcher knowledge of hypotheses is not present in the final results. These observations did differ significantly based on whether or not researchers were blind to hypotheses, with those who were familiar with hypotheses identifying more goal-tracking behavior during block 4 of the CS period ($F = 7.725$, $p = .01$, $\eta^2 = .15$). As described in the Results section below, results using behavioral observation data based on both blind and unblinded researchers did not differ from those calculated using other measurement methods.

Automated data from pilot testing also showed that learning, indicated by a change in response over blocks and trials resulting in a consistent response pattern in the final block, was not readily apparent. We chose to add a fourth block of ten more trials to increase the number of trials over which an observable learned response pattern might develop. Since participants during pilot testing did not express fatigue or appear inattentive after three blocks, we determined that the benefit of additional data merited adding a fourth block.

As the primary aim of the current study was to test feasibility, initial pilot participants were also included in the final sample in order to maintain power to observe true effects. For these participants, block three served as the final block. As the only changes to the automated data collection method were the addition of a fourth block and small changes to the range of the ITI duration (from 10-30s to 8-32s), we do not expect that data from initial pilot participants differed meaningfully from that of participants who completed the final version of the protocol.

Measures

Aim 1 measures.

Pavlovian Conditioned Approach (PCA) index. To determine conditioned response based on contacts to the Pavlovian conditioning apparatus, we calculated a Pavlovian

conditioned approach (PCA) index, based on that developed by Meyer and colleagues (2012). This index is calculated for each trial, and consists of the average of three measures: response bias (i.e., the probability of contacting the CS versus the US), probability difference score (i.e., probability of contacting the CS minus the probability of contacting the US), and latency difference score (i.e., latency to contact US minus latency to contact CS for each trial). The PCA index score ranges from -1.0 to 1.0, with -1.0 representing pure goal-tracking behavior and 1.0 representing pure sign-tracking behavior. Based on initial pilot testing, the probability difference score yielded less useful information than in the animal models, as most participants contacted each stimulus at least once per trial. Thus, we also elected to calculate a modified version of the PCA index consisting of the average response bias (number of CS contacts – number of US contacts / total number of contacts) and latency difference scores (latency to contact US – latency to contact CS) only. The PCA index was calculated separately for the CS-period (i.e., while the lever is being presented) and the ITI-period in order to capture difference in behavior based on trial period.

Behavioral observation and video data. In order to assess phenotypic behavior that does not include contact to the apparatus, we coded proximity and orientation behavior using behavioral observation data. During the study, a researcher recorded whether the child was in closer proximity to the CS or the US on each trial. If the child was equidistant to both stimuli, proximity was recorded as neutral, indicating that they were not displaying engagement with either stimulus. If the child moved back and forth between stimuli equally, proximity was recorded as both, indicating that they were engaging with both stimuli without showing a preference for one over the other. The researcher also recorded whether the child's head was oriented towards the CS (lever) or US (candy) for each trial. If the child was not facing towards

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either stimulus, head orientation was recorded as neutral. If the child faced equally towards both stimuli, head orientation was recorded as both. While this dissertation initially proposed calculating scores ranging from 0.0 to 1.0 separately for CS and US, we elected to combine these into a single score ranging from -1.0 to 1.0 to be consistent with the PCA index. Thus, for both proximity and head orientation, trials in which behavior was oriented solely towards the CS were assigned a value of 1, while those in which behavior was oriented solely toward the US were assigned a value of -1. These values were used to calculate response bias scores for both the CS and ITI trial periods. A full PCA index was not calculated for behavioral observation data, as latency data was not obtained for this measurement method. Scores were calculated both as a total across all trials and separately for each block.

Video data was coded by trained undergraduate research assistants who were blind to study hypotheses. Behavior was coded in 20-second blocks divided into 10 2-second increments. While this coding was initially proposed to be done in 8-second increments, children displayed enough variation in behavior in a short period of time that we elected to code in 2-second increments in order to capture finer detail. Five 20-second blocks were coded for each block of the study, with 45 seconds in between each block. During each 2-second increment, coders indicated whether the lever was out (CS period) or in (ITI period), as well as the direction of the participant's proximity, head orientation, and any touching behavior. The video coding protocol (described below) was developed and detailed in a coding manual by the study PI, who then trained two lab managers as lead coders. Trained coders all coded the same two training videos, which were closely checked by either the PI or one of the lead coders. Coders were asked to re-code training videos if they did not achieve acceptable reliability with lead coders. Once they had achieved acceptable reliability, coders were assigned participant videos to code. As video coding

was time-consuming, most videos were coded by a single coder to increase feasibility by reducing excessive workload. A selection of videos was coded by two different coders to assess interrater reliability, which was calculated using Cohen's kappa. Kappa values were at least .75 for all coders, with an average kappa of .86 across all coding pairs.

Video data was captured from an overhead angle and included proximity and head orientation as described above. Video codes also included the additional measure of touching behaviors. If the child was touching any part of the CS apparatus (including, but not limited to the lever), touching was coded as CS, while if the child was touching any part of the US apparatus (including, but not limited to the candy tray), touching was coded as US. Again, while we initially proposed calculating scores ranging from 0.0 to 1.0 separately for CS and US, we elected to combine these into a single score ranging from -1.0 to 1.0 to be consistent with the PCA index. Thus, for each behavior (i.e., proximity, head orientation, and touching), trials in which behavior was oriented solely towards the CS were assigned a value of 1, while those in which behavior was oriented solely toward the US were assigned a value of -1. These values were used to calculate response bias scores for both the CS and ITI trial periods. A full PCA index was not calculated for video data, as latency data was not obtained for this measurement method. Scores were calculated both as a total across all 2-second increments and separately for each block.

Aim 2 measures.

Relative Reinforcing Value of food and toys (RRV) task. To assess how each phenotype is related to food motivation, we used an RRV task, similar to that described in Chapter II. In the current study, participants responded with a number of button presses on a computer to earn tickets that could be used to obtain fun-size servings of candy (e.g., Twix bars, Starbursts) and

small toys (similar to those dispensed from a gumball machine), with toys serving as the alternate reinforcer. Participants were allowed to move back and forth between stations as they wished, and continued the task to earn as many tickets as they wished. The task ended when the participant chose to stop playing.

Participants received one ticket to be used toward the relevant reinforcer for every point earned, and redeemed their tickets for prizes after they indicated they were finished earning tickets. Points were earned on a variable ratio reinforcement schedule beginning at 10 button presses (VR10), and doubling each time they earned a point (i.e., VR20, VR40, VR80, VR160, VR320, VR640, VR1280, VR2560). The ratio randomly varied from 50% to 150% of the schedule for the current point (e.g., the first point could be earned by any number of button presses ranging from 5 to 15). Food and toy RRV were determined by the highest variable ratio schedule completed for each reinforcer.

Children's Eating Behaviour Questionnaire (CEBQ). To measure responsivity to food, we used the Food Responsiveness scale from the CEBQ (Wardle, Guthrie, Sanderson, & Rapoport, 2001). This 35-item parent-report measure yields eight subscales assessing different aspects of eating behavior in children. It has been found to have good internal validity and good test-retest reliability when assessing children ranging from early childhood to school-aged. In the current sample, the Food Responsiveness subscale showed good internal consistency ($\alpha = .80$).

Go/No-go Zoo Task. Inhibitory control was assessed using a child-friendly version of the Go/No-go task called the Zoo Game (Grammer et al., 2014). In this game, children are instructed that zoo animals have escaped from their cages, and that they are to assist in catching them by pressing the spacebar when they see an image of a zoo animal (Go trials). Additionally, the children are shown three images of orangutans, and told that the orangutan friends are helping,

and thus do not need to be caught (No-go trials). Following a 12-trial practice block consisting of nine Go trials and three No-go trials, participants completed eight 40-trial blocks, each consisting of 30 Go trials and 10 No-go trials. Reaction times, the number and percentage of commission errors (i.e., responses to No-go trials), and the number and percentage of omission errors (i.e., failing to respond to Go trials) were calculated to assess inhibitory control.

Peg-tapping Task. To provide an additional behavioral index of inhibitory control, the Peg-tapping Task (Diamond & Taylor, 1996; Luria, 1966) was added approximately halfway through data collection (after 26 participants had engaged in the protocol). In this task, children were instructed to tap a wooden peg once when the experimenter taps twice, and to tap twice when the experimenter taps once, requiring them to remember multiple rules and inhibit the response to directly mimic the experimenter's action. The task consists of 16 trials, with each correct trial receiving a score of 1 and each incorrect trial receiving a score of 0. The total score indicates the child's level of inhibitory control, with higher scores indicating greater inhibitory control.

Behavior Rating Inventory of Executive Functioning (BRIEF). We collected parent-report data on inhibitory control using the BRIEF (Gioia, Isquith, Guy, & Kenworthy, 2000). This measure yields eight subscales, two composite scores, and a global summary score (Global Executive Composite; GEC). For assessing inhibitory control in the current study, we used the Behavioral Regulatory Index (BRI) composite, which is composed of the Inhibit, Shift, and Emotional control subscales, and the GEC. The BRIEF has been validated in children aged 5-18, showing good internal consistency and good test-retest reliability. In the current sample, The BRIEF scales used showed acceptable to excellent consistency (α = .73 - .94).

Inhibitory Control Composite. In order to obtain a global measure of inhibitory control, we created a composite using scores on the Go/No-go task (commission error percentage), peg-tapping, and the BRIEF (BRI, GEC). We calculated z-scores for each of these measures, and computed a composite by taking the mean of each individual's z-scores.

Summary

The current study aimed to recruit children aged 5-7 to test the feasibility of the paradigm described above. Participants were recruited from the community through flyers posted in locations frequented by parents of children of the appropriate age, as well through online postings. Participants were screened via phone to determine eligibility. Eligible participants came in to the lab for one visit, during which they engaged in the Pavlovian conditioning task, consisting of four blocks of ten trials each, followed by the RRV task and the Go/No-go Zoo game. During the Pavlovian conditioning task, number of contacts to each stimulus was recorded by the apparatus, and video and behavioral observation data were used to capture non-contact approach and orientation behavior. Parents completed survey measures of their children's food responsiveness and impulsivity. We indexed sign-tracking and goal-tracking behavior using the PCA index calculated using the automated data, and response bias scores calculated using the automated, video, and behavioral observation data.

Data Analytic Plan

Statistical analyses were conducted using IBM SPSS 25 (IBM, 2017). We used frequencies to examine the distributions of all variables of interest and to check for missing data. Missing data is described in detail in the Results section below under Child Engagement. Food and Toy RRV were both skewed (skewness > 1.0), so we performed a log transformation on each of these variables for further analyses. There were two participants with outlier data (>2 SD

above the mean) in commission errors, so we excluded these cases only from analyses involving Go/No-Go performance. One participant had outlier data in peg-tapping, and was excluded only from analyses involving peg-tapping performance.

In order to determine the study's feasibility (Aim 1), we assessed whether children appeared to be tolerating and engaging with the task, whether they showed variability in their conditioned response, and whether they showed learning of a conditioned response to the CS. In initial pilot testing, child engagement with the Pavlovian conditioning task appeared to be good, as most children interacted freely with the task and did not express boredom or inattention. To assess whether participants showed variability in conditioned response, we examined the PCA index for the automated measures and response bias for the automated, video, and behavioral observation measures. Each score was calculated by block for each participant. As we still expected learning to be occurring during blocks 1 and 2, we used an average of performance during blocks 3 and 4[2] to create an index for each measure (i.e., automated PCA, automated response bias, video response bias, behavioral observation response bias). We elected to use this average rather than final block only in order to allow the index to be informed by a greater number of data points. We expected that for each measurement index, we would see a range encompassing both sign-tracking and goal-tracking behavior (i.e., ranging from -1.0 to 1.0) across participants. While all indices were calculated separately for both the CS and ITI periods, analyses focused on behavior during the CS-period, as the sign-tracking and goal-tracking model is concerned with behavior while the CS is being presented.

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[2] The decision to use an average of performance during blocks 3 and 4 rather than simply the final block was made after the study was pre-registered and after data analysis had begun, and thus differs from the analytic plan stated in the pre-registration.

In addition to examining these measures continuously, as proposed initially, we also classified participants into phenotypic groups based on each measurement index to facilitate group comparisons. First, participants with scores >0.50 were classified as ST, those with scores <-0.50 were classified as GT, and those with scores ranging from -0.49 to 0.49 were classified as intermediate (IR), consistent with categorization used in animal models (Yager, Pitchers, Flagel, & Robinson, 2015). This categorization method resulted in very few individuals being identified as GTs (two based on behavioral observation, one based on automated response bias). Given that the sample appeared to be skewed toward sign-tracking, we divided individuals into two groups: sign-trackers (STs; >0.50) and non sign-trackers (nSTs; <0.50) for remaining analyses. Chi-square analyses were conducted to compare classification of individuals as ST or nST across different measurement methods (i.e., automated, video, behavioral observation). To examine whether learning is occurring, we assessed whether the participant displayed a consistent CR (i.e., sign-tracking, or non-sign-tracking) during the final block of the protocol, defined as having an automated PCA index indicative of the same CR on ≥70% of trials during their final block. We then compared their response pattern during the first and final blocks, to determine whether their CR became more consistent over time. We expected an increase in consistency during the final block compared to the first to be indicative of learning a stable behavioral response.

To test Aim 2, we conducted one-way ANOVA to test whether performance on the Go/No-go and RRV tasks and scores on the CEBQ, BRIEF, and inhibitory control composite differed significantly by phenotype (i.e., ST, nST). We conducted these tests separately for each of the measurement indices (i.e., automated PCA index, automated, video, and behavioral observation response bias). We also conducted bivariate correlations to test the association between degree of sign-tracking or goal-tracking behavior according to each measurement index

and performance on the Go/No-go and RRV tasks and scores on the CEBQ, BRIEF, and inhibitory control composite.

Hypotheses

1. We expected that the above-described paradigm would allow us to observe distinct sign-tracking and goal-tracking phenotypes. We expected that the automated PCA index across participants would show a range from -1.0 (goal-tracking) to 1.0 (sign-tracking). We also expected that automated, video and behavioral observation response bias scores would range from -1.0 to 1.0, showing a full range of behavior. We initially expected that two distinct phenotypes (ST, GT) and an intermediate group (IR) would be observed when grouping participants based on automated PCA index and automated, video, behavioral observation response bias scores from the final two blocks. When this did not occur, we expected that distinct ST and nST groups would be observed.

2. We expected that the ST group would have lower inhibitory control (assessed by the Go/No-go task, peg-tapping, and BRIEF scores) and higher food motivation (assessed by the RRV task and CEBQ FR scores) compared to the nST group. Similarly, we expected that behavior consistent with sign-tracking (i.e., PCA index and response biases closer to 1.0) would be associated with lower inhibitory control and higher food motivation. We expected that behavior consistent with goal-tracking (i.e., PCA index and response biases closer to -1.0) would be associated with greater inhibitory control and lower food motivation.

Aim 1

  Child Engagement. Children showed moderate to good engagement with the study

tasks, with 34 (53%) children completing the entire protocol (i.e., Pavlovian conditioning task,

RRV task, and Go/No-go task[3]). Of those who did not complete the entire protocol,

eight (12.5%) did not complete the Pavlovian conditioning task; four (6%) due to technical

issues with the apparatus and four (6%) due to child request to end early, thought to reflect low

engagement. Twenty-five (39%) did not complete the Go/No-go task, 14 (22%) due to technical

issues with the task and 11 (17%) due to child request to end early. All participants completed

the RRV task.

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[3] The peg-tapping task was added to the protocol after 26 participants had completed the study. All 38 children who participated after the addition of the peg-tapping task completed the peg-tapping task, and 57.8% of participants after this point completed the entire protocol, including the peg-tapping task.

Table III.1

Means and Standard Deviations of Block Scores by Index

Index Type	Time Period	Block	ST	nST
Automated PCA-index	CS	1	0.70 (0.23)	0.36 (0.32)
		2	0.78 (0.23)	0.30 (0.36)
		3	0.81 (0.12)	0.11 (0.27)
		4	0.78 (0.14)	0.16 (0.27)
	ITI	1	-0.49 (0.38)	-0.46 (0.46)
		2	-0.46 (0.38)	-0.48 (0.40)
		3	-0.46 (0.35)	-0.54 (0.26)
		4	-0.56 (0.33)	-0.52 (0.27)
Automated Response Bias	CS	1	0.80 (0.27)	0.42 (0.37)
		2	0.86 (0.25)	0.35 (0.42)
		3	0.91 (0.10)	0.12 (0.33)
		4	0.89 (0.15)	0.18 (0.31)
	ITI	1	-0.37 (0.52)	-0.32 (0.62)
		2	-0.35 (0.55)	-0.43 (0.50)
		3	-0.36 (0.53)	-0.54 (0.44)
		4	-0.58 (0.44)	-0.58 (0.33)
Video Response Bias	CS	1	0.61 (0.32)	0.43 (0.40)
		2	0.72 (0.30)	0.32 (0.40)
		3	0.70 (0.34)	0.19 (0.45)
		4	0.65 (0.39)	0.17 (0.38)
	ITI	1	-0.09 (0.14)	0.03 (0.32)
		2	-0.07 (0.25)	-0.04 (0.30)
		3	-0.07 (0.26)	-0.18 (0.27)
		4	-0.07 (0.20)	-0.12 (0.34)
Behavioral Observation Response Bias	CS	1	0.77 (0.26)	0.46 (0.47)
		2	0.76 (0.30)	0.38 (0.45)
		3	0.83 (0.27)	0.26 (0.50)
		4	0.76 (0.30)	0.19 (0.53)
	ITI	1	-0.35 (0.32)	-0.31 (0.36)
		2	-0.24 (0.18)	-0.34 (0.36)
		3	-0.26 (0.27)	-0.37 (0.35)
		4	-0.32 (0.32)	-0.37 (0.36)

Response Variability. Children displayed a full range of behavior according to each index calculated. Group mean scores for each index broken down by block are shown in Table III.1. Overall, automated PCA-scores during the CS-period ranged from -0.39 to 0.94, with an average of 0.48. Automated PCA-scores during the ITI-period ranged from -.94 to 0.75, with an average of -.50. Automated response bias during the CS-period ranged from -0.57 to 1.0, with an average of 0.54. Automated response bias during the ITI-period ranged from -1.0 to 0.95, with an

average of -0.44. Video coding response bias during the CS period ranged from -0.78 to 1.0, with an average of 0.45. Video coding response bias scores during the ITI-period ranged from -0.80 to 0.74, with an average of -0.03. Behavior coding response bias during the CS-period ranged from -0.70 to 1.0, with an average of 0.56. Behavior coding response bias during the ITI-period ranged from -1.0 to 0.60, with an average of -0.30.

Phenotypic Groups and Index Reliability. We classified participants into groups (i.e., ST or nST) based on each measurement index (i.e., automated PCA-index, automated response bias, video response bias, behavioral observation response bias). The number of individuals in each group based on each measurement method is presented in Table III.2. Chi-square analyses were conducted to compare groupings based on each measurement method (see Table III.3). There was significant agreement among all measures in group composition (all p's <.003). Correlations among sign-tracking and goal-tracking indices are shown in Table III.4. Indices for all measurement methods (automated PCA-index, automated response bias, video response bias, and behavioral observation response bias) showed a significant positive correlation with each other (all p's <.001). To further investigate reliability of the automated PCA index, we conducted a split-half analysis, testing the correlation between performance during odd and even numbered trials during the blocks used to calculate groupings. Odd and even trial performance was strongly correlated ($r = .91$, $p < .001$), suggesting that the automated PCA index showed excellent internal consistency.

Table III.2

Phenotype Categorizations by Measurement Index

	Automated PCA-index	Automated Response Bias	Video Response Bias	Behavioral Observation Response Bias
ST	22 (45.8%)	19 (47.5%)	20 (48.8%)	27 (57.4%)
nST	26 (54.2%)	21 (52.5%)	21 (51.2%)	20 (42.6%)

Table III.3

X^2 *Tests of Association between Measurement Indices*

	Automated PCA index	Automated Response Bias	Video Response Bias	Behavioral Observation Response Bias
Automated PCA index	_	32.00***	12.22***	8.53**
Automated Response Bias	32.00***	_	12.22***	8.53**
Video Response Bias	12.22***	12.22***	_	24.89***
Behavioral Observation Response Bias	8.53**	8.53**	24.89***	_

Note: *p <.05 ** p <.01 *** p <.001

Learning. We examined both the originally hypothesized three CRs (i.e., ST, GT, or IR) and the two CRs (i.e., ST or nST) to determine whether learning had occurred. When examining three CRs, 33 (67.3%) participants with automated data displayed a consistent CR during their final block of the CS-period according to automated PCA index, 25 (61.0%)[4] participants

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[4] We were unable to calculate ITI PCA-index for eight participants who engaged in the Pavlovian conditioning task due to latency data for the ITI-period not being printed, thus PCA data involving the ITI-period is from a sample of 41.

displayed a consistent CR during their final block of the ITI-period according to PCA index, and 19 (46.3%) of participants displayed a consistent CR according to PCA index during both the CS-period and ITI-period. We followed this by comparing consistency during the final block to that seen during the first block. Eighteen (36.7%) participants had greater CS-period consistency in their final block than in their first block, and an additional six (12.2%) participants had a consistent CR across all trials in both blocks. Seventeen participants (41.5%) had greater ITI-period consistency in their final block than in their first block, and an additional two (4.9%) participants had a consistent CR across all trials in both blocks. When examining two CRs, 39 (79.6%) participants with automated data displayed a consistent CR during their final block of the CS-period according to automated PCA index, 41 (100%) participants displayed a consistent CR during their final block of the ITI-period according to PCA index, and 33 (80.5%) participants displayed a consistent CR according to PCA index during both the CS-period and ITI-period. Sixteen (32.7%) participants had greater CS-period consistency in their final block than in their first block, and an additional five (10.2%) participants had a consistent CR across all trials in both blocks. Seventeen participants (41.5%) had greater ITI-period consistency in their final block than in their first block, and an additional two (4.9%) participants had a consistent CR across all trials in both blocks.

Table III.4

Correlation Coefficients between Measurement Indices

	Automated PCA index	Automated Response Bias	Video Response Bias	Behavioral Observation Response Bias
Automated PCA index	–	.99***	.63***	.69***
Automated Response Bias	.99***	–	.63***	.69***
Video Response Bias	.63***	.63***	–	.91***
Behavioral Observation Response Bias	.69***	.69***	.91***	–

Note: *$p < .05$ ** $p < .01$ *** $p < .001$

Aim 2

Means and standard deviations of all variables of interest are presented in Table III.5. We conducted Aim 2 analyses using each of the four measurement indices. Given that the automated PCA index showed high agreement with the other measures, and is most similar to the measure used in animal models, results using that index will be reported. Overall, results using the other measurement indices were in agreement with those using the automated PCA index.[5] Correlation coefficients of associations between automated PCA-index and all outcome variables are shown in Table III.6.

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[5] The only variable that differed in significance level between automated PCA index and other measures was GNG commission error reaction time, which differed significantly between STs and nSTs when categorized using video response bias ($F = 4.60$, $p = 0.03$, $\eta^2 = .35$) and behavioral observation response bias ($F = 4.98$, $p = 0.02$, $\eta^2 = .32$).

Table III.5

Means and Standard Deviations of Variables of Interest

	ST	nST	$\underline{F}$	$\underline{p}$	η^2
	$\underline{M\ (SD)}$	$\underline{M\ (SD)}$			
Total GNG omission errors	23.50 (21.67)	23.29 (20.72	0.00	.98	.00
GNG omission error proportion	.09 (.08)	.09 (.08)	0.00	.98	.00
GNG Go trial reaction time (ms)	606.63 (51.18)	609.26 (78.91)	0.01	.91	.00
Total GNG commission errors	2.81 (4.94)	2.24 (3.33)	0.16	.70	.01
GNG commission error proportion	.09 (.24)	.04 (.05)	0.67	.42	.02
GNG No-go error reaction time (ms)	563.27 (214.38)	403.97 (208.38)	3.25	.09	.13
Peg-Tapping score	15.45 (0.69)	15.24 (1.09)	0.92	.35	.03
RRV VRC Candy	2.40 (0.43)	2.29 (0.48)	0.62	.44	.02
RRV VRC Toy	2.25 (0.32)	2.22 (0.47)	0.06	.82	.00
CEBQ Food Responsiveness	3.00 (0.91)	2.62 (0.71)	2.70	.11	.06
BRIEF Behavioral Regulation Index	49.32 (7.31)	48.23 (9.61)	0.19	.66	.00
BRIEF Global Executive Composite	127.23 (17.03)	122.62 (21.80)	0.65	.43	.01
Inhibitory Control Composite	0.06 (0.55)	-0.00 (0.77)	0.10	.75	.00

Note. RRV values represent log-transformations of raw scores.

Food Motivation. STs and nSTs did not differ significantly on the highest ratio completed for either candy ($F = 0.62$, $p = .44$, $\eta^2 = .02$) or toys ($F = 0.06$, $p = .82$, $\eta^2 = .00$). Automated CS-period PCA index was not significantly correlated with highest ratio completed for either candy ($r = .18$, $p = .28$) or toys ($r = .09$, $p = .58$).

STs and nSTs did not differ significantly on CEBQ Food Responsiveness ($F = 0.62$, $p = .44$, $\eta^2 = .02$). However, there was a significant positive correlation between automated PCA index and CEBQ Food Responsiveness ($r = .34$, $p = .02$).

Table III.6	
Correlation Coefficients of Automated PCA-index and Outcome Variables	
Outcome Variable	Automated PCA-index
GNG Go errors	.035
GNG Go error %	.035
GNG Go RT	-.092
GNG NG errors	-.027
GNG NG error %	.023
GNG NG error RT	.431*
Peg-Tapping	.064
VRC Candy	.179
VRC Toy	.094
CEBQ FR	.340*
BRIEF BRI	.013
BRIEF GEC	-.088
Inhibitory Control Composite	-.04

Note: *$p <.05$ ** $p <.01$ *** $p <.001$

Inhibitory Control. STs and nSTs did not significantly differ in number or percentage of Go/No-go commission errors, omission errors, or reaction time on Go trials (all p's > .43). There was a non-significant trend-level difference in reaction time on No-go error trials, with STs having a marginally slower reaction time than nSTs ($F = 3.25$, $p = .09$, $\eta^2 = .13$). STs and nSTs did not significantly differ on peg-tapping ($F = 0.35$, $p = .56$, $\eta^2 = .01$), BRIEF BRI ($F = 0.19$, $p = .67$, $\eta^2 = .00$), BRIEF GEC ($F = 0.65$, $p = .43$, $\eta^2 = .01$), or the inhibitory control composite ($F = 0.10$, $p = .75$, $\eta^2 = .00$).

Automated PCA-score was positively correlated with reaction time on No-go error trials ($r=.43$, $p=.04$), indicating that children displaying more sign-tracking behavior had slower reaction times when making commission errors. However, this result did not survive Bonferroni correction for multiple comparisons of measures of inhibitory control. Automated PCA-score

was not significantly correlated with number or percentage of Go/No-go commission errors, omission errors, reaction time during Go-trial correct responses, peg tapping, BRIEF BRI or GEC, or the inhibitory control composite (all p's >.55).

Discussion

The current study aimed to develop and test the feasibility of a novel paradigm to identify sign-tracking and goal-tracking behavior in human children. We designed and built an apparatus capable of running a Pavlovian conditioning paradigm and recording response data. To supplement automated data reported by the apparatus, we also collected video and *in vivo* behavioral observation data. For each method of measurement, we calculated response bias scores, and for automated measures we calculated a modified PCA index similar to that used in animal models. Scores on all measurement indices were significantly associated with one another, as were categorizations as ST or nST based on each index. Automated PCA-index is most similar to the index used in animal models (Meyer et al., 2012), thus we chose to use this measure as the primary predictor variable for Aim 2 analyses.

While video measures appeared to add some information not captured by the automated PCA index, the significant correlation between data obtained using these two measures suggests that the additional cost and analysis time needed to collect and code video data may exceed any added value of video data. While we focused on CS-period data for the current analyses, video response bias appeared to be slightly higher during the ITI-period, indicating greater sign-tracking behavior during the ITI–period, than did other indices. However, video response bias during each of these blocks showed a significant positive correlation with each of the other index scores for corresponding blocks, with the exception of block 3 of automated PCA index and

automated response bias. If future studies are interested in behavior during the ITI specifically, it may be useful to include video assessment methods to ensure all relevant behavior is captured.

While we observed a full range of sign-tracking and goal-tracking behavior across the sample, we were not able to fully observe distinct phenotypes, due to limited goal-tracking behavior in our sample. This was inconsistent with our hypothesis, and merits further examination in future research studies. During initial pilot testing, it was apparent that our ability to observe goal-tracking behavior using automated measures was limited, particularly during the CS-period. While video and behavioral observation data allowed us to observe slightly more goal-tracking behavior, this was still not enough to constitute a full phenotypic GT group. It is possible that limited goal-tracking behavior is due to the young age of our sample, as younger children tend to be lower in inhibitory control (Williams, Ponesse, Schachar, Logan, & Tannock, 1999). As sign-tracking behavior is associated with greater impulsive action in animal models (Lovic et al., 2011), there may have been a developmental impact on our ability to observe the goal-tracking phenotype. Of note, neither age nor inhibitory control was significantly associated with sign-tracking behavior in the current sample. Still, given the small sample size and limited age range in the current study, further research is needed to elucidate any effect of age or developmental stage.

Aspects of the protocol design may also have contributed to the lack of a distinct GT group in the current study. When designing our Pavlovian conditioning apparatus, we elected to make the stimuli child-friendly, in order to encourage child engagement with the task. However, it is possible that our stimuli were too attractive to participants, resulting in increased interaction with the lever used as the CS. As interaction with the CS was considered sign-tracking, this may have artificially inflated the number of children identified as STs and reduced our ability to

observe true GTs. Future studies examining children should be cautious that stimuli are not so attractive as to promote interaction above and beyond that which would be expected from true STs. Additionally, a previous study with a sample of adult humans was able to identify individuals as both STs and GTs by using eye-tracking software while participants engaged in a monetary Pavlovian conditioning task involving stimuli presented on a computer screen (Garofalo & di Pellegrino, 2015). This methodology may have improved ability to capture goal-tracking by limiting the method of interaction with the stimuli to visual attention. The current study included multiple ways that participants could interact with the stimuli (e.g., touching, head orientation, moving towards or away from each stimulus), which may have added excessive noise, thus limiting the ability to observe distinct phenotypes. It is possible that simplifying the paradigm and limiting the methods by which participants are able to interact with the stimuli may improve ability to observe goal-tracking behavior.

Recent research using animal models has shown that ITI duration may also impact the likelihood of displaying each CR. Sign-tracking behavior appears to be more likely during a longer ITI and goal-tracking behavior more likely during a shorter ITI (B. Lee et al., 2018). Lee and colleagues suggest this is due to a weakened association between contacting the location of the US and receiving a reward. Participants in the current study appeared to display more goal-tracking behavior during the ITI, and more sign-tracking behavior during the CS-period, and unlike animals continued to interact with the CS to some extent during the ITI-period (i.e., when the lever was retracted). While our ITI durations were shorter than those typically used in animal models, it may have felt long to our child participants due to limited attention span appropriate to this developmental stage (Betts, McKay, Maruff, & Anderson, 2006; Levy, 1980). This may have inhibited participants' learning of a strong association between contacting the location of

the US and receiving a reward, whereas the CS in this protocol may have been more instantly

rewarding.

Contrary to our hypothesis, STs and nSTs did not differ significantly on Go/No-go

commission or omission errors, Peg-tapping, BRIEF BRI or GEC, or the composite combining

these measures. In animal models, sign-tracking has been robustly associated with greater

impulsivity and lower attentional control (Lovic et al., 2011; Paolone et al., 2013), which could

be indicative of a possible pathway to problematic consumption in STs (T. E. Robinson et al.,

2014). If our paradigm had the ability to validly identify individuals as STs or GTs, we would

expect these phenotypic groups to differ in constructs shown to be strongly associated with sign-

tracking and goal-tracking, such as impulsivity. Thus, the lack of a significant association

between sign-tracking behavior and inhibitory control in the current study is a source of

considerable concern about the validity of the current paradigm. It is important to note that the

current study was not able to observe both ST and GT phenotypes, and it is possible that the ST

and nST groups did not vary enough to show significant differences in inhibitory control. It is

possible that with a paradigm able to reliably identify both ST and GT phenotypes, significant

group differences in inhibitory control might be observable.

Inconsistent with our hypothesis, food motivation as measured by the RRV task was not

significantly associated with sign-tracking or goal-tracking behavior, suggesting that sign-

tracking does not appear to be contributing to overeating through increased food motivation. As

animal models have shown STs to be more likely to show cue-induced food-seeking behavior

(Yager & Robinson, 2010), we hypothesized that STs would also show increased motivation to

obtain and consume palatable foods. However, additional research has shown that sign-tracking

and goal-tracking may each contribute to excessive consumption through different pathways,

with STs being at increased risk in the presence of discrete, localizable cues and GTs being at increased risk in cue-rich contexts (Saunders et al., 2014). The current study did not employ a food consumption task in a cue-rich context, and it is possible that in such a context, goal-tracking rather than sign-tracking would be correlated with overeating-related constructs (e.g., palatable food motivation, craving, consumption). Examining conditioned response to food cues employing both a cue-rich context (e.g., similar to that used in Study 1) and discrete, localizable cues (as used in the current study) would help clarify the type of risk that may be relevant for each phenotype. Additionally, in the current protocol, the RRV task took place after the Pavlovian conditioning paradigm, which involved consumption of M&Ms. It is possible that children achieved satiety during the Pavlovian conditioning task, and thus were less motivated to obtain additional food during the RRV task. Future studies should account for satiety, for example by counterbalancing task order, to fully understand any association between sign-tracking and goal-tracking behavior and food motivation.

While the current paradigm appeared to be moderately well-tolerated by children, several limitations suggest modifications would be needed before using this paradigm to validly identify individuals as STs or GTs. A primary concern is the lack of individuals identified as GTs. In order to better capture goal-tracking behavior, future designs may employ less attractive stimuli to reduce interaction with the CS in individuals who may not otherwise be considered STs. Other methodological changes may include employing measurement methods such as eye-tracking that have been successful in capturing goal-tracking in adult humans. An additional concern is that contrary to our hypotheses, sign-tracking behavior was not significantly associated with any of our inhibitory control measures (GNG, Peg-tapping, BRIEF). Sign-tracking is thought to contribute to compulsive behavior through impulsive action (Lovic et al., 2011), thus association

with inhibitory control would be a strong indicator that true sign-tracking behavior is being captured. As discussed above, the attractiveness of the CS may have added excessive noise to our observations of behavior consistent with sign-tracking. A modified apparatus allowing for cleaner measurement of sign-tracking behavior may result in more valid identification of STs and GTs and thus show expected relationships with measures such as inhibitory control. We also did not observe strong evidence of learning a particular CR during the protocol, suggesting that additional blocks may be needed to allow learning to occur. Alternatively, compared to rats, human children may learn more rapidly and develop a particular CR following only a few trials, which may lead to a less detectable learning response curve over multiple blocks. Finally, aspects of the current sample included some limitations that should be addressed in future studies. The sample size was relatively small, which may have inhibited our ability to observe effects due to being underpowered. Additionally, the current sample was somewhat homogenous, being recruited from a fairly affluent, highly educated area. It is possible that a more diverse sample may have also produced more variable responses to our task.

The current study developed and tested a novel Pavlovian conditioning apparatus and paradigm in efforts to identify sign-tracking and goal-tracking behavior in human children. While this study was unsuccessful at validly identifying STs and GTs, it provided useful lessons on design considerations when attempting to identify these phenotypes in humans. Given the robust association shown in animal models between sign-tracking and compulsive consummatory behaviors (Flagel et al., 2008; H. M. Jenkins & Moore, 1973; Tomie & Sharma, 2013), understanding how these phenotypes may manifest in humans would provide valuable information on the identification of individuals who may be at risk. Though the current study did not fully elucidate how sign-tracking and goal-tracking may present in humans, it did appear that

we were able to observe behavior consistent with sign-tracking, indicating that this risky profile

may be relevant at an early developmental stage. It is hoped that the lessons learned from the

development of the current study will help inform future research into the assessment of sign-

tracking and goal-tracking behavior in humans, leading to the development of improved

prevention and intervention efforts for those at risk for compulsive consummatory behaviors.

CHAPTER IV

Neurobiological Correlates of Overeating and Obesity in Adolescents

Behavioral studies of overeating and obesity illustrate the important role of individual differences in cue responsivity, impulsivity, and food reinforcement or motivation to eat. Food-related cues in the environment can trigger increased craving and feelings of hunger, contributing to overeating (Joyner, Kim, & Gearhardt, 2017). Animal models also suggest that some individuals may be both more attuned to these cues and more likely to act impulsively in their presence (Flagel et al., 2009; T. E. Robinson et al., 2014). Functional neuroimaging research has underscored the importance of sensitivity to food reward and ability to inhibit response to food cues, finding overeating and obesity to be related to patterns of activation in areas related to reward processing and executive function (for review, see Carnell et al., (2012) and Reinert, Po'e, and Barkin (2013)). For example, obese compared to lean adults show greater activation in areas related to reward, (e.g., nucleus accumbens [NAcc], caudate, putamen, orbitofrontal cortex [OFC]), and areas related to EF, (e.g., medial prefrontal cortex [mPFC], and anterior cingulate cortex [ACC]), when presented with visual cues for high-calorie foods (Carnell et al., 2012). Research in children has also shown that those who are obese compared to lean not only show increased PFC activation in response to food images before a meal, but also show smaller reductions in PFC and NAcc activation following the meal (Bruce et al., 2010). Studies on adolescents show similar findings, finding activation in the putamen and OFC in response to

pictures of appetizing food to be positively correlated with BMI (Reinert et al., 2013; Stice, Yokum, Bohon, Marti, & Smolen, 2010).

While task-based neuroimaging research illustrates alterations in reward processing and EF in obese compared to non-obese individuals, research examining whether these variations reflect an intrinsic underlying difference in their neural systems is nascent. Resting state functional connectivity (rsFC) analysis, which examines connections between neural regions during a period of rest, provides a tool with which to understand neural differences that are inherent as opposed to occurring only in response to a task (Damoiseaux et al., 2006; M. H. Lee, Smyser, & Shimony, 2013). rsFC can elucidate neural networks, or areas of the brain that tend to be activated together, developing stronger connections even when not engaged in a task (Guerra-Carrillo, Mackey, & Bunge, 2014; Taubert, Lohmann, Margulies, Villringer, & Ragert, 2011). Examination of these networks can provide information above and beyond analysis of activation in single regions, as many neural regions are involved in a variety of functions, and many processes involve simultaneous activation of functionally connected regions (Anderson, Kinnison, & Pessoa, 2013). Thus, rsFC analysis allows one to investigate abnormalities at the network-level that may contribute to impaired function. Common rsFC analytic approaches include independent component analysis (ICA), which uses algorithms to extract spatially and temporally independent components which may reflect intrinsic neural networks, and seed-based analysis, which examines connectivity between pre-determined regions-of-interest (ROIs; Smitha et al. (2017)).

rsFC research has consistently identified a number of intrinsic networks representing functionally-related neural regions (van den Heuvel & Hulshoff Pol, 2010). For example, the default mode network (DMN) consists of areas such as the posterior cingulate cortex (PCC),

precuneus, medial frontal regions, and inferior parietal regions which show increased activity when at rest compared to when engaged in a task, and is thought to be involved in processes such as self-monitoring (Greicius, 2008; van den Heuvel & Hulshoff Pol, 2010). The DMN has been found to show increased connectivity in individuals with depression, which could reflect the neural correlates of symptoms such as rumination (Berman et al., 2011; Greicius, 2008). Another network, consisting of limbic and paralimbic regions (e.g., dACC, OFC, insula), has been termed the salience network (SN), and is thought to be involved in processing of information related to emotion, reward, and homeostatic regulation (Barrett & Satpute, 2013; Seeley et al., 2007). Alterations in this network could be informative in psychopathologies involving maladaptive attribution of salience to stimuli, such as addiction (Sutherland, McHugh, Pariyadath, & Stein, 2012). rsFC studies have also identified an executive function network (EFN), which consists of prefrontal regions (bilateral dorsolateral PFC [dlPFC], ventrolateral PFC [vlPFC], dorsomedial PFC [dmPFC]) and appears to be involved in executive functions such as working memory and attentional control (Seeley et al., 2007). Deficits in the EFN have been found in children with attention-deficit/hyperactivity disorder (ADHD), suggesting that impairment in intrinsic networks may be underlying some of the attentional and inhibitory control difficulties associated with the disorder (de Celis Alonso et al., 2014). As discussed in Chapters II and III, overeating and obesity may also be marked by impairments in salience attribution and inhibitory control. Thus, comparing intrinsic networks related to these functions in individuals who are obese compared to lean may increase understanding of any deficits in these networks associated with obesity, thereby providing insight into functions that may be potential targets for intervention.

The literature examining rsFC in obesity is in its early stages, but the extant research appears to support the existence of weight-related differences in rsFC, particularly between

networks involved in determining the salience of stimuli and the interpretation of homeostatic signals. For example, Garcia-Garcia and colleagues (2013) found that adults who were obese compared to lean displayed stronger connectivity in the salience network (SN), most prominently between the left putamen nucleus, involved in motor function and motivation, and other SN regions. This may suggest that stronger connectivity between SN and the putamen nucleus could be underlying increased sensitivity to and motivation to approach rewarding food cues in obese individuals, in turn contributing to overeating (Garcia-Garcia et al., 2013). This connectivity pattern was also found to be related to slower performance on cognitive tasks assessing processing speed (i.e., Grooved Pegboard and Symbol-Digit Modalities Test), specifically components involving motor execution and selective attention (Garcia-Garcia et al., 2013). This may suggest that that extra recruitment of the left putamen nucleus into salience processing tasks may hinder its ability to efficiently control motor functions.

Individuals who are obese compared to lean also appear to have altered rsFC in regions related to the interpretation of homeostatic signals. Research has found obesity to be related to reduced connectivity between SN areas including the insula and hypothalamus (Contreras-Rodriguez et al., 2018; Kullmann et al., 2012; Wijngaarden et al., 2015),, which may contribute to deficits in the ability to interpret and respond to interoceptive signals of hunger. Alterations in rsFC between homeostatic regions also appear to be impacted by whether one is in a fasted or satiated state. In a fasted state, obese compared to lean adults displayed stronger rsFC between the hypothalamus and regions of the EFN (e.g., frontal pole) and SN (e.g., dACC, bilateral caudate nucleus, putamen, insula). (Lips et al., 2014). This may indicate that when hungry, these individuals are more attuned to rewards, and may have to exert greater cognitive control, for example in the presence of food rewards.

Fewer studies have examined rsFC at earlier developmental stages, however early research suggests that in adolescence, the imbalance between neural regions related to EF and reward processing may be particularly important. Adolescence as a developmental stage is characterized by risk for impulsive and risky behavior (Steinberg, 2007). While neural reward regions (e.g., striatum) are fully developed by adolescence, EF regions (e.g., PFC) continue to develop until adulthood (Casey et al., 2008). Thus, adolescents have a structural imbalance between EF and reward, resulting in less capacity for inhibitory control and a greater influence of reward on decision-making (Luna et al., 2001; Somerville, Hare, & Casey, 2011; Steinberg, 2007). Further, adolescents compared to adults have more difficulty inhibiting a response to food cues (Guerrieri, Nederkoorn, & Jansen, 2008; Teslovich et al., 2014). This imbalance between EF and reward processing could thus leave adolescents more susceptible to overeating and obesity. Obese compared to normal-weight adolescents appear to show an even more pronounced imbalance between EF and reward regions in task-based functional magnetic resonance imaging (fMRI) paradigms (Batterink et al., 2010; Black et al., 2014; Bruce et al., 2013; Yokum, Ng, & Stice, 2011). For example, an fMRI study utilizing a food-specific Go/No-go task, in which participants are instructed to respond to certain stimuli while inhibiting responses to other stimuli, found that teens who were obese compared to those who were lean showed reduced activation in EF regions, (e.g., frontal gyrus) and increased activation in reward regions (e.g. mid-insula) during the task (Batterink et al., 2010). This elevated reward response combined with lower EF likely contributes to excess food consumption and obesity by making it difficult to inhibit the desire to eat foods one finds rewarding.

Studies examining rsFC in lean-to-obese children and adolescents do suggest that obesity is related to altered connectivity between EFN and reward-related SN regions. Some studies have

found obesity to be related to greater rsFC between the EFN and areas in the SN (Black et al., 2014; Moreno-Lopez, Contreras-Rodriguez, Soriano-Mas, Stamatakis, & Verdejo-Garcia, 2016). This may suggest that children who are obese may be more heavily influenced by rewarding stimuli, and that reward motivation may hold more influence over decision-making and attempts to execute self-control(Black et al., 2014). Other research on rsFC in adolescents has found obesity to be related to greater connectivity between SN regions and weaker connectivity between the SN and EFN (Martín-Pérez, Contreras-Rodríguez, Vilar-López, & Verdejo-García, 2018). This may indicate that these individuals are prone to find rewarding stimuli particularly salient, and less prone to exert executive control in the context of rewards. Further, in those with excess weight, greater connectivity between regions in the SN was associated with greater stress response as measured by the Trier Social Stress Task (TSST), which may suggest that those who are obese may show greater sensitivity to reward when in a state of stress. Overall, in adolescents with excess weight, regions related to salience determination and reward processing appear to be particularly influential and regions related to inhibitory control appear to have relatively lower influence.

In sum, a few patterns emerge from the extant literature on rsFC and obesity. From childhood through adulthood, obesity appears to be related to alterations in the connectivity both among regions implicated in EF and between EF regions and other regions (Black et al., 2014; Kullmann et al., 2012; X. Li et al., 2016; Lips et al., 2014; Martín-Pérez et al., 2018; Moreno-Lopez et al., 2016). Further, in both adolescents and adults, excess weight is related to an imbalance in connectivity between regions implicated in salience and reward processing and those implicated in executive function (Black et al., 2014; Kullmann et al., 2012; Martín-Pérez et al., 2018; Moreno-Lopez et al., 2016; Sharkey et al., 2019). However, the directionality of

connectivity strength varies across studies, with some finding obesity to be related to stronger

rsFC between EF and salience/reward regions (Black et al., 2014; Garcia-Garcia et al., 2013),

and others finding obesity to be related to weaker rsFC between these areas (Martín-Pérez et al.,

2018; Moreno-Lopez et al., 2016; Wijngaarden et al., 2015). Given that adolescents have

reduced EF capacity compared to adults, (Casey et al., 2008; Luna et al., 2001; Spear, 2000)

rsFC between EF and reward-related regions is likely most important to focus on in this

developmental stage. Further, in the adult literature, regions implicated in the processing of

hunger and satiety appear to play an important role (Garcia-Garcia et al., 2013; Lips et al., 2014;

Wijngaarden et al., 2015). The few rsFC studies on adolescents have not controlled for

participant hunger (Black et al., 2014; Moreno-Lopez et al., 2016), which may be an important

consideration given the varying rsFC patterns between fasted and satiated states found in the

adult literature (Lips et al., 2014; Wijngaarden et al., 2015).

In order to fully understand how rsFC patterns relate to obesity, it is also important to

investigate associations with traits and behaviors related to obesity. In adults, Garcia-Garcia and

colleagues (2013) found rsFC patterns associated with obesity to be related to deficits in EF,

showing that greater rsFC between the putamen nucleus and the rest of the SN was related to

poorer performance on processing speed tasks. In adolescents, Moreno-Lopez et al. (2016)

examined the relationship between rsFC patterns and personality traits, finding that in

adolescents with excess weight, reward sensitivity was negatively correlated with rsFC between

the insula and right superior frontal gyrus, and with rsFC between the middle temporal cortex

and left frontal operculum. In contrast, these correlations were positive in lean adolescents.

These results may indicate sensitization of reward-related regions as opposed to cognitive

regions in obesity. Altered rsFC between regions implicated in reward processing was also found

to be associated with greater impulsivity and stress response (Martín-Pérez et al., 2018; Sharkey et al., 2019). While these studies provide initial support for the theory that rsFC patterns are associated with behavioral phenotypes of obesity, the extant research is limited and further studies are needed.

Investigating the association between connectivity patterns and behavioral outcomes is necessary to fully understand the role these neural regions play in obesity. Often, neural regions are implicated in multiple different functions (Anderson et al., 2013). While testing associations to obesity alone allows us to theorize what function is at play, examining associations to both obesity and behavioral outcomes will allow us to refine this interpretation. Further, if alterations in rsFC in obese individuals are related to behavioral differences, it is possible that by targeting the relevant behavioral outcomes, we can induce changes in rsFC, strengthening adaptive connections. There is evidence that behavioral intervention can have a significant effect on functional networks in the brain. For example, one study of overweight children showed that an aerobic exercise intervention strengthened neural networks related to cognitive control (Krafft et al., 2014). Thus, identifying the neural connectivity patterns associated with obesity and related behaviors may be the first step toward the development of novel interventions.

Relating rsFC patterns to behavioral phenotypes can provide information about effective targets for intervention. For example, alterations in rsFC between reward and executive function regions may be indicative of problems with top-down control, such as finding food to be excessively motivating or reinforcing. Alternatively, these alterations could indicate deficits in bottom-up processing of stimuli, such as inhibiting response to food and its cues. For each of these potential phenotypes, different intervention approaches may be most effective. Interventions such as changing temporal focus, or training people to think about the future, has

been found to decrease cravings for cigarettes and food (Kober et al., 2010). Training individuals to inhibit their response to a type of stimuli, for example by using a Go/No-go task, has shown some effectiveness in reducing alcohol use and overeating behavior (Houben, Nederkoorn, Wiers, & Jansen, 2011; Lawrence et al., 2015). Depending on the mechanisms underlying overeating, each type of intervention may be more or less effective for certain people or in certain situations. Now that prior research has elucidated neural networks that are likely involved in overeating and obesity, relating connectivity patterns to behavioral outcomes is the next step towards identifying behavioral phenotypes of obesity in a way that can inform tailored interventions.

The current study aimed to address some of these gaps in the literature by examining rsFC in a large sample of 164 adolescents aged 13-16 ranging from normal-weight to obese, and evaluating the association of rsFC with impulsivity and food reinforcement, controlling for variability in hunger. We employed seed-based analyses, to test hypothesized connections between specific ROIs included in the DMN, SN, and EFN, as well as an exploratory ICA analysis, to identify neural networks that may be associated with differences in weight status and the outcome variables of interest in our sample. Further, while the literature examining rsFC in obesity is in its early states, studies examining task-based neural activation are more numerous. The current study involves secondary analysis of data from a project employing both resting-state and task-based fMRI scans. This provides a unique opportunity to examine the added utility of rsFC analysis. This paper will also discuss the rsFC findings of the current analyses in the context of the task-based results of the parent study. This research will provide insight into behavioral outcomes associated with obesity-related connectivity patterns, in turn elucidating potential effective targets for interventions aimed at addressing overeating and obesity.

Specific Aims

1. Test whether obese compared to normal-weight adolescents exhibit differences in rsFC between regions in the DMN, EFN, and SN.

2. Test whether rsFC between the DMN, EFN, and SN is associated with food consumption, food motivation, and inhibitory control.

3. Test whether elevated food consumption, food motivation and reduced inhibitory control mediate an association between obesity and rsFC strength between the DMN, EFN, and SN.

Methods

Participants

Participants were recruited through flyers posted in the community and online advertisements to participate in a study on how advertisements impact the brain (which was the aim of the parent study). A total of 193 participants ranging from lean to obese took part in the study. Of those, 177 completed the full resting state scan protocol and were thus included in the current analyses. Nine participants were found to have excessive motion during the resting state scan (i.e., more than three total minutes with motion >0.2mm) and were excluded. Quality assurance (QA) checks found that four additional participants had problems in their imaging data following preprocessing (e.g., unsuccessful coregistration) and were excluded. Thus, the final sample size for the current analyses is 164. The final sample included 77 (47.0%) males and 87 (53.0%) females. Participants ranged in age from 13 to 16 (mean = 14.30, SD = 1.03). BMI ranged from 16.20 to 44.50 (mean = 24.11, SD = 5.35). zBMI (which z-scores participants' BMI based on gender-specific developmental trajectories) ranged from -1.24 to 2.87 (mean = 0.86, SD = 0.94). Eighty-eight (53.7%) participants were categorized as normal-weight, 40 (24.4%) as

overweight, and 36 (22.0%) as obese. Exclusion criteria included contraindications to fMRI (e.g., metal implants, braces), major psychiatric or neurological disorders (e.g., major depressive disorder, autism spectrum disorder), dietary restrictions preventing them from consuming the foods served in the study, serious medical problems (e.g., diabetes), habitual or recent illicit drug use, and current pregnancy.

Procedure

The University of Michigan Institutional Review Board approved this study. Parents provided written informed consent, and adolescent participants provided written assent. Participants were compensated $150 for their time. Participants who were traveling from greater than 30 miles from the study site were compensated an addition $20 for travel. Participants completed two laboratory visits. During the baseline assessment (Visit 1), participants completed an RRV task assessing food motivation, a food Go/No-go task assessing inhibitory control, and BMI/body composition measures. During the second visit (Visit 2), participants were taken to the fMRI laboratory, where they completed a rsFC paradigm and a functional task investigating neural response to food advertising (functional task pertains to the aims of the parent study). Immediately following the scan session, they were taken to a simulated fast-food restaurant laboratory (see Chapter II) to complete a food consumption task.

Measures

Aim 1 measures.

Body mass index (BMI). Age- and sex-adjusted zBMI scores were used to assess participants' adiposity. BMI (kg/m^2) was calculated using height and weight measured in the lab, then converted to z-scores using age- and sex-adjusted BMI growth curves. Participants were classified as overweight with a zBMI cutoff of >+1SD, and obese with a zBMI cutoff of >+2SD.

Resting state paradigm. Upon arrival at the fMRI laboratory, participants completed an 8-minute rsFC scan and a high-resolution anatomical scan. During the rsFC scan, participants were instructed to focus on a fixation cross and to think about nothing in particular. During the scan, participants' eyes were visible to research staff, enabling visual confirmation that their eyes were open and they had not fallen asleep.

Aim 2 and 3 measures.

Hunger. Hunger was assessed during Visit 2, before the fMRI scan. Participants rated their hunger using a visual analog scale (VAS) ranging from 0 ("Not hungry at all") to 100 ("It's all I can think about"). Hunger was included as a covariate in all rsFC analyses.

Post-scan food consumption. Food consumption was assessed in the simulated fast-food laboratory immediately following the fMRI scan during Visit 2. Menu options available to participants resembled foods available in fast-food restaurants, and included both unhealthy (cheeseburger, French fries, non-diet soda, milkshake) and healthy (chicken sandwich, salad, iced tea, fruit smoothie) options. Participants were instructed that they had unlimited time to eat any foods they wanted to order, and that they could not take any food home with them. All food was weighed pre- and post-participant access to measure the amount of food consumed. Calories consumed by participants were calculated using the weight of the food in grams and the calorie content of each food served.

Relative Reinforcing Value (RRV) task. The RRV task, performed in Visit 1 of the current study, measures the reinforcing value of food by allowing participants to make an increasing number of button presses to gain access to food. In the current study protocol, participants earned points on a fixed-ratio scale beginning at FR4 and doubling each time a point was earned (i.e., FR4, FR8, FR16, etc.). For each point earned, participants immediately received

a 50-calorie portion of snack food (e.g., M&Ms, Lay's potato chips). Participants were instructed

that they were able to perform the task and earn access to snack food as long as they desired.

Food motivation was operationalized as the highest fixed ratio schedule completed.

Food Go/No-go task. An adapted version of the food Go/No-go task (Batterink et al.,

2010) was employed during Visit 1 to assess food-related inhibitory control deficits. This task

requires participants to respond to "go" signals (75% occurrence) and occasionally inhibit

responses to "no-go" signals (25% occurrence), measuring the ability to inhibit a pre-potent

tendency to respond. Participants were presented with images of healthy and unhealthy foods.

The pictures were arranged in 2-picture blocks, and rapidly presented one-by-one with a

presentation time of 300 milliseconds and an inter-stimulus interval of 900 milliseconds. The

number of commission errors (i.e., responses to distractors), the number of omission errors (i.e.,

failing to respond to target pictures), and reaction time (RT) for both Go-trials and commission

errors were calculated separately for the healthy and unhealthy food pictures.

Analysis

fMRI scanner and data acquisition. MRI images were acquired using a GE Discovery

MR750 3T scanner with an 8 channel head coil located at the UM Functional MRI Laboratory

(http://www.umich.edu/~fmri/). Foam padding, a vacuum pillow, and tape (sticky side away

from head) were used to limit head movement. Participants completed all scanning in one 60-

minute session. Spiral imaging was used to measure BOLD signal as an indication of cerebral

brain activation. To improve BOLD signal detection and minimize susceptibility-based distortion

effects for regions subject to signal distortions (e.g., OFC, amygdala), we used a protocol that

utilizes a high readout bandwidth and a shorter echo time. Functional data were acquired using a

spiral sequence with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) =

30 ms, TI = 500 ms, flip angle = 90^0, field of view (FOV) = 22 x 22 cm^2, acquisition matrix = 64 x 64, 3-mm slice thickness with no gap, 43 axial slices. Anatomical scans were acquired using a high-resolution T1-weighted spoiled-gradient-recalled acquisition (SPGR; TR = 12.3 ms, TE = 5.2 ms, TI = 500 ms, flip angle = 15^0, FOV = 22 x 22 cm^2, slice thickness = 1.0mm). Slices were prescribed parallel to the AC-PC line (same locations as structural scans). Images were reconstructed into a 64x64 matrix. Slices were acquired contiguously, which optimizes the effectiveness of the movement post-processing algorithms. Images were reconstructed off-line using processing steps to remove distortions caused by magnetic field inhomogeneity and other sources of misalignment to the structural data, which yields excellent coverage of subcortical areas of interest.

Preprocessing of neuroimaging data. fMRI data was analyzed using SPM12 (Wellcome Department of Imaging Neuroscience; Institute of Neurology, University College of London, London UK) and the *CONN* toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). Functional images were realigned to the scan immediately preceding the anatomical T1 image. Anatomical and rsFC images were normalized to the Montreal Neurological Institute (MNI) T1 template brain. Functional images were smoothed with a 6mm FWHM isotropic Gaussian kernel. We used Artifact detection toolbox (ART; https://www.nitrc.org/projects/artifact_detect/), a composite movement measure derived from the linear (X, Y, Z) rotational (roll, pitch, yaw) motion parameters, to detect motion artifacts greater than 0.2mm. Participants were excluded if ART motion detection showed that they had more than three total minutes of scan time during which movement was greater than 0.2mm. In the analyzed participants, motion was included as a first-level covariate in order to control for any frames with excess motion. Upon completion of

the above spatial preprocessing steps, quality assurance (QA) plots were examined to check for successful normalization to the template and co-registration of structural and functional images.

During denoising, potentially confounding BOLD effects due to white matter, CSF, motion, and main effects of rest were regressed out. A high-pass filter (128s) and band-pass filter (.01 Hz - .1 Hz) were applied to remove low frequency noise and signal drifts. Following the above temporal preprocessing steps, QA plots showing voxel-to-voxel functional connectivity values and BOLD timeseries both before and after denoising were visually examined to determine successful reduction of effects due to noise.

Statistical analyses

First- and second-level analyses were conducted using the *CONN* toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012). In first-level analyses, motion parameters detected by ART for each subject were included as nuisance covariates. In second-level analyses, we conducted ROI-to-ROI analyses using a general linear model (GLM) to examine how rsFC strength between neural regions implicated in reward and EF (e.g., OFC, NAcc, DLPFC) differed by weight status and was associated with behavioral outcomes of interest. ROIs previously established as comprising the networks of interest (i.e., DMN, SN, EFN) were included in the current ROI-to-ROI analyses (see Table IV.1 for a list of all ROIs tested). ROIs were defined using masks derived from the Montreal Neurological Institute AAL template (Tzourio-Mazoyer et al., 2002).

Table IV.1	
Networks and Source ROIs	
Network	Source ROIs
DMN	
	Hippocampus
	Inferior parietal lobe (IPL)
	Middle frontal gyrus (MFG)
	Parahippocampus
	Posterior cingulate cortex (PCC)
	Precuneus
	Ventrolateral prefrontal cortex (vlPFC)
	Ventromedial prefrontal cortex (vmPFC)
SN	
	Amygdala
	Anterior cingulate cortex (ACC)
	Caudate
	Insula
	Nucleus accumbens (NAcc)
	Olfactory tubercle
	Orbitofrontal cortex (OFC)
	Pallidum
	Putamen
	Thalamus
EFN	
	Dorsolateral prefrontal cortex (dlPFC)
	Ventrolateral prefrontal cortex (vlPFC)

We also conducted group-level ICA to examine differences between obese and lean subjects in rsFC within and between networks. Data were reduced to 11 dimensions, and 11 independent components were estimated for ICA analysis, similar to a study with similar aims, comparing rsFC in obese and lean adults (Garcia-Garcia et al., 2013). We examined the correlation coefficient between each of the components from the ICA and established masks (Fox et al., 2005) of networks of interest (DMN, SN, EFN). We controlled for sex, age, hunger, and handedness by including these as second-level covariates. Effects were considered

significant at $p<0.05$, family-wise error corrected (FWE) for ICA analyses, or analysis-level false

discovery rate (FDR) corrected for ROI-to-ROI analyses (Genovese, Lazar, & Nichols, 2002).

We also applied a Bonferroni correction for multiple comparisons in analyses involving multiple

measures of the same construct (i.e., food consumption, inhibitory control). Significant

connectivity coefficients were extracted for each subject. Then, rsFC correlation maps were

converted to z-scores using Fisher's r-to-z transformation. Each participant's rsFC z-score was

exported to SPSS 25.0 (IBM, 2017) to conduct Aim 3 mediation analyses.

The distributions for all outcome variables for Aim 2 and 3 analyses were examined for

skewness and outliers (>3 SD from the mean). Variables for food and game RRV were highly

skewed (skewness > 3.0), so we performed a log transformation of these variables. Six

participants were found to have outliers in the Go/No-go task data, and thus were excluded from

Go/No-go analyses. Twelve participants were found to have outliers in food consumption data,

and thus were excluded from food consumption analyses.

For Aim 1, we conducted second-level group analyses in CONN comparing rsFC

strength between ROIs and within the extracted independent components to test the hypothesis

that rsFC patterns would differ in individuals who were obese and overweight compared to lean.

For Aim 2, we tested the hypothesis that greater connectivity between EF- and reward-related

regions would be associated with greater food consumption, food motivation, and lower

inhibitory control. To do so, we used CONN to conduct second-level regression analyses

between rsFC strength and number of both healthy and unhealthy calories consumed during the

food consumption task (food consumption), individual performance on the RRV task (food

motivation), and performance on the food Go/No-go task (inhibitory control). For Aim 3, we

tested the hypothesis that food motivation and inhibitory control mediate the relationship

between obesity and rsFC. To do so, we planned to use the PROCESS macro (A. F. Hayes, 2012) to conduct mediation analyses. To test the hypothesized mediation models (e.g., weight status → food motivation → rsFC z-scores), we planned to employ the bootstrapping method with 10000 samples described by Preacher and Hayes (2008), which yields a 95% confidence interval. To compare the effect sizes of statistically significant indirect effects, we planned to use the completely standardized indirect effect (ab_{cs}) (Preacher & Kelley, 2011). Effect sizes can be interpreted as small (.01), medium (.09), or large (.25) (Kenny, 2014).

Power analysis. Previous research has found a large effect size ($d = 1.538$) when comparing rsFC between adult obese and normal weight groups. We used this effect size as a starting estimate to conduct a power analyses. We applied standard power estimation procedures based on N = 180 and assuming 2-tailed alpha of .05. While effect sizes may differ in our adolescent sample, based on the estimate we will have a power of > 99% to detect the effect size of 1.538 achieved in an adult sample.

Hypotheses

1. We expected that individuals who were obese compared to lean would show significant differences in rsFC between regions in the SN (e.g., OFC, striatum) and those in the DMN and EFN (e.g., PCC, DLPFC). We also expected that independent components analysis would show differences in the rsFC network strength between obese and lean participants, particularly with regard to the executive function network (EFN) and salience network (SN).

2. We expected that greater connectivity between reward and EF regions and greater rsFC network strength in the EFN and SN would be associated with greater food consumption, greater food motivation, and lower inhibitory control.

3. We expected that food consumption, food motivation, and inhibitory control would mediate the association between weight status and rsFC strength in the DMN, EFN, and SN.

Results

Aim 1

Table IV.2

Between-group ROI-to-ROI rsFC Differences

Contrast	Seed/Source	T	pFDR
Obese > Normal weight			
	Caudate		
	Precuneus	3.16	.027
	Hippocampus	-4.11	.0001
	Hippocampus		
	Caudate	-4.11	.002
	OFC		
	Olfactory Tubercle	3.18	.050
	Olfactory Tubercle		
	OFC	3.18	.050
	Parahippocampus		
	L NAcc	-2.87	.047
	Olfactory Tubercle	-2.74	.047
	ACC	-3.09	.047
	Amygdala	-2.85	.047

Table IV.2 shows between-ROI connectivity values that significantly differed by weight status, and Figure IV.1 displays significant connectivity patterns. Individuals who were obese compared to normal-weight showed stronger connectivity from the caudate to the precuneus and between the OFC and the olfactory tubercle. They showed stronger negative connectivity from the parahippocampus to regions in the SN, including the ACC, amygdala, OFC, and left NAcc, and between the caudate and hippocampus. Individuals who were overweight did not show any significant differences in rsFC compared to those who were either normal-weight or obese.
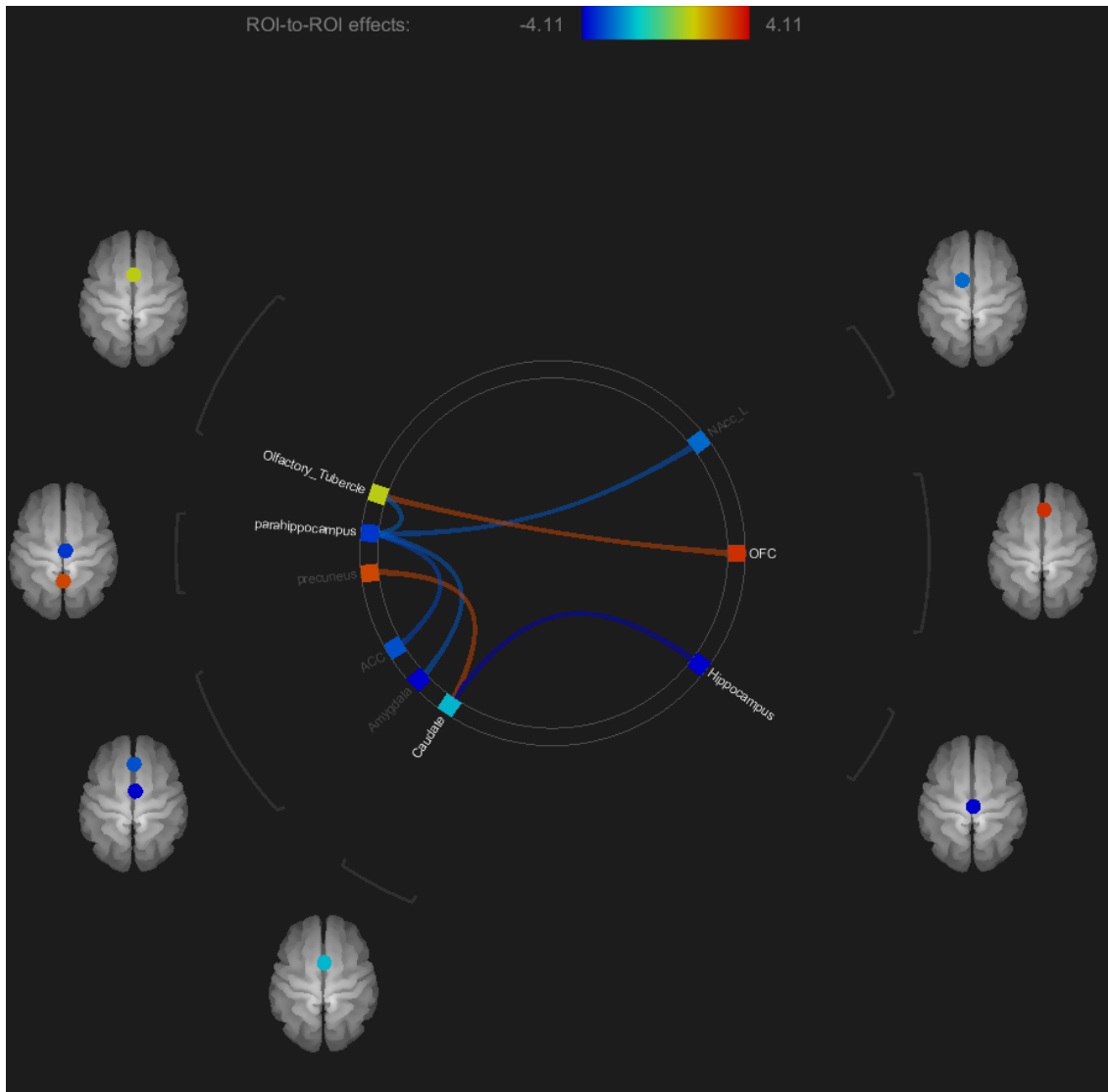
Figure IV.1. ROI-to-ROI connectivity patterns that significantly differed between the obese and normal weight groups.

Aim 2

Table IV.3 shows all significant associations between rsFC and outcome variables. After correcting for multiple comparisons, neither healthy nor unhealthy food consumption was significantly associated with rsFC between any of the regions tested.

Table IV.3

Associations between ROI-to-ROI Connectivity and Outcome Variables

Outcome Variable	Seed/Source	T	pFDR
Food RRV			
	R NAcc		
	Putamen	3.04	.042
	Posterior VLPFC	3.02	.042
	PCC		
	VMPFC	-3.32	.032
	VMPFC		
	PCC	-3.32	.032
Healthy commission error RT			
	L NAcc		
	Precuneus	3.95	.003
	Precuneus		
	L NAcc	3.95	.003

Food motivation. Higher food RRV was associated with stronger positive connectivity from the right NAcc to the putamen and the posterior VLPFC. Higher food RRV was associated with stronger negative connectivity between the PCC and the VMPFC (see Figure IV.2). Game RRV was not significantly associated with rsFC strength between any of the tested ROIs.
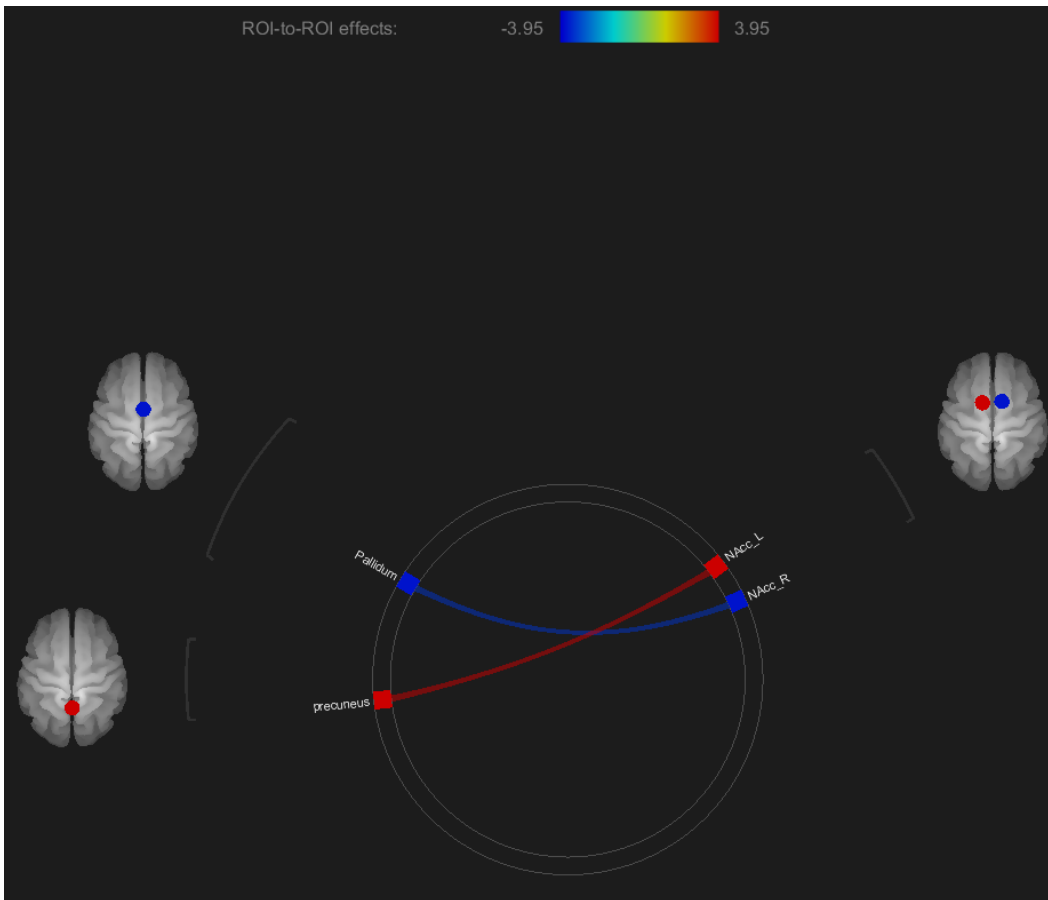
91

Figure IV.2. ROI-to-ROI connectivity patterns significantly associated with food RRV.

Inhibitory control. Slower reaction time during healthy commission errors was significantly associated with stronger positive connectivity between the left NAcc and the precuneus, and with stronger negative connectivity between the right NAcc and the pallidum (see Figure IV.3). Number of healthy omission and commission errors made was not significantly associated with connectivity strength between any of the tested ROIs. Number of unhealthy omission errors, commission errors, and RT during unhealthy commission errors were not significantly associated with connectivity strength between any of the tested ROIs.
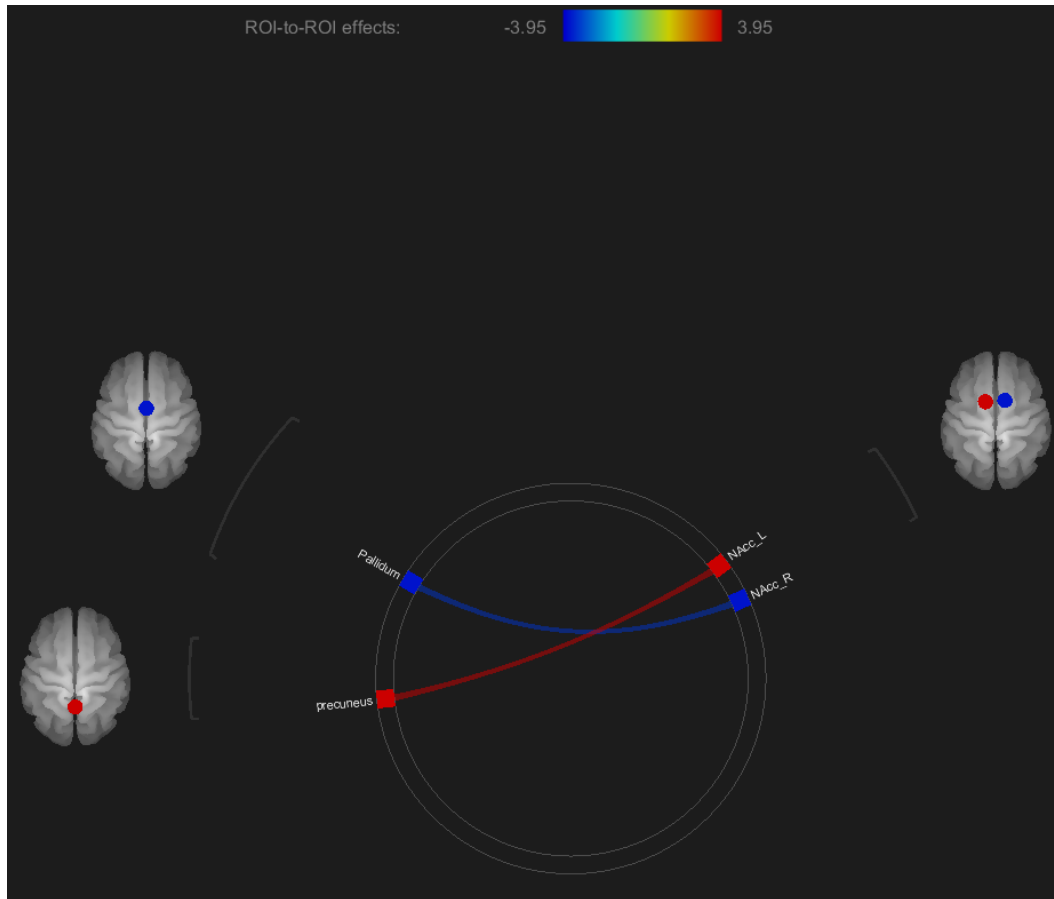
Figure IV.3. ROI-to-ROI connectivity patterns significantly associated with reaction time on healthy Go/No-go commission error trials.

Aim 3

For mediation analyses, we focused on connections and potential mediators that significantly differed by weight status (for group means, see Table IV.4). Number of healthy calories ordered and number of healthy calories consumed were found to differ significantly by weight status. Post-hoc analyses showed that only the normal-weight and overweight groups significantly differed from one another, with the overweight group ordering and consuming a greater number of healthy calories than the normal-weight group. Since there were no rsFC differences between the normal-weight and overweight groups, the requirements for mediation were not met. Thus, we did not conduct mediation analyses.

Independent Components Analysis (ICA)

We extracted 11 components using ICA. We then examined correlation coefficients showing overlap with masks of established networks, and visually inspected the components to determine whether they appeared to be reasonably matched to existing networks. One component appeared to be a match to the DMN ($r = .32$), containing regions including the precuneus and medial frontal cortex. None of the extracted components appeared to show strong overlap with the EFN or SN. Thus, we only included the component identified as the DMN in subsequent analyses. The extracted DMN component did not significantly differ by weight status, and was not significantly associated with any of the tested outcome variables.

Table IV.4
Means and Standard Deviations of Outcome Variables

	Normal-weight $M (SD)$	Overweight $M (SD)$	Obese $M (SD)$	F	p	η^2
Healthy calories ordered	207.75 (202.08)	338.23 (269.84)	222.03 (245.87)	4.43	.01**	.06
Healthy calories consumed	156.56 (178.92)	269.22 (244.24)	176.81 (242.72)	3.84	.02*	.05
Healthy calories consumed to ordered	0.70 (0.27)	0.77 (0.25)	0.65 (0.33)	1.20	.31	.02
Unhealthy calories ordered	714.10 (470.68)	524.52 (471.94)	689.51 (555.38)	2.05	.13	.02
Unhealthy calories consumed	605.69 (388.95)	455.12 (414.05)	597.03 (496.09)	1.82	.17	.02
Unhealthy calories consumed to ordered	0.87 (0.14)	0.87 (0.16)	0.87 (0.15)	0.01	.99	.00
Total calories ordered	954.50 (429.05)	903.12 (393.03)	953.04 (516.95)	0.20	.82	.00
Total calories consumed	794.23 (358.57)	762.83 (351.17)	812.77 (465.89)	0.16	.85	.00
Total calories consumed to ordered	0.84 (0.15)	0.85 (0.13)	0.84 (0.17)	0.09	.91	.00
Food RRV (log 2)	5.78 (2.57)	4.93 (1.82)	5.17 (2.51)	1.92	.15	.02
Game RRV (log 2)	6.24 (2.66)	6.62 (1.96)	6.40 (2.26)	0.33	.72	.00
Healthy omission errors	1.22 (1.87)	1.35 (1.67)	1.97 (2.56)	1.74	.18	.02
Healthy Go-trial RT (ms)	484.28 (51.04)	485.04 (42.95)	506.48 (53.03)	2.62	.08	.03
Healthy commission errors	6.44 (3.89)	6.17 (3.76)	5.32 (3.81)	1.02	.36	.01
Healthy commission error RT (ms)	444.85 (88.30)	435.88 (63.18)	445.10 (72.05)	0.19	.82	.00
Unhealthy omission errors	1.73 (1.92)	1.93 (2.14)	2.71 (2.96)	2.35	.10	.03
Unhealthy Go-trial RT (ms)	523.38 (48.27)	528.83 (44.80)	535.25 (47.60)	0.79	.45	.01
Unhealthy commission errors	9.62 (5.80)	9.40 (5.30)	9.94 (6.01)	0.08	.92	.00
Unhealthy commission error RT (ms)	480.79 (66.92)	484.64 (77.99)	494.96 (88.82)	0.43	.65	01

Discussion

The current study sought to examine the relationship between weight status, eating-related outcomes, and resting state functional connectivity. Individuals who were obese compared to normal-weight were found to have differences in rsFC strength between regions in the SN and DMN. While rsFC strength was associated with food motivation, participants who were obese compared to normal-weight did not exhibit differences in any of the behavioral outcomes, thus we did not conduct mediation analyses as the requirements for mediation were not met.

Weight-related Differences in rsFC

Individuals who were obese compared to normal weight were found to have stronger positive connectivity between the caudate and precuneus. The caudate, part of the SN, has been suggested to play a role in goal-directed action and the evaluation of reward-related outcomes (Grahn, Parkinson, & Owen, 2008), and the precuneus, often identified as a part of the DMN, has been implicated in functions such as mental imagery (Cavanna & Trimble, 2006). The incentive-sensitization theory of addiction suggests that increased motivation to obtain and consume food is heavily influenced by increased sensitivity to rewarding cues, and the assignment of excessive reward value to food-related stimuli (Berridge, 2012; T. E. Robinson & Berridge, 2000). Stronger connectivity between neural networks implicated in determining the salience of rewards and mental imagery at rest may suggest that rewarding stimuli are likely to become salient and engage cognitive resources during a period of rest, which could contribute to increased motivation and goal-directed action toward food consumption. Future research may do well to examine the relationship between rsFC and cue responsivity in individuals who are obese.

Participants who were obese compared to normal-weight also showed stronger rsFC between the OFC and the olfactory tubercle, both areas in the SN. The OFC is thought to play a role in emotional decision-making and the learning of cue-outcome associations, particularly assessing the value of a reward (Kerr & Zelazo, 2004; McDannald, Jones, Takahashi, & Schoenbaum, 2014). OFC dysfunction is also theorized to be involved in addiction, particularly compulsive drug-taking (Volkow & Fowler, 2000). Meanwhile, the olfactory tubercle is thought to be involved in mediating the effects of rewarding stimuli (e.g., cocaine) (Ikemoto, 2003, 2007). This rsFC pattern may suggest that the rewarding properties of stimuli are particularly salient in individuals who are obese, which may influence learning of paired cues and decision-making about consumption of food, possibly contributing to excessive consumption.

The obese compared to normal-weight group also showed stronger negative connectivity between areas in the DMN (hippocampus, parahippocampus) and the SN (caudate, amygdala, ACC). This connectivity pattern is in contrast to the stronger rsFC between the caudate and precuneus found in individuals who are obese compared to normal-weight. This may be due to differing functions between the specific DMN regions. The precuneus has been shown to be activated in response to cues (Burger & Stice, 2014; Carnell, Benson, Pantazatos, Hirsch, & Geliebter, 2014), while the hippocampus and parahippocampus have been shown to be involved in memory encoding (Eichenbaum, Otto, & Cohen, 1994). Greater negative connectivity between the SN and these DMN regions could suggest reduced salience of memories at rest in general. However, we cannot definitively conclude that this is the case, as the current study did not include analysis of memory-related task-based activation.

The overweight group did not show any significant connectivity differences from either the obese or normal-weight group. In the current study, we calculated weight status using zBMI,

to limit variability stemming from rapid developmental changes in this age range (Adair, 2008). Still, pubertal and muscle development in adolescents results in wide variability even in zBMI scores that may particularly impact the overweight category (Loomba-Albrecht & Styne, 2009). For example, individuals who are particularly muscular for their age may be categorized as overweight, along with those who have excess body fat. This combination of body composition within the same category may limit our ability to observe differences between the overweight group and either the obese or normal-weight group.

Behavioral Outcomes and rsFC

We found that higher food motivation was related to greater connectivity between reward-related SN regions (e.g., NAcc, putamen). This may suggest that individuals highly motivated by food may have a more integrated reward system, leading them to be more attentive to and highly influenced by reward. Higher food motivation was also associated with negative connectivity between DMN regions (e.g., PCC, VMPFC). Reduced integration of the DMN might suggest that these individuals experience intrusion of other functions while at rest, likely reward functions, given the strong rsFC between reward-related regions of the SN.

Slower reaction time during healthy trial No-go errors was associated with negative rsFC between reward-related SN regions (e.g., NAcc, pallidum) and stronger positive rsFC between the areas in the SN (NAcc) and DMN (precuneus). Faster RT during commission error trials could indicate lower inhibitory control, thus the current results appear to suggest that greater salience of reward overall and reduced intrusion of reward at rest may contribute to lower ability to inhibit prepotent response. However, this relationship was only present during healthy food trials, so further research is needed to fully understand the implications for palatable food consumption.

The observed associations between rsFC and behavioral outcomes provide insight into the practical implications of differences in neural architecture. Stronger connectivity within salience and reward circuitry and weaker connectivity within DMN areas may contribute to excessive motivation to obtain and consume palatable food. Additionally, stronger connectivity between these regions may be related to more successful inhibitory control, which could be viewed as protective when it comes to the overconsumption of palatable foods. These findings suggest that excessive food motivation may be marked by excessive influence of salience and reward processing intruding on DMN functions at rest.

While the current study found associations between rsFC and both weight status and the behavioral outcomes of consumption, food motivation, and inhibitory control, the obese and normal-weight groups did not differ on any of the behavioral outcomes, thus not meeting the requirements for mediation. Participants who were obese or overweight may have displayed reactivity to engaging in a study examining eating behaviors, being less likely to overconsume unhealthy food (and in the case of the overweight group, possibly consuming more healthy food) due to sensitivity to how consumption of different types of food may be perceived based on their weight status. Individuals who are overweight and obese often experience stigma due to their weight, and this stigma may have especially negative impacts during adolescence (DeJong, 1980; Falkner et al., 2001; Goldfield et al., 2010). In the current study, methodological efforts were made to reduce demand characteristics (e.g., participants were left alone during food consumption in order to minimize observation-related alterations in eating behavior). However, participants who were overweight or obese may have shown some reactivity due to engaging in a study about eating behaviors and obesity, thus inhibiting their consumption of foods recognized as unhealthy or consciously consuming more healthy food options. In future research, it would

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be important to understand how the experience of stigma may impact eating behavior (particularly food consumption) in the study setting for individuals who are overweight or obese.

It is also possible that in this age group, some individuals are beginning to display problem eating behaviors that place them at risk for obesity as they age, but have not yet experienced excessive weight gain. As the rsFC differences associated with problem eating behaviors were not specific to obese participants, this underscores the importance of early intervention and prevention efforts. Future studies may employ longitudinal design to fully understand the timing and development of rsFC changes, problem eating behaviors, and excessive weight gain.

Comparison with Task-based Results

The current rsFC findings appear to complement and build upon task-based findings from the parent study, which examined BOLD activation in response to commercials for healthy foods, unhealthy foods, and phones, as well as associations between BOLD activation and behavioral outcomes (food consumption, RRV, Go/No-go). In the parent study, greater BOLD activation in the right thalamus and right NAcc during unhealthy compared to phone commercials was found to be associated with greater unhealthy intake. Stronger rsFC between the right NAcc and the putamen, involved in learning and reward, was associated with greater food motivation as measured by the RRV task. This may suggest that a greater propensity to become attuned to rewarding stimuli and learn rewarding associations could be involved in higher motivation to obtain palatable foods, which could in turn result in greater unhealthy food consumption.

While the parent study did not find any significant relationships between task-based BOLD activation and performance on the RRV task, the current study did find associations

between rsFC and performance on this task. Understanding how behavioral tasks associated with constructs important in overeating and obesity relate to neural activity can bridge the gap between neuroimaging findings and clinical intervention. The relationship of RRV performance to resting-state, but not task-based connectivity in the current study suggests that food motivation may be driven by differences in basic functional architecture of the brain, rather than differences in how the brain responds to a particular task. This indicates that study of rsFC is a worthwhile addition to research on the neural correlates of overeating and obesity, as it can provide information beyond that yielded by task-based neuroimaging research.

Limitations and Future Directions

The current study had some substantial strengths making it a useful contribution to the literature on neural correlates of overeating and obesity. The relatively large sample size provided sufficient power to observe effects that may not be visible in a smaller sample. The current study also had participants across a wide BMI range, which allowed for the observation of differences across weight status groups. Additionally, the inclusion of behavioral tasks provided the opportunity to examine how rsFC differences may impact specific behaviors related to overeating and obesity. Still, some limitations of the current study provide ideas for future research directions. The current analyses employed a cross-sectional design, preventing us from making conclusions about how the observed effects may change over time. Examining changes in rsFC over time, particularly as related to task performance, will further elucidate the impact behavior may have on functional neural architecture and could inform the development of potential interventions. Though we controlled for individual differences (e.g., hunger), we did not specifically manipulate hunger and satiety in the current study. Given that a state of hunger versus satiety has been shown to impact the association between rsFC and obesity in adult

samples (Lips et al., 2014; Wijngaarden et al., 2015), manipulating this in adolescent samples would be an important future direction. With the exception of the DMN, independent components corresponding to established neural networks were not observable in the current study. While we were able to make inferences based on ROI-to-ROI connectivity, further research is needed to fully understand the influence of network-level rsFC differences on overeating and obesity in adolescents.

Conclusions

The current study provides support for the hypothesis that obesity and related behaviors are associated with alterations in rsFC in areas related to SN and DMN function. Specifically, stronger connectivity within the SN and negative connectivity within the DMN appears to be associated with greater motivation to consume food, and negative connectivity between the SN and DMN appears to be associated with obesity. These findings are consistent with existing adolescence research illustrating greater influence of reward and salience determination over other functions at rest (Black et al., 2014; Moreno-Lopez et al., 2016; Sharkey et al., 2019). Further, the current study's rsFC findings complement and extend findings from the parent study's task-based analyses, furthering our understanding of how neural architecture at rest is associated with obesity-related behaviors. Given that reward processing is highly influential in individuals who are obese and in those highly motivated by food, training adaptive response to rewarding stimuli is likely an important aspect of interventions aimed at reducing intake of unhealthy foods. It is hoped that the current study provides support for continued understanding of the impact of rsFC on overeating and obesity, and for the development of interventions aimed at strengthening the role of functions other than reward in adolescent eating behavior.

CHAPTER V

Integration and Conclusions

This dissertation aimed to provide a fuller understanding of environmental, psychological, and neurobiological influences on overeating and obesity. Study 1 examined the impact of a cue-rich context on food motivation and consumption, finding that an environment rich in food cues led to elevated food motivation, craving, feelings of hunger, and consumption of palatable foods. Study 2 sought to test a novel paradigm identifying sign-tracking and goal-tracking phenotypes of cue responsivity in children. While this paradigm did not reliably capture multiple phenotypes or find a relationship between sign-tracking and inhibitory control or food motivation, the development of this protocol provided valuable information for future study of these phenotypes in humans. Study 3 examined the association of rsFC with obesity and related constructs (inhibitory control, food motivation, and food consumption), finding that alterations in the connectivity within and between areas in the SN and DMN were related to differences in weight status, food motivation, and inhibitory control.

The above findings provide support for the targeting of elevated motivation to consume food, particularly in the presence of food-related cues, in interventions aimed at reducing problematic consumption. Studies 1 and 3 illustrated two potential pathways by which one might experience elevated food motivation: through cues in the environment, and through greater rsFC in the SN. Interventions aimed at teaching people to cope with craving have been shown to be

effective in reducing food and alcohol consumption (Bowen & Marlatt, 2009; K. T. Jenkins &

Tapper, 2014; Katterman, Kleinman, Hood, Nackers, & Corsica, 2014; Levoy, Lazaridou,

Brewer, & Fulwiler, 2017). Beyond the scope of this dissertation is the question of how such

training may impact neural connectivity, however there is evidence that behavioral intervention

targeted at craving in internet gaming disorder can change the structure and functional

architecture of the brain (Zhang et al., 2016). Future research might investigate whether such

changes might also occur in response to interventions targeted at food craving (e.g., mindfulness

training, urge surfing), thus reducing both behavioral and biological risk for overconsumption.

Study 3 also illustrated how individuals who are obese and those who are highly

motivated by food show greater rsFC within the neural salience network. Additionally, a highly

integrated SN was often accompanied by reduced connectivity between the SN and DMN,

suggesting that salience and reward processing is intruding at rest. This pattern could be

indicating that these individuals are engaging in cognitive processing of rewards such as food

even at rest, which may result in greater susceptibility when presented with rewarding stimuli.

While Study 2 was unable to observe distinct phenotypes of conditioned responses to cues, it is

possible that stronger SN rsFC is a marker of increased cue responsivity. Examining the

relationship between rsFC within the SN and cue responsivity phenotypes could be a promising

direction for future research aimed at understanding mechanisms underlying overconsumption of

palatable foods.

Study 1 of this dissertation also underscored the power of environmental cues in driving

eating behavior. We found cues to increase consumption even in a relatively healthy sample. For

participants at higher risk, for example due to increased cue responsivity or neurobiological

vulnerabilities, inhibiting a cue-driven urge to consume could be even more difficult. In addition

to training individuals to respond effectively to cravings, changing the food environment to reduce the omnipresence of cues would be a powerful intervention. The parent study to Study 3 found elevated activation in reward regions during commercials for unhealthy foods to be related to increased consumption of unhealthy foods, illustrating the impact of cues such as advertising on overconsumption for vulnerable individuals. Policies restricting the presence of unhealthy food cues (e.g., advertisements, fast food restaurants) may be effective in reducing excessive consumption and rates of obesity.

The current dissertation illustrates the impact of food motivation across development, from childhood into young adulthood. While a relationship between food motivation and cue responsivity phenotypes was not observed in childhood in Study 2, the illustration of how environmental cues impact craving and subsequent consumption in Study 1 highlights the necessity of understanding how food motivation develops. rsFC differences associated with elevated food motivation were observable in adolescence in Study 3, and if not addressed, elevated "wanting" may lead to more problematic consumption as individuals develop into adulthood and make increasingly independent food choices. It is possible that a connection between food motivation and problem consumption is not yet apparent early in development, however the impact as children age into adolescence and adulthood suggests that early intervention efforts are worthwhile.

In sum, the findings of the current dissertation have provided insight into factors contributing to overeating and obesity, as well as potential treatment targets. Increased food motivation and craving may arise due to both biological predisposition and environmental cues. Interventions aimed at coping with cravings and responding adaptively to rewarding stimuli can address both behavioral and biological risk factors, reducing vulnerability to overeating and

becoming obese. Additionally, environmental interventions aimed at reducing exposure to food-related cues could result in lower risk of engaging in cue-driven problem eating behaviors. It is hoped that the current dissertation provides rationale for continued research on such intervention and prevention efforts, thus contributing to the reduction of obesity rates and associated health problems.

APPENDIX

Text of Study 2 Pre-registration

As Predicted: *"Sign-tracking and associated traits in human children"* **(#18607)**

Created: 01/15/2019 05:21 PM (PT)

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1) Have any data been collected for this study already?

It's complicated. We have already collected some data but explain in Question 8 why readers
may consider this a valid pre-registration nevertheless.

2) What's the main question being asked or hypothesis being tested in this study?

We expect that children aged 5-7 classified as sign-trackers (ST) will be higher in impulsivity
and reward-driven eating compared to those classified as non-sign-trackers (nST).

3) Describe the key dependent variable(s) specifying how they will be measured.

Impulsivity will be measured using the Go/No-go (GNG) task, requiring individuals to inhibit

prepotent response to stimuli. Task outcomes are percentage of Go errors (i.e., misses) and No-go errors (i.e., false positives), and reaction time for both Go trials and No-go errors. Impulsivity will also be assessed using parent-report measures, including the subscales making up the Behavioral Regulation index (BRI; includes Inhibit, Shift, and Emotion Control) of the Behavior Rating Inventory of Executive Functioning (BRIEF), as well as the Attentional Focusing, Impulsivity, and Inhibitory Control subscales of the Children's Behavior Questionnaire-Revised (CBQ-R). Reward-driven eating will be assessed behaviorally using the Relative Reinforcing Value of Food (RRV) task, requiring subjects to make a progressively higher number of responses to gain access to a food reward. A higher number of responses made indicates higher food motivation. We will also use the Food Responsiveness subscale of the Children's Eating Behaviour Questionnaire (CEBQ), a parent-report measure.

4) How many and which conditions will participants be assigned to?

Due to the observational design, there are no experimental conditions. Participants were classified as ST or nST based on responses to the final block of a Pavlovian conditioning task, measured by automated data, video observation, and in vivo behavioral observation. Behaviors during this task are coded numerically, to calculate Pavlovian Conditioned Approach Index (PCA-index; for automated data only) and response bias (for automated, video, and response bias) scores, which range from -1.0 to 1.0. Individuals with scores ranging from 0.5 to 1.0 will be considered ST, while those with scores below 0.5 will be considered nST. Groups will be calculated separately for each measurement method.

5) Specify exactly which analyses you will conduct to examine the main

question/hypothesis.

2-sample t-tests will be conducted to test whether scores on each of the dependent variables differ significantly between the ST and nST groups. These analyses will be conducted separately for each measurement method.

6) Describe exactly how outliers will be defined and handled, and your precise rule(s) for excluding observations.

Participants who complete at least 2 but fewer than the full 4 blocks of the Pavlovian conditioning task will be classified as ST or nST based on performance during their final block. Those who do not complete at least 2 blocks will be excluded. Participants will be excluded if technical malfunction prevents collection of valid data from the Pavlovian conditioning task. Finally, participants who are missing data for any of the dependent variables will be excluded from analyses involving that variable only.

7) How many observations will be collected or what will determine sample size? No need to justify decision, but be precise about <u>exactly</u> how the number will be determined.

We planned to end data collection at the end of June 2018 or upon reaching 70 child participants, whichever came first. Data collection ended on 6/24/18 with a total sample of 64 child participants.

8) Anything else you would like to pre-register?

(e.g., secondary analyses, variables collected for exploratory purposes, unusual analyses

planned?)

Due to the novelty of the current study design, with no clear analogue in this type of sample, the investigators conducted preliminary descriptive analyses to determine how best to categorize individuals as ST or nST. Group comparisons with the dependent variables have not yet been conducted.

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